SCREENING OF INDIGENOUS MEDICINAL PLANTS IN NORMAL AND ALLOXAN DIABETIC RABBITS FOR ANTIDIABETIC ACTIVITY

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DEPARTMENT OF ANIMAL SCIENCES

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This thesis is submitted in partial fulfillment of the thesis requirement for the Degree of Doctor of Philosophy.

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IN THE NAME OF ALLAH

The Most Merciful,
The Most Gracious

Dedicated to

My Beloved Deceased

Parents

CERTIFICATE

This thesis submitted by Mr. Abdul Mateen Khan is accepted in its present form by the Department of Animal Sciences as satisfying the thesis requirement for the degree of Doctor of Philosophy in Endocrinology.

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In the name of ALLAH, the beneficent, the Merciful.
Praise to be ALLAH, Lord of the world,

The beneficent the Merciful:
Owner of the day of judgment

These (alone) we worship:
Thee (alone) we ask for help

Show us the straight path

The path of those whom thou has favour.
Not (the path) of those who earn
Thine anger,
Nor of those who go astray.

(*Al- Fateha*)

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ABDUL MATEEN KHAN

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AIDS	Auto Immune Deficiency Syndrome
ALT	Alanine Aminotransferase
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CCl_4	Carbon tetrachloride
CM	Centimeter
CMC	Carboxymethylcellulose
CNS	Central Nervous System
COX	Cyclooxygenase
CR	Catharanthis voscus
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
FFA	Free Fatty acids
GLP	Glucagons-like peptide
GLUT	Glucose Transporters
GTF	Glucose Tolerance Factor
HDL	High Density Lipoprotein
HLA	Human Leukocyte Antigen
IDDM	Insulin Dependent Diabetes Mellitus
IGT	Impaired Glucose Tolerance
KG	Kilogram
LADA	Latent Autoimmune Diabetes of Adulthood
LDL	Low Density Lipoprotein
MG	Milligram
NIBGE	National Institute of Biotechnology and Genetic Engineering
NIDDM	Non Insulin Dependent Diabetes Mellitus
NIH	National Institute of Health
NPH	Neutral Protamine Hagedorn
NS	Non Significant
NSAIDs	Non-steroidal anti-inflammatory drugs
NWFP	North West Frontier Province
RDA	Recommended Daily Allowance
RNA	Ribonucleic Acid
PG	Psidium guajava
SEM	Standard Error of Means
STZ	Streptozotocin
UK	United Kingdom
USA	United State of America
USP	United States Pharmacopoeia
WHO	World Health Organization

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ABSTRACT

The study was purposefully designed to investigate the *hypoglycaemic* effects of the indigenous medicinal plants *Cassia sophera, Caralluma tuberculata* and *Achillea santolina* allegedly used in ethnomedicine for the remedy of diabetes mellitus since time immemorial in India and Pakistan. First four experiments were designed to find out the *hypoglycaemic* effects of *Cassia sophera* powdered seeds. Effects of powdered seeds on blood glucose levels in normal non-diabetic rabbits were carried out in increasing doses i.e. 2, 3, 4g/kg body weight at 0, 2, 4, 8, 12 and 24 hours intervals. It was found that there was no significant decrease at 4 hours interval with 2g/kg body weight but significant decrease occurred at 8 and 12 hours intervals, while highly significant decrease in mean blood glucose level were observed at 4, 8 and 12 hours with both 3g/kg and 4g/kg body weight.

The effects of methanolic and aqueous extracts equivalent to 4g/kg body weight of *Cassia sophera* powdered seeds were also studied in normal rabbits which showed significant decrease ($p<0.05$) at 8 hours and 12 hours intervals. However, the methanolic extract of *Cassia sophera* seeds produced better *hypoglycaemia* as compared to aqueous extract. The effects of these extracts were comparable to Acetohexamide one of the oral *hypoglycaemic* agent.

The same study was also done in alloxan-induced diabetic rabbits and it was noticed that increasing doses 2, 3 and 4g/kg body weight of *Cassia sophera* powdered seeds caused significant decrease in blood glucose level and highly significant decrease ($p<0.001$) in blood glucose level was noticed with methanolic extracts at 4 hours interval which continue up to 8 hours while treatment with aqueous extract with *Cassia sophera* seeds showed highly significant decrease at 12 hours time. It seems that powdered seeds of *Cassia sophera* contained more than one type of *hypoglycaemic* principles, which have exerted a significant and consistent *hypoglycaemic* effects in normal as well as in diabetic rabbits. No change in the normal behavioral pattern in the treated animals or toxic adverse effects were observed during the study.

The next four experiments were designed to study the *hypoglycaemic* effects of both *Caralluma tuberculata* and *Achillea santolina,* which are also used as traditional

medicine for treating diabetes mellitus in this subcontinent. The study was designed to investigate the *hypoglycaemic* effects of these medicinal plants together. The experiments were carried out with crude extract and carbon tetrachloride (CCl_4) fractions in capsule and cooking oil forms of both plants and compared in different dosage forms. In the first experiment, the crude extract 200mg/kg body weight of *Caralluma tuberculata* and *Achillea santolina* showed highly significant decrease ($p<0.001$) in mean blood glucose level at 2, 4, 8 and 12 hours. But these *Caralluma tuberculata* vs *Achillea santolina* showed highly significant ($p<0.001$) difference at 2, 4, 8 and 12 hours times. The metformin 500mg/kg body weight was also given to compare its effects with the plant crude extracts, which was more or less intermediate between that of crude extracts of *Caralluma tuberculata and Achillea santolina.*

Both crude extracts of both plants caused highly significant decrease ($p<0.001$) in blood glucose levels in diabetic rabbits. It was also noticed that Metformin (glucophage) appears to be less effective compare to that with *Caralluma tuberculata* and *Achillea santolina.*

The diabetic rabbits were treated with carbon tetrachloride (CCl_4) fractions 100mg/kg body weight of *Caralluma tuberculata* and *Achillea santolina* in capsule form, blood glucose level dropped markedly at 2 hours and further more at 4 hours. These effects were compared with Metformin, which showed that *Achillea santolina* produces significantly low blood glucose level quite late as compared to Metformin.

The diabetic rabbits were also treated with carbon tetrachloride (CCl_4) fractions 100mg/kg body weight of both *Caralluma tuberculata* and *Achillea santolina* in cooking oil. Highly significant decrease ($p<0.001$) in blood glucose levels occurred in 2, 4, 8 and 12 hours intervals. However, it showed that *Caralluma tuberculata* administered in cooking oil leads to higher reduction in blood glucose level than that administered in capsule form.

It is therefore, concluded that *Caralluma tuberculata* is one of the better *hypoglycaemic* agent to be used as herbal medicine for the treatment of diabetes mellitus. It is also known well for its vegetables usages since centuries, causing no apparent adverse effects.

INTRODUCTION

PLANTS AND MEDICINES

Plants have been in the use of human being since ages for food, shelter and clothing, which are primary needs of mankind. Plants have influenced man's religious expressions; his architecture and his use of ornaments and in large measure have influenced degree of civilization, which he has attained.

All the higher forms of life (animals) depend upon directly or indirectly on plants for their food. The human food usually consists of cereals, legumes, fruits etc. Similarly, leafy vegetables and the stems of the plants like cabbages, cauliflower, asparagus and sugar cane etc are popular food substances. The roots used as food include beet, reddish, carrot, turnip and sweet potato etc.

Since time immemorial, plants have been used for healing purposes (Buchman, 1980). Even as modes of medicine changed throughout the centuries, plants continued to be mainstay of country medicine and passed down from family to family and within communities. Thus, tribes, villages, towns and sometime entire countries tended to have similar styles in healing (Ali and Nasir, 1983). Many people know nothing of chemistry, pharmacognosy or pharmacology have ears better tuned to catch remedies, that nature has secretes in the roots, stem, bark, juice, flowers and seeds of plants some of them are now world wide remedies (Buchman., 1980).

Knowledge of medicinal plants has origin in the 4^{th} to 5^{th} centuries B.C under the patronage of Hippocrates in Greece and from there this system moved to Iran and in the middle of 8^{th} century, it came to Baghdad and from 750 A.D to 850 A.D, learned Muslims, Christians and other scholars translated a large number of important work of philosophy '*Tibb*' and different sciences into Arabic (Buchman, 1980). The mixture of '*Tibb*' and Ayrovedic medicine is called "Eastern medicine" (Said, 1991).

In the early 1990s, herbs and herbal preparations constituted a substantial portion of the pharmacopoeia of the United States. Many pharmaceuticals in common use today had their origins in herbal preparations (aspirin from willow bark, digitalis from foxglove etc (Brent and Bauer, 2000).

As the Science, progressed, the whole crude drugs were replaced by their extracts in the modern era. Moreover, the chemical nature of the active constituents in crude drugs, their isolation, purification and their synthesis has tremendously advanced the medical science. Most of recent medicines are either synthetic or prototypes of the natural drugs, which though are effective but very costly and have many serious side effects. These facts along with present socio-economic and industrial factors convinced the mankind to revert to "Nature" (Gilani *et al.*, 1992).

Many if not most pharmacological classes of drugs include a natural product prototype. Morrphine, digoxin, quinine, atropine, reserpine, physostingmine, pilocarpine, vincristine and vinblastine are just a few examples of what medicinal plants had given in the past. Furthermore, some alkaloids have limited applications in modern medicines. But they are valuable as pharmacological "tools" for elucidating the modes of action of other drugs or investigation for basic physiological mechanisms. Some examples are atropine, (antimuscarinic agent) Cocaine, (a non-epinephrine uptake inhibitor) reserpine and yohimbine, (a specific adrenergic antagonist). A very recent addition in the discovery of yohimbine, an alkaloid derived from the plant *Galbulimima baccata,* as a cardio–selective antimuscarinic agent and is a novel pharmacological tool for the study of muscarinic receptor subtypes in cardiac and smooth muscles (Gilani *et al.*, 1992).

Biguanides includes the drug metformin, which was originally derived from a medicinal plant, *Galega officinalis*. Metformin reduces plasma glucose via inhibition of hepatic glucose production and increase of muscle glucose uptake (Bailey and Day, 1989).

In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries

because of their natural origin and less side effects. Many tradional medicines in use are derived from medicinal plants, mineral and organic matter (Grover *et al.*, 2002).

An active principle artemisinine has been isolated from a Chinese indigenous medicinal plant *Artemisia annua* crude extract of which has been used against malaria for 2000 years in China. "Qinghaosu" has been evaluated for activity against *Plasmodium falciparum* and potentially has significant clinical utility against chloroquine resistant *falciparum* malaria even better than pyrimethamine-sulfadoxine combination and quinine (Van Agtmael *et al.*, 1999).

A considerable number of *hypoglycaemic* plants and herbs are known through ethnomedicine but their introduction into modern therapy await pharmacological testing by modern methods (Volkovic, 1975).

Augusti and Benaaim (1974) have reported that *Allium cepa* (onion) and its extract exert distinct slowly developing *hypoglycaemic* action. The alkaloids obtained from *Trigonella foenum graceum* (*Maithee*) and *Lupinus termis* (*Turmnas*) exert *hypoglycaemic* activity in normal and alloxan diabetic rabbits. Akhtar *et al.*, (1981) and Akhtar (1982) have reported that whole dried *Momordica charantia* fruit (*Karela*) exerts significant and consistent *hypoglycaemic* effect in normal and diabetic rabbits. Similarly, Akhtar and Ali (1985) have observed that various doses of *Cumum nigrum* (*Kala zera*) produce a significant *hypoglycaemic* effect in normal as well as in diabetic rabbits. Akhtar and Riffat (1986) have reported that various doses of powdered fruit of *Cassia fistula Linn* (*Amaltas*) produce a significant *hypoglycaemic* effect in normal and alloxan diabetic rabbits at different time intervals. Akhtar (1992) has reviewed 26 indigenous medicinal plants and described several plants drug, which possess *hypoglycaemic* effects both in normal and diabetic rabbits. He has observed that the *hypoglycaemic* principals in these plants and in their aqueous and methanolic extracts not only exerts an insulin releasing effects but also direct insulin like effect in the normal rabbits. A water-soluble compound from the leaves of *Gymnema sylvestre*, which reduced the insulin requirements in the patients with insulin dependent diabetes mellitus. *Gymnema sylvestre* aqueous extract

appears to enhance endogenous insulin, possibly by revitalization of the residual β cells in insulin dependent diabetes mellitus (Shanmugasundram *et al.*, 1990).

The extracts of leaves and roots of *Cococinia indica* showed significant *hypoglycaemic* effect in normal rats. Pectin administration resulted in significant reduction in blood glucose levels. The reduction may be due to the decreased absorption of glucose from intestine, higher rate of glycolysis because of higher amount of liver glycogen in pectin-administered groups. The ethanolic extract of *Cococinia indica* has *hypoglycaemic* properties but alkaloids extracted from it have no such activity. Leaf preparation of this plant significantly lowers the blood glucose levels (Kumar *et al.*, 1993).

The ethanol extract of the whole plant *Swetia chirayita*, administered with different doses and effect recorded at different times showed the maximum lowering of blood sugar in albino rats was highly significant after 3 hours of administration (Bajpai *et al.*, 1991). Water-soluble mucilages from plants of *Liliaccae*, *Malvacae*, *Discoreacae*, *Orchidacae*, *Sacifragacae*, *Amaryladacae* and *Plantaginacae* for their *glycaemic* activity (Tomoda *et al.*, 1987). Plants from *Malvaceae* showed considerable lowering of blood glucose compared to plants from other families. *Mucilages* obtained from *Malvaceae* posses trisaccharide structural unit in their main part. These factors may have trisaccharides, which show high *hypoglycaemic* activity.

The properties of *Momordica charantia* (bitter gourd) with water extract of the fruit on alloxan diabetic rats (Srivastava *et al.*, 1993). The fall of blood sugar with water extract was more effective in diabetes after 3 weeks treatment than the dried powder of fruit. They found highly significant effect of aqueous extract of fruit than powder of fruit the later showed only 25% fall of blood sugar, which was not significant. Khanna *et al.*, (1985) showed the presence of polypeptide in the fruit of bitter gourd. Srivastava *et al.*, (1993) gave clinical trials with water extract of bitter gourd for 2, 3, 4 and 7 weeks until the level stabilized or returned to normal limits. They observed that aqueous extract of the fruit induces a better adaptation against diabetes with control of *glycaemia* as reflected by delay in the appearance of cataract. They found that different doses of bitter gourd juice fed with high fat diet significantly lowered energy efficiency and tended to

have less visceral fat mass. They also observed rats fed on high fat diet plus bitter gourd juice gained significantly less weight and visceral fat than the control.

Herbal plants like *Allium sativum* (Garlic), *Ocmum sanctum* (Tulsi), *Azadirachta* (Neem) and *Momordica charantia* (Bitter gourd) not only posses *hypoglycaemic* activity but also they are hypotensive, hepatoprotective and blood purifier (Grover *et al.*, 2002). Mahdi *et al.*, (2003) injected water extracts as well as dried leaves powder of these herbal plants in streptozotocin induced diabetic rats. They observed significant increase in lipid peroxide levels in streptozotocin induced diabetic rats compared to normal. However, there was significant decrease in lipid peroxide levels in diabetic rats treated with herbal preparations of above plants. They found in part through their fibre, vitamin or mineral contents and some secondary metabolite. Herbal deficiencies are common in diabetic patient, which aggravates insulin deficiency. Several minerals found in some medicinal plants have been reported to be cofactors that signal intermediaries of insulin action and key enzymes of glucose metabolism (Day *et al.*, 1990).

Oral *hypoglycaemic* activity of some Sri Lankan medicinal plants, most widely used Like *Salacia reticulate* (*Calestracea*), *Aegle normals* (*Rutaceae*), *Momordica charantia* (*Cucurlitacae*) were investigated by Karunanayake *et al.*, (1984). Their results indicated that aqueous extracts of these plants had significant *hypoglycaemic* effect. The magnitude of this effect showed time related variation with these plants. The highest oral *hypoglycaemic* effect was associated with the extract of *Momordica charantia*, but least however, significant effects were observed with *Salacia reticulate*. This study also shows time related variation in *hypoglycaemic* effect of the extracts from these plants used. The use of medicinal plants for their anti-diabetic activity seems to be universal, but not restricted to Pakistan and India. In different countries of the World, many medicinal plants have been used to control diabetes and complications caused due to their disorder. Mehmet *et al.*, (2006) have used medicinal plants *Astemisia herba-alba* and *Tencruim plium* for therapy of diabetes mellitus in turkey. They observed that these plants produced significant hypoglycaemic effect in normal and diabetic rabbits, but insignificant effect due to *Teucrium polium*. Abu El-Soud NH *et al.*, (2007) in Egypt investigated the effect

of alkaloid extract of fenugreek-dried seeds (*Trigonella foenum – graceum*). They suggested that mode of action of these plants may be caused by their contents of alkaloids through reducing blood glucose level, thereby preventing *hyperglycaemia* during diabetes and reducing lipid profile almost normal and reducing the oxidative stress together with converting liver and kidney pathology caused of diabetes to normal pattern (Abdul-Barry *et al.*, 1997)

Fourteen medicinal plants from different families and localities in South Africa. They investigated four of these fourteen plants, which were frequently recommended by traditional healers and rural dwellers. These plants were *Herichryseem odoratissimum*, *Herricheysum patrilore*, *Heypasin hemocallidea* and *Hypoxin colchilcifolia*.

The effects of crude garlic and its two organic extracts on blood glucose level of rabbits were studied by Nyyer *et al.*, (1989). It was found that the petroleum ether extract and crude garlic juice had some *hypoglycaemia* in the experimental animals, where as no effect was observed on the group fed on ethanol extract.

Allium sativum (Garlic) and *Allium cepa* (Onion) have been used for several thousand years to flavour food and for their medicinal properties have been claimed to be beneficial in infection, diabetes, hypertension, hyperlipidaemia, hypercholestrolaemic and atherosclerosis. Garlic should also be avoided in patients on aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) because it enhances antiplatelet activity. Three main antiplatelet constituents, namely adenosine, allicin and paraffinic polysulfide are present in both garlic and onion. Adenosine and allicin both inhibit platelet aggregation without affecting cyclo-oxygenase and lipo-oxygenase metabolites of arachidonic acid. The trisulfides inhibit platelet aggregation as well as thromboxane synthesis along with induction of new lipoxygenase metabolites. The data indicate that the observed in vivo antiplatelet effects of ingesting onion and garlic are attributable more to the adenosine than to the allicin and paraffinic polysulfide constituents (Makheja and Bailey, 1990).

Zingiber officinale (Ginger) has been widely used in India and China as both a spice and a medicine for at least 2500 years. The tuberous rhizome part of ginger is used for the treatment of headaches, colds, digestive and appetite problems, and rheumatological conditions. In Western herbal medicine practice, ginger is used primarily in the prevention of motion sickness and postoperative nausea and vomiting. It is also used as anti-inflammatory. The anti-inflammatory properties of ginger have been known and valued for centuries. During the past 25 years, many laboratories have provided scientific support for the long-held belief that ginger contains constituents with anti-inflammatory properties. The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed. This discovery identified ginger as an herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger suppresses prostaglandin synthesis through inhibition of cyclo-oxygenase- 1 and cyclo-oxygenase-2 (Grzanna *et al.*, 2005).

It has been studied that in Olive oil a higher proportion of monounsaturated fatty acid like oleic acid is present which is linked with a reduction in the risk of coronary heart disease. Olive oil has favourable effects on cholesterol regulation and LDL cholesterol oxidation and it exerts anti-inflammatory, antithrombotic, antihypertensive and hypoglycaemic effect both in animals and humans (Ferrara *et al* 2005 and Parillo *et al.*, 1992). It has been suggested that long-term consumption of small quantities of this compound from olive oil may be responsible in part for the low incidence of heart disease associated with a Mediterranean diet (Bogania *et al.*, 2007).

The World Health Organization (WHO) has listed 21000 plants, which are used for medicinal purposes around the World. Among these, 2500 species are in India out of which 150 species are used commercially on a fairly large scale (Seth and Sharma, 2004).

The medicinal usage of plants, their extracts and even pure chemical compounds isolated from natural products have been widely used all over the globe both in human beings and animals. Innumerable indigenous medicinal plants have been employed to treat various diseases since centuries. Several such plants have been tested by the pharmacological

methods and have now been found to posses active principles useful for treating various diseases (Panday, 1997).

Herbal European community legislation defines a "Medicinal Product" as any substance or combination of substances which may be administered to human being or animal with a view to making diagnosis or to resorting correcting or modifying physiological functions in human beings or animals is like wise considered as "Medicinal Product" (Brent and Bauer, 2000). The legal status of herbal products is complicated by the fact that in most of the countries including Pakistan; they are available both as medicinal products and as food supplement (Cranz, 1994).

There has been resurgence of scientific interest in medicinal plants in the Western world during the past 20 years. The interest has been rekindled by the worldwide importance of medicinal plants and crude drugs in tradional medicine. Moreover, there have been attempts at more-precise studies on such medicinal plants. Besides the use of purified natural substances, a growing number of crude plant extracts are now being utilized in naturopathic remedies. These remedies are an integral part of what today is called phytotherapy. It should be borne in mind that it is not necessary for the active component to be isolated and the structures established for a plant extract to be active. Indeed, it is possible that structurally different compounds present in a crude extract may have synergistic effect. Thus, there is a potential today for the industrial development of Tradional herbal remedies and plant extracts for the treatment or prevention of disease. The complexity of these 'drugs' and their inherent biological variation, makes it necessary, but also very difficult, to evaluate their safety, efficacy and quality. There are several problems associated with the development of plant-based drugs (Gilani *et al.*, 1992).

DIABETES MELLITUS

Clinical features similar to *Diabetes mellitus* were described 3000 years ago by the ancient Egyptians. The term "Diabetes" a Greek word, means "to run through a siphen" was first coined by "*Aretaeus of Cappodocia*" (81-133 AD) to describe the plyurea.

Later, the word "mellitus" (honeyed or sweet) was added by 'Cullen' in 1675 after rediscovering the sweetness of urine and blood of patients. It was only in 1776 that Dobson (Britain) firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness. In modern time, the history coincided with the emergence of experimental medicine. An important milestone in the history of diabetes is due to excess glucose production Claude Bernard (France) in 1857. The role of the pancreas in pathogenesis of diabetes was discovered by Merning and Minkowaski (Austria) in 1921 (Ahmad *et al.*, 1993).

Diabetes mellitus is a syndrome with disordered metabolism and inappropriate *hyperglycaemia* due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate.

Diabetes mellitus is a complex disorder that is characterized by *hyperglycaemia* due to insufficient insulin secretion and/or insulin action both resulting in impaired metabolism of glucose, lipids and proteins. Some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic (Akhtar, 1995).

Diabetes is a major public health problem worldwide. It currently affects 246 millions people and this is expected to increase to 380 million by the year 2025. Pakistan is a South-Asian country with a population of approximately 160 millions. Diabetes prevalence Pakistan is high: 12% of people above 25 years of age suffer from the condition and 10% have ***impaired glucose tolerance (IGT).*** When one considers the associated risk factors present in Pakistani society, the large number of people with diabetes is no surprise. Obesity tops the list. Figures based on ***Body mass index (BMI)*** show 37% of men with diabetes and 79% of women were obese. According to a Diabetic Association of Pakistan study into chronic complications, involving 500 people with diabetes, eye damage (retinopathy) affected 43% of the people, kidney disease (nephropathy) 20%, and nerve damage (neuropathy) 40% (Shera *et al.*, 2002).

The prevalence of diabetes in Pakistan is 6.0% in men and 3.5% in women in urban and 6.9% in men and 2.5% in women in the rural areas. IGT in the urban areas versus rural

areas is 6.3% in men and 14.2% in women against 6.9% in men and 10.9% in women. The over all prevalence of glucose intolerance, including diabetes mellitus and IGT is 20.04% in urban and 17.15% in rural areas (Shera *et al.*, 2007).

Type-1: Diabetes Mellitus

Type 1 diabetes in 90% of cases is due to pancreatic islet β cells destruction predominantly by an autoimmune process (Eisenbarth, 1986 and Bach, 1994). These patients are prone to ketoacidosis. Type 1 diabetes accounts for only about 5—10% of all cases of diabetes; however, its incidence continues to increase worldwide. The disorder has a strong genetic component, mostly triggered by viruses. Most commonly arises in children and young adults with a peak incidence before school age. Circulating insulin is virtually absent, plasma glucagon is elevated, and the pancreatic β cells fail to respond to all insulinogenic stimuli (Daneman, 2006). Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis, reduce the *hyperglucagonemia* and reduce blood glucose.

Fewer than 10% of subjects, most of these are of Asian or African origin (Imagawa *et al.*, 2000) has no evidence of pancreatic β-cells autoimmunity to explain their insulinopenia and ketoacidosis. This subgroup has been classified as "idiopathic type-1 diabetes".

Type 2 Diabetes Mellitus

Type 2 diabetes is the more prevalent form and results from insulin resistance with a defect in compensatory insulin secretion, is milder form of diabetes that occurs predominantly in adults but occasionally in juveniles. More than 90% of all diabetes in the United States is included under this classification. Circulating endogenous insulin is sufficient to prevent ketoacidosis but is inadequate to prevent *hyperglycaemia* due to tissue insensitivity. In most cases of this type of diabetes, the cause is unknown. However, genetic factor, which is aggravated in time by additional enhancers of insulin resistance such as aging, a sedentary life style and abdominal-visceral obesity. In addition, there is an accompanying deficiency in the response of pancreatic β cells to glucose (Stumvoll, 2001).

Diabetic patients with abdominal obesity displayed peripheral insulin resistance in combination with defective insulin secretion, whereas non-obese diabetic patients showed only a secretory defect to glucose; however, it can be elicited in response to other insulinogenic stimuli such as acute intravenous administration of sulfonylureas glucagons or arginine (Arner *et al.*, 1991). Thus, type 2 diabetes in obese and non-obese elderly male subjects may take two forms where the cause of *hyperglycaemia* differs (Allison *et al.*, 2001).

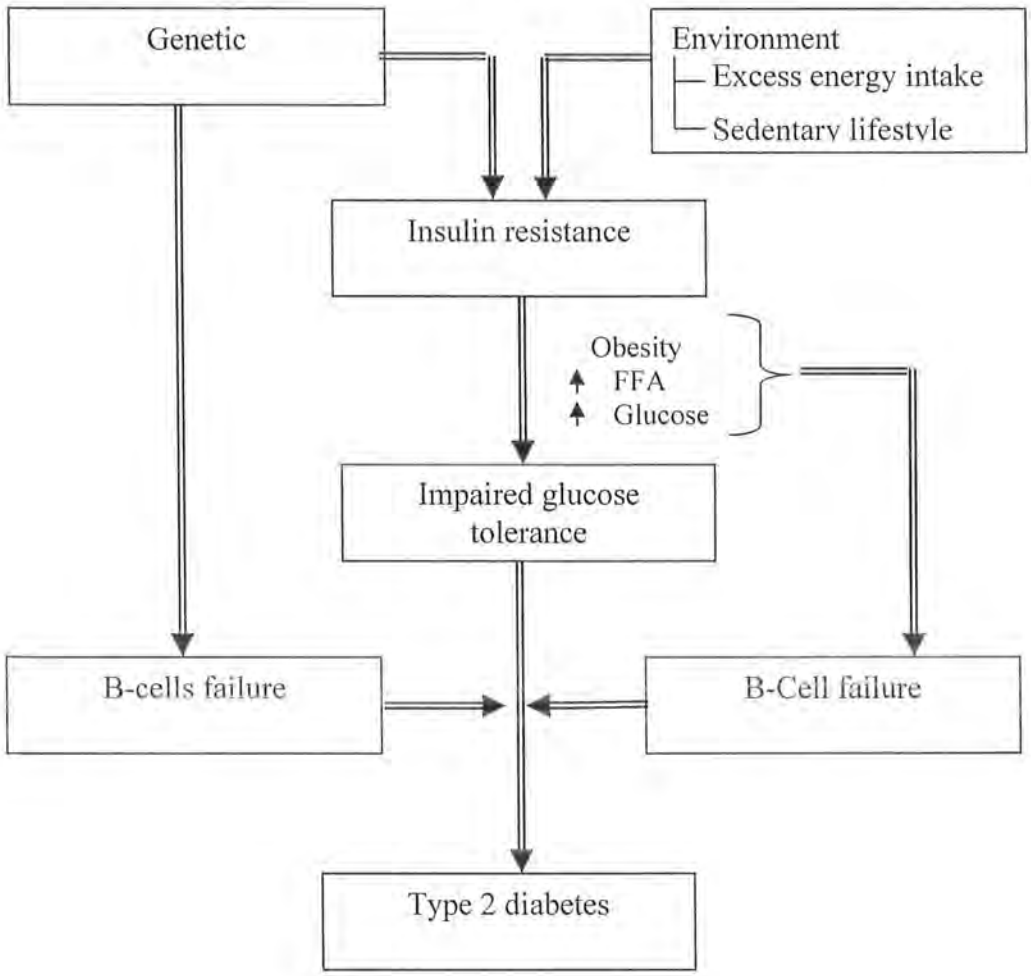

Figure 1: Overview of the pathogenesis of type 2 diabetes mellitus (Alice *et al.*, 2005).
(FFA = Free Fatty Acid)

TREATMENT REGIMENS

DIET

A well-balanced, nutritious diet remains a fundamental element of therapy. However, in more than half of cases, diabetic patients failed to follow their diet. In prescribing a diet, it is important to relate dietary objectives to the type of diabetes. In obese patients with mild *hyperglycaemia*, major goal of diet therapy is weight reduction by caloric restriction, low carbohydrate and lipids intake. In non-obese diabetics emphasis on timing of meals or periodic snacks, all of which are so essential in the treatment of such patients.

Addition of soluble fibers in diet such as gums and pectins found in bean, oatmeal etc, prevent glucose absorption and *hyperglycaemia* may slightly diminish in diabetics (Chandalia *et al.*, 2000). The ADA recommends food such as oatmeal, cereals and beans with relatively high soluble fiber contents as staple components of the diet in diabetics. High soluble fiber content in the diet may also have a favorable effects on blood cholesterol levels (Vinik and Jenkins, 1988).

Similarly, Nutritive sweeteners such as sorbital and fructose. However, Sorbital may cause acute diarrhea and fructose induces only slight increase in plasma glucose levels but raises serum cholesterol (Usitupa, 1994).

ORAL DRUG TREATMENT FOR HYPERGLYCAEMIA

A). Sulfonylureas

Sulfonylureas, stimulate insulin secretion remain the most widely prescribed drugs for treating *hyperglycaemia.* They bind the receptors closing potassium channels, resulting in depolarization of the β cells (Malaisse and Lebrum, 1990). This depolarized state permits calcium to enter the cell and activity promotes insulin release.

Sulfonylureas are generally contraindicated in patients with hepatic or renal impairment. Idiosyncratic reactions are rare, with skin rashes or hematologic toxicity (leucopenia, thrombocytopenia) occurring in less than 0.1% of patients.

First-generation sulfonylureas (tolbutamide, tolazamild, acetobexamide, chlorproamide) Tolbutamide is the safest sulfonylureas to use if liver function is normal. However, severe *hyperglycaemia* may occur suddenly after prolonged therapy with one of these sulfonylurea drugs or soon after the initiation of treatment (Joseph *et al.*, 1964).

Second-generation sulfonylureas Glyburide, Glipizide, gliclazide, and glimepiride are more potent than tolbutamide. Fasting is well tolerated among the elderly patients with type 2 diabetes treated with sulfonylureas. However, these drugs should be used with caution in patients with cardiovascular disease patients, in whom prolonged *hyperglycaemia* would be especially dangerous (Mark *et al.*, 1998 and Enrique *et al.*, 2004).

Meglitinide analogs, repaglinide and nateglinide also stimulate insulin secretion. Repaglinide acts by binding to the sulfonylureas receptor and closing the ATP-sensitive potassium channel. It is rapidly absorbed from the intestine and then undergoes complete metabolism in the liver to inactive biliary products giving it a plasma half-life of less than 1 hour. The drugs therefore cause a brief but rapid pulse of insulin. Repaglinide can be used in combination with metformin. *Hypoglycaemia* is the main side effect. Like the sulfonylureas also, repaglinide causes weight gain. The drug may be useful in patients with renal impairment or in the elderly (Schmitz *et al.*, 2002 and Yale, 2005).

Drugs that alter insulin action—

Metformin: alter insulin action primarily in the liver. It is used either alone or in combination with other oral agents or insulin, in the treatment of patients with type 2 diabetes. It reduces both the fasting level of blood glucose and postprandial *hyperglycaemia* in patients with type 2 diabetes but has no effect on fasting blood glucose in normal subjects (Bailey and Day, 1989).

Metformin is particularly effective in reducing hepatic gluconeogenesis by interfering with lactate oxidation, glucose uptake by the liver, slows down gastrointestinal absorption of glucose and increases glucose uptake by skeletal muscle. Because of its very high concentration in intestinal cells after oral administration, metformin increase

glucose to lactate turnover, which may account for a reduction in *hyperglycaemia* (Cusi, 1998). Metformin has a half-life of 1.5—3 hours, is not bound to plasma protein and is not metabolized in human beings excreted unchanged by the kidneys.

Metformin (Knowler, 2002) may be used as an adjunct to diet for the control of *hyperglycaemia* and its associated symptoms in patients with type 2 diabetes, particularly those who are obese or are not responding optimally to maximal doses of sulfonylureas. It also improves both fasting and postprandial *hyperglycaemia* and *hypertriglyceridemia* in obese diabetics without the weight gain associated with insulin or sulfonylureas therapy. Metformin is not indicated for patients with type 1 diabetes and is contraindicated in diabetes with high serum creatinine levels or a propensity to develop tissue hypoxia.

The most frequent side effects of metformin are anorexia, nausea, vomiting, abdominal discomfort, diarrhea, which occur in up to 20% of patients. These effects are dose-related, tend to occur at onset of therapy and often are transient. However, in 3—5% of patients, therapy may have to be discontinued because of persistent diarrhea. *Hypoglycaemia* dose not occur with therapeutic doses of metformin. Lactic acidosis has been reported as a side effect but is uncommon with metformin in contrast to phenformin (Shelley *et al.*, 2003 and Safadi *et al.*, 1996).

Thiozolodinedione, rosiglitazone and pioglitazone are agents sensitize peripheral tissues like skeletal muscle and adipose tissue to insulin. They bind a nuclear receptor called peroxisome proliferators-activated receptor gamma (PPAR-Y) and affect the expression of a number of genes and regulate the release of the adipokines—resistin and adiponectin—from adipocytes. Adiponectin secretion is stimulated, which sensitizes tissues to the effects of insulin (Yki-Jarvinen, 2004). Observed effects of thiazolidinediones include increased glucose transporter expression (GLUT 1 and GLUT 4), decrease free fatty acid levels. Decreased hepatic glucose output, and increased differentiation of preadipocytes into adipocytes. Like metformin, this class of drugs does not cause *hypoglycaemia* (Mudaliar, 2005).

When used as monotherapy, these drugs lower HbA1c by about 1 or 2 percentage points. When used in combination with insulin, they can result in a 30—50 % reduction in insulin dosage, and some patients even do not need insulin completely (Perez *et al.*, 2009).

3. Drugs that affect absorption of glucose

The α-glucosidase inhibitors, acarbose and miglitol principally affect absorption of glucose. By inhibiting the α–glycosidase enzymes in the gut that digest dietary starch and sucrose. They are potent inhibitors of glucoamylase, α–amylase, and sucrase but have less effect on isomaltase and hardly any on lactase. Acarbose binds 1000 times more avidly to the intestinal disaccharidases than do products of carbohydrate digestion or sucrose (Biscohoff, 1994). A fundamental difference between acarbose and miglitol is in their absorption. Miglitol, however, has a structural similarity with glucose and is absorbable. Both drugs delay the absorption of carbohydrate and lower postprandial *glycaemic* excursion. For maximal benefit on postprandial *hyperglycaemia*, acarbose should be given with the first mouthful of food ingested. In diabetic patients, it reduces postprandial *hyperglycaemia* by 30—50% and its overall effect is to lower the HbA1c by 0.5%.

The major adverse effect, seen in 20—30% of patients, is flatulence. This is caused by undigested carbohydrate reaching the lower bowel, where gases are produced by bacterial flora. In 30% of cases, troublesome diarrhea occurs. This gastrointestinal discomfort tends to discourage excessive carbohydrate consumption and promotes improved compliance of type-2 patients. When acarbose given alone, there is no risk of *hypoglycaemia*. However, if combined with insulin or Sulfonylurea, it might increase the risk of *hypoglycaemia* from these agents. A slight rise in hepatic aminotransferase has been noted in clinical trials with acarbose and particularly with doses>300 mg/dl). The levels generally return to normal on stopping the drug (Holman *et al.*, 1999).

b. Miglitol—Miglitol is similar to acarbose in terms of its clinical effects. It is indicated for use in diet- or Sulfonylurea- treated Patients with type-2 diabetes. Therapy is initiated at the lowest effective dosage of 25 mg three times a day. The usual maintenance dose is

50 mg three times a day, although some patients may benefit from increasing the dose to 100mg three times a day. Gastrointestinal side effects occur as with acarbose. The drug is not metabolized and is excreted unchanged by the kidney. Miglitol should not be used in renal failure, when its clearance would be impaired (Shelley *et al.*, 2003).

INSULIN

Insulin is indicated for type 1 diabetes as well as for type 2 diabetic Patients with Insulinopenia whose *hyperglycaemia* does not respond to diet therapy either alone or combined with oral *hypoglycaemic* drugs (Owens, 2001).

With the development of recumbent technology highly purified human insulin preparations, immunogenicity such as insulin allergy, immune Insulin resistance and localized lipoatrophy at the injection site has been markedly reduced. However, the problems of achieving optimal insulin delivery remain unsolved with the present state of technology (Edelman and Steven 2005).

Human insulin is produced by recombinant DNA techniques (Johnson, 1983) (biosynthetic human insulin) as Human (*Eli Lilly*) and as Nivolin (Novo Nordisk). Types of regular insulin are: (i) NPH (N), (ii) lente (L) and (iii) ulterlente (U).

NPH insulin Neutral Protamine Hagedorn or isophane insulin is intermediate-acting insulin whose onset of action is delayed by combining two parts soluble crystalline zinc insulin with 1 part protamine zinc insulin (Michael and Fowler, 2008).

Analogs human Insulin: Insulin lispro is rapid-acting insulin (Humalong) (Brunelle *et al.*, 2000) is an Insulin analog produced by recombinant technology, wherein two amino acids near the carboxyl terminal of the B chain have been reversed in position: Proline at position B28 has been moved to B29 and lysine has been moved from B29 to B28 (Prarlcin *et al.*, 1996). Another desirable feature of Insulin lispro is that its duration of action remains at about 4 hours irrespectively of dosages. This is contrary with regular Insulin, whose duration of action is prolonged when larger doses are used (Tsui *et al.*, 2001).

Insulin aspart is a single substitution of proline by aspartic acid at position B28; the analog quickly dissociate into monomers and are absorbed very rapidly, reaching peak serum values in 1 hour—in contrast considerably more time to dissociate and become absorbed (Raskin *et al.*, 2000).

Insulin glargine is insulin analog in which the asparagines at position 21 of the A chain of the human insulin molecule is replaced by glycine and two arginines are added to the carboxyl terminal of the B chain (Heinemann, 2000).

Insulin glargine is a clear insulin which, when injected into the neutral pH environment of the subcutaneous tissue, forms microprecipitates that slowly release the insulin into the circulation. It lasts for about 24 hours without any pronounced peaks and is given once a day to provide basal coverage while the short-acting insulins are used to cover the glucose rise associated with meals (Lepore, 2005).

Short-acting regular Insulin—Regular Insulin is short-acting soluble crystalline zinc insulin whose effect appears within 30 minutes after subcutaneous injection and lasts 5—7 hours when usual quantities are administered. Intravenous infusions of regular insulin are particularly useful in the treatment of diabetic ketoacidosis (Guillermo *et al.*, 2004).

Intermediate-acting insulins—Lente insulin is a mixture of 30% semilente (an amorphous precipitate of insulin with zinc ions) with 70% ultralente insulin (an insoluble crystal of zinc and insulin). Its onset of action is delayed for up to 2 hours and because its duration of action after is less than 24 hours (with a rang of 18—24 hours) most patients require at least two injections daily to maintain a sustained insulin effect. Lente insulin had its peak effect in most patients between 8 and 12 hours (Edelman *et al.*, 2005).

If the herbal drugs after thorough investigations prove to be really effective in controlling blood glucose levels of the diabetes. This would go a long way in the management of the diabetic patients at this time of "Diabetes Explosion"

HYPOGLYCAEMIC ACTIVITY IN PLANTS

Interestingly many of the indigenous medicinal plants have been traditionally used to treat *hyperglycaemia* and *Diabetes Mellitus* (Ali *et al.*, 1983). Several plant species have been described as *hypoglycaemic*. These include *Opuntia streptacantha Lem, Trigonella foenum graecum L, Momordica charantia L, Polygalasenega L., Gymnema sylvestre R., Allium sativum, Citrullus colocynthis, Myrrh, fenugreek, aloe* and *Artemisia*. However, few plants have received scientific or medical scrutiny (Dey *et al.*, 2002).

There are about 75 plants, indigenous to the subcontinent, which posses *hypoglycaemic* activity. Various studies have revealed that plants insulin (P-insulin) has a consistent *hypoglycaemic* effect in patients with diabetic mellitus (Khanna *et al.*, 1985). The work done in 75 Indians plants for their anti-diabetic activity (Nagurgem *et al.*, 1982). About 32 plants are locally used for lowering the blood glucose in Bangladesh (Yusaf *et al.*, 1994). Anti-hyperglycaemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids etc that are frequently implicated as having antidiabetic effect (Baldwa *et al.*, 1977).

A study to evaluate the effects of aqueous extract of *Tecoma stans* on the blood sugar levels were conducted by Nash *et al.*, (1950). The extract was found to produce some *hypoglycaemia* in normal rabbits. However, when the extract was tried on the alloxan diabetic mice and rats, no antidiabetic activity could be demonstrated.

The seeds of fruit *Blighial sapida* (*Ackee*) contain two toxic components hypoglycin A and B. The *hypoglycaemic* activity was observed by Hessal *et al.*, (1954). This was most striking pharmacological effect of the plant. Blood glucose level was found to decrease to less than 20mg/100ml before death in normal albino rats.

The effect of *Cryptostegia grandiflora* on blood sugar of normal and alloxan diabetic rabbits were demonstrated by Sharma *et al.*, (1967). The extract exerted significant

hypoglycaemic effect in normal rabbits. The extract failed to reduce the blood sugar of the alloxan diabetic rabbits.

Memoridica charantia fruit (*Karela*) exerts significant *hypoglycaemic* effect in normal and alloxan diabetic rabbits (Akhtar *et al.*, 1981). They observed that maturity onset diabetic patients receiving whole dried and powdered fruit of *Memoridica charantia* for seven days showed considerable improvement. Some of the patients remained free of symptoms even after discontinuation of drug for about 1-2 months. No adverse effects were observed in any of these patients (Akhtar, 1982).

The *hypoglycaemic* effect of orally administered extracts of fruits of cultivated *Momordica charantia* (*Karela*) was examined by Day *et al.*, (1990), in normal and streptozotocin diabetic mice. In normal mice, an aqueous extract (A) lowered the *glycaemic* response to both oral and intraperitoneal glucose, without altering the insulin response. This aqueous extract (A) and the residue after alkaline chloroform extraction (B) reduced the *hypoglycaemia* in diabetic mice at 1 hour. The data suggested the presence of at least two types of orally active *hypoglycaemic* principles in "*Karela*": a more rapidly effective water-soluble component, and a more slowly acting component possibly an alkaloid. The oral *hypoglycaemic* effect of "*Karela*" was independent of intestinal glucose absorption and insulin secretion.

The *hypoglycaemic* properties of *Memoridica charantia* (bitter gourd) water extract produced a significant fall of blood sugar in the diabetic rats after 3 weeks treatment with aqueous extract of fruits of the herb. The aqueous extract of fruit was more effective in diabetes (fall of blood sugar 54% after 3 week's therapy) than the powder of the dried fruit (fall 25% nonsignificant). *Hypoglycaemic* effects in diabetic patients were found to be highly significant ($p<0.001$) at the end of the trial but were cumulative and gradual, unlike that produced by insulin. Adaptogenic properties are indicated by the delay in the appearance of cataracts, the secondary complications of diabetes and relief in neurological and other common symptoms even before the *hypoglycaemia* occurred (Srivastava *et al.*, 1993). Chen *et al.*, (2003) used bitter gourd freeze-dried juice at different doses fed with high fat diet to rats. They found that different doses of bitter

gourd juice fed with high fat diet significantly lowered energy efficiency and tended to have less visceral fat mass. They also observed rats fed on high fat diet plus bitter gourd juice gained significantly less weight and visceral fat than the control.

The effects of daily oral feeding of *Memoridica charantia, Eujania jambolana* and *Mucuna pruirer* fruits by Grover *et al.*, (2001) and they found significant *hypoglycaemic* effects and prevented polyuria in streptozotocin diabetic rats. Similarly, Grover *et al.*, 2002 studied plasma glucose lowering concentration in induced diabetic rabbits by *Eujania jambolana, Mucuna pruiriens* and *Tinospora cordifolia* leaves (Wadood N, 1991).

The antioxidative potential of *Momordica charantia, Azadirachta indica, Allium sativum* and *Ocimum sanctum* was assessed in streptozotocin induced diabetic rabbits. They observed significant increase in lipid peroxide levels in streptozotocin induced diabetic rats compared to normal. However, there was significant decrease in lipid peroxide levels in diabetic rate treated with above herbal *hypoglycaemic* agents. They found in part through their fibre, vitamin or mineral contents and some secondary metabolite. Diabetes mellitus aggravates in patients on herbal deficient diet. Several minerals found in some medicinal plants have been reported to be cofactors that signal intermediaries of insulin action and key enzymes of glucose metabolism (Mahdi *et al.*, 2003).

The leaves of *Cococinia indica* (*Kauduriki beal*) improved the glucose tolerance considerably while placebo showed no improvement. This study showed (Khan *et al.*, 1980) that the drug might be useful for the oral treatment of patients with type 2 diabetes especially there were no adverse effects during six weeks of use.

Two fractions from cabbage, both of which were found to affect the blood sugar level when given by mouth one of the fractions caused an increase of blood sugar level in rabbits and the other fractions produced *hypoglycaemia* in normal rabbits. The later was founded to be apparently replaced by insulin in the depancreatised dogs. They have proposed the name of vigulin for this *hypoglycaemic* substance (MacDonald and Wislicki 1938).

The oral *hyperglycaemic* activity of (a) *Salacia reticulate*, (b) *Aegle marmelos* and (c) *Momordica charantia* were evaluated by Karunanayake *et al.*, (1984). The result indicated that the aqueous decoctions of all three plants possess significant *hypoglycaemic* effect. The highest oral *hypoglycaemic* activity and the maximum improvement of the oral glucose tolerance were associated with the extract of *Momordica charantia* while the least but significant effects were shown by *Salacia reticulate*.

It was observed by Lamela *et al.*, (1986), significant *hypoglycaemic* activity of extracts of *Liythrum salicaria* stems and flowers in rat with glucose and epinephrine induced *hyperglycaemia*. These extracts were also found to be active in alloxan and streptozotocin diabetic rats and alloxan-diabetic mice.

A significant *hypoglycaemic* effect of dried roots of *Onosma echioides* (*Ratan jot*) in both normal as well as diabetic rabbits was observed by Akhtar and Riffat (1986). Further screening was the alleged *hypoglycaemic* activity of *Europhorbia prostrata* Lit (*Doudi khurd*) a Common herb, indigenous to Indo-Pakistan and have also analyzed clinically and found that substantial amount of manganese (Mn^{++}) are present in it (Akhtar and Irfan 1986). Therefore, it is possible that the *hypoglycaemic* effects of *Europhorbia prostata* may at least in part be due to its high contents of Manganese (Mn^{++}) one of the trace elements, which can exert blood glucose lowering effect.

Twenty water soluble mucilages from plants in the *Liliaceae, Amarlyidaceae, Dioscoreaceae, Orehidaceae, Saxifragraceae, Malvaceae* and *Plantaginaceae* families and tested for the *hypoglycaemic* activity by Tomoda *et al.*, (1987) after administration to normal mice. Considerable activity was observed for most of the mucilages isolated from plants in the *Malvaceae* family. The deacetylated product of Plantago-mucilages A, the main mucilage present in the seed of *Plantago asiatica*, also showed remarkable *hypoglycaemic* activity.

The blood glucose levels of the normal and alloxan-diabetic male albino rabbits were determined by Akhtar *et al.*, (1987) after oral administration of various doses of the

powdered root of the asparagus racemosus and fruit of *Lodoicea sechellarum*, comm. It was concluded that the powder of *A. racemosus* root and *L. sechellarum* fruit produced significant *hypoglycaemic* effect only in the normal rabbits. In the alloxan-treated rabbits, both the medicinal plant drugs did not affect the blood glucose levels. Acute toxicity studies and records of behavioral patterns carried out in rabbits and rats respectively showed no adverse effects of A. Racemosus and L. Sechellarum in the dosage tested. It was conceived that these plants contain some *hypoglycaemic* principle(s), which act probably by initiating the release of insulin from pancreatic β cells of normal rabbits.

The effects of tormentic acid, a natural product isolated from *Poterium ancistroides*, in normoglycaemic, *hypoglycaemic* and streptozotocin diabetic rats were studied by Ivorra *et al.*, (1988). This principle lowered the fasting plasma glucose level with a corresponding increase in circulating insulin levels. Moreover, it improved the glucose tolerance test by increasing insulin secretory response to glucose. However, tormentic acid did not change the insulin and glucose levels in streptozotocin induced diabetic rats. These results suggest that tormentic acid, like glibenclamide, may act by increasing insulin secretion from the islets of Langerhans.

The alcoholic extract of the leaves of *Eriobotrya japonica* caused significant *hypoglycaemic* effect in normal rabbits. Most probably powdered seeds of *Eriobotrya japonica* when administered 2, 3 and 4g/kg body weight to normal rabbits induced *hypoglycaemic* effect by initiating release of insulin from pancreatic β cells (Wadood *et, al.*, 1988). The leaves of *Eriobotrya japonica*, a small tree commonly known as "*Loquat*" are documented for use as folk medicine for the treatment of diabetes mellitus. DeTommasi *et al.*, (1991) reported the *hypoglycaemic* effects of extract of leaves of *Eriobotrya japonica* (*Loquat*) and isolated sesquiterpene glycosides and polyhydroxylated triterpenoids reduced blood glucose levels in genetically diabetic mice and normoglyceamic rats interestingly.

The *hypoglycaemic* activity of olive oil leaf. Maximum *hypoglycaemic* activity was obtained from samples collected in the winter months. One of the compounds responsible for this activity was oleurospeoside, which showed activity at a dose of 16mg/kg. This compound also demonstrated antidiabetic activity in animals with alloxan-induced-diabetes. The *hypoglycaemic* activity of this compound may result from two mechanisms: (a) potentiation of glucose-induced insulin release and (b) increased peripheral uptake of glucose (Gonzalez *et al.*, 1992).

The flowers of *Puniea granatum* are used for the treatment of diabetes mellitus in '*Unani*' medicines. Oral administration of its aqueous-ethanolic extract led to significant blood glucose lowering effect in normal, glucose-fed hyperglycaemic and alloxan-induced diabetic rats. This effect was maximum at 400mg/kg body weight (Jaffri, 1996).

Magnifera indica (Mango): the leaves of this plant are used as an antidiabetic agent in Nigerian folk medicine. The results indicate that aqueous extract of *Magnifera indica* possesses *hypoglycaemic* activity. This may be due to an intestinal reduction of the absorption of glucose (Aderibigbe *et al.*, 1999).

The seeds of *Cuminum nigrum* were screened phytochemically and were found to contain 8% flavonoids. When studied for their effect on blood glucose levels, oral administration of the flavonoids contents of the plant caused a *hypoglycaemic* activity effect started 24 hours after drug administration reaching maximum with 4—8 hours and blood glucose level returned close to normal within 24 hours of drug administration (Ahmad *et al.*, 2000).

The *hypoglycaemic* activity of twenty-four medicinal plants like *Cococinia indica, Tragia involucrate, Gymnema sylvestre, Ptefonuim-graecum, Eugenia jambolana, Momordica charantia* etc were investigated by Kar *et al.*, (2003) and confirmed definite blood glucose lowering effects in alloxan diabetic albino rats.

Artemisia herba-alba and *Teucrium polium* are used as conventional therapy for diabetes mellitus. Mehmet Iriadam *et al.*, (2005) observed a significant ($p<0.05$) *hypoglycaemic* effect in normal and diabetic rabbits by *Teucrium polium* had significant ($p<0.05$) effect and found useful *Artemisia herba-alba* in preventing *hyperglycaemia* by having insulin-like action and can significantly reduce the blood glucose in normoglycaemic and *hyperglycaemic* rabbits.

The effects of aqueous extract of *Ananona squamosa* leaf on blood glucose were studied by Kaleem *et, al.* (2006) and found useful in controlling the blood glucose level, improves the plasma insulin, lipid metabolism and is beneficial in preventing diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats; therefore it could be useful for prevention or early treatment of diabetes mellitus.

Capparis deciduas produced *hypoglycaemic* effect in alloxanized rats when the rats were fed with 30% extracts of *Capparis deciduas* fruit powder for 3 weeks (Manisha Modak *et al.*, 2007).

The leaves of three herbs *Murraya koenigii (MK), Psidium guajava (PG)* and *Catharanthus roseus (CR)* were used to test their antidiabetic activity in streptozotocin (STZ) induced diabetic albino rats. Body weight showed significant increase (MK and PG: $p<0.05$, CR and GBC: $p<0.001$) after 15 days of treatment with herbal extract when compared with the control. Blood glucose level on 15^{th} day of treatment become significantly low ($p<0.05$). At the termination of the experiment (on 15^{th} day) the urine glucose and ketone were absent in herbal treated group, which was present in the diabetic control. The most significant findings of the present study is that the aqueous leaves extract of MK, PG and CR at the dose of 500mg/kg body weight for 15 days have shown beneficial effect not only on blood glucose but also on body weight, glucose and ketone levels of urine and tissue of pancreas in streptozotocin induced diabetic rats. Results obtained from the present study are very much promising and comparable with glibenclamide, a standard drug used to treat diabetes mellitus (Prasad *et al.*, 2009).

Enough information is available which indicates that a large variety of compounds obtained from several plants families were found to be responsible for the *hypoglycaemic* activity. For instance, glycoside isolated from the families *Caesalpiniaceae*, *Papaveraceae*, *Ranunculaceae*, *Rhamnaceae* and *Scrophulariaceae* afforded active principles, which lowered the blood glucose in test animals. Similarly, glycans of *Ranunculaceae* and *Granmineae* also showed *hypoglycaemic* activity. Certain triterpenes from plants belonging to the family Ranunculaceae also showed *hypoglycaemic* activity. In family *Liliaceae*, this property was attributed to various types of sulfides molecules. Polysacharides, oils and vitamins from the family graminae also showed pharmacological activity by decreasing blood glucose sugar level in animals (Dixit *et al.*, 2006). Alkaloids of *Apocyanaceae*, *Papaveraceae*, *Rhamnaceae* and *Zygophyllaceae* were particularly effective in diabetes. Saponins from *Malvaceae*, peptides, amino acids and protein from *Papolionceae* and *Rubiaceae* showed beneficial effects in reducing the blood sugar (Loew and Kaszkin, 2002).

ROLE OF TRACE ELEMENTS IN DIABETES MILLITUS

A metal can influence the activity of an enzyme by its sheer physical presence in the active center of the enzyme. It is not unlikely that even when a metal is attached to an enzyme at some distance from its active centre the structural alteration may affect the activity of the enzyme. They concluded that the decisive factor for the maintenance of several vital structures and functions in an organism is the presence of traces of metals (Cotzias and Foradorin, 1969).

Less than 0.02% or so i.e. (11g) of the reference, man is made up of trace elements, at least 24 found in amounts large enough to be detected. Eleven of the twenty-four are of vital importance for his health, without them, he is sick more often and dies, for they are very spark plugs of life. He also explained that most of these trace elements act as catalysts, causing chemical reactions, which would ordinarily not proceed to take place at low temperature. They are all joined to proteins, making metallo-enzymes. Magnesium e.g. activates 67 enzymes having to do with energy, as well as many others. Various clinical and pathological disorders arise as a consequence of trace element deficiencies

and excesses (Schroeder, 1976). Man can exist on a deficiency or even absence of certain vitamins but cannot exist on an extreme deficiency or an absence of certain minerals in such a way that particular element creates problem while conducting deficiency experimentation (Donsbach, 1982).

CHROMIUM

Severe chromium depletion led to fasting *hyperglycaemia*, *glycosuria* and impaired growth rates in rats an ability to handle glucose efficiency resulted in a mild state of diabetes (Schroeder and Balassa, 1968). Functions as a cofactor in all insulin-regulating activities (Offenbacher and Pi-Sunyer, 1980). Chromium facilitates insulin binding and subsequent uptake of glucose into the cell. Supplement chromium has been shown to decrease fasting glucose levels, improve glucose tolerance, lower insulin levels, and decrease total cholesterol and triglycerides, while increasing HDL cholesterol in normal, elderly and type 2 diabetic subjects (Mooradian *et al.*, 1994 and Baker, 1982) without chromium, insulin's action is blocked and glucose levels are elevated. Chromium (Cr^{3+}) is an essential micronutrient for humans. Its main action is thought to be the regulation of blood sugar, because chromium deficiency is associated with diabetic-like symptoms, and chromium supplementation is correlated with increased glucose tolerance and insulin sensivity. Some Portuguese aromatic plants are utilized as tisanes by diabetic people as medicinal plants. Their active principle is not yet known, and the importance of their chromium content in the claimed therapeutic properties should not be discarded. Therefore, determination of chromium in some Portuguese medicinal plants was performed by flameless atomic absorption (Castro, 1998).

ZINC (Zn^{++})

Abnormalities in glucose utilization that occur with severe Zn^{++} depletion are related to impaired release of insulin from the pancreas and to a decline in peripheral tissue sensitivity to insulin. Today, there is considerable evidence to show that Zinc deficiency may accompany Diabetes mellitus (Hurber and Gershoff, 1973 and Kirchgessner *et al.*, 1976 and Wolman 1979).

Oral Zinc is able to increase the intestinal absorption of glucose and that the absorption of Zinc is significantly stimulated by the presence of glucose. Important interaction occurs between Zinc and insulin metabolism. Zinc not only promotes formation of hexamers from insulin monomers, but also alters bindings of iodoinsulin to the hepatic cells and may, exert a stimulatory effect on lipogenesis, an action similar to that of insulin. In view of important role of Zinc in the activity of many enzymes, it has been hypothesized that Zinc participate, in the synthesis and storage of insulin in the β cells and that the amount of insulin stored during Zinc deficiency is decreased. Alternatively, increase degradation of insulin due to Zinc deficiency may also account for glucose tolerance (Lee *et al.*, 1989).

MANGANESE (Mn^{++})

Rats fed on manganese deficient diet for two months showed a diabetic or per-diabetic glucose curve and it stayed up there, the body was not utilizing glucose in the absence of adequate manganese. However, when the rats were put back on a manganese adequate diet their glucose tolerance curves returned to normal (Donsbach, 1982). Alleged *hypoglycaemic* activity of *Europhorbia prostrata* (*Doudi khurd*) a common herb contains substantial amount of manganese (Mn^{++}) are present in it. Therefore, it is possible that the *hypoglycaemic* effect of *Europhorbia prostrata* plant many at least in part be due to its high contents of manganese (Mn^{++}), one of the trace elements, which has blood glucose lowering effect (Akhtar and Irfan 1986).

Many tradional treatments have been recommended in the complimentary and alternatives system of medicine for treatment of diabetes mellitus. It has been attributed that the anti-hyperglycaemic effect due to their ability to restore the functions of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to be facilitation of metabolites in insulin dependent process. Hence, treatment with herbal drugs has an effect in β cells and smoothing out fluctuation in glucose levels (Jia *et al.*, 2003 and Elder, 2004).

Although a number of medicinal plants are traditionally used for over thousand years for the treatment of diabetes mellitus. These three plants *Cassia sophera, Caralluma tuberculata* and *Achillea santolina* have been known for their anti-diabetic effects in Ayurverdic medicine "*Kitab-ul-mufridat Khawas-ul-adwiya*". The seeds of *Cassia sophera* and aerial parts of *Caralluma tuberculata* and *Achillea santolina* were used to investigate the *hypoglycaemic* effects of these plants both in normal and alloxan-induced diabetic rabbits.

Pharmacological screening of indigenous medicinal plants in normal and alloxan diabetic rabbits for antidiabetic activity

In the present study, three indigenous medicinal plants i. *Cassia sophera*, ii. *Caralluma tuberculata* and iii. *Achillea santolina* were used for their *hypoglycaemic* potential affecting normal and alloxan-induced diabetic rabbits. *Cassia sophera* grows in India and also in Sindh, Pakistan. Its seeds have been used since time immemorial in ethno medicine for many varied medicinal purposes including as a traditional remedy for diabetes mellitus. The *hypoglycaemic* activity of the seeds of this plant has been evaluated and compared with an oral *hypoglyaemic* agent acetohexamide in both normal and diabetic rabbits. Further to explore the *hypoglycaemic* effects by different fractions its methanolic and aqueous extracts equivalent to 4g/kg body weight of the powdered seeds producing maximum response were also administered to normal as well as diabetic rabbits.

The *hypoglycaemic* effects of *Caralluma tuberculata* and *Achillea santolina* were also determined. *Caralluma tuberculata* is a wild herb, grows in most of the parts of hilly areas of Baluchistan, and is used as vegetable since centuries in Pakistan. While *Achillea santolina* is an ornamental plant cultivated in gardens and is also used as treatment for diabetes mellitus in Baluchistan by traditional healer.

In these plants crude and carbon tetrachloride (CCl_4) extracts were administered to normal and alloxan diabetic rabbits. Also the extracts were given in capsule form and in cooking oil. The results of these three plants were also compared with Metformin.

Materials and Methods

ANTI DIABETIC ACTIVITY STUDY IN *CASSIA SOPHERA*

PLANT MATERIAL USED

***Cassia sophera* seeds**

Botanical Name:	*Cassia sophera*
Family Name:	Caesalpiniaceae
English Name:	Senna
Common Name:	Kasunda, Kala Kasonji, Banar

This plant is found throughout India and in most tropical countries. It is common in waste lands, on road sides and in the forests. Root bark in used for preparation of the medicine. It has been used by ancient Indian physicians for its efficacy in respiratory disorders. It has also been recommended in common cold, asthma and osteoarthritis (Kiritikar and Basu, 1984). The aerial parts of the plant are shown in figure 2.

The root is useful in thirst, urinary discharges; cures tumours, skin disease and asthma. The leaves are anthelmintic; good for ulcers, leprosy, skin diseases. The flowers are used in urinary discharges, nocturnal emissions, diabetes mellitus, and throat troubles. The seed is alexipharmic; used in ophthalmia, diabetes mellitus, dysentery (Ayurveda) (Dhanukar and Thatte, 1989).

Cassia sophera seeds used in the present research were donated by Prof. Dr. Noor Ahmad Shahani, University of Agriculture, Tando Jam, Sindh, Pakistan.

Cassia sophera

1. Preparation of seeds powder

The seeds were powdered finely with a grinder at room temperature. The powdered material was stored in well-closed glass bottles at 4°C in the refrigerator.

2. Preparation of aqueous extract

Hundred grams of powdered seeds were kept for maceration in distilled water in a round neck well closed flask. It was agitated occasionally and kept for a period of one day (Osol, 1955). The drug was then filtered through a fine filter. The process of maceration was done at room temperature. The extract thus obtained was then dried in Petri dish in an oven at a temperature not more than 40°C (Vats *et al.*, 2002).

3. Preparation of methanolic extract

Methanolic extract was prepared by using Soxhlet's apparatus. (Figure. 3) Hundred grams of powdered form of seeds of plant were put into the thimbles made of special filter paper and 1000ml of methanol was taken into the glass flasks. The extract obtained was evaporated by slow heating at 40°C and continuous stirring. The process of evaporation was continued till complete evaporation of methanol was ensured. The dried methanol extract so obtained was dissolved in distilled water just before administration to rabbits.

EXPERIMENTAL ANIMAL USED:

Healthy male rabbits of *Oryctologus cunniculus* species weighing 1000-1500g were used in these experiments. The animals were kept under observation for one week before experimentation in the animal room. The animals were fed green fodder and fresh water was also supplied ad libitum. The animals were used to be treated at the early hours of the day mostly between 08:00AM to 09:00AM.

PREPARATION OF ALLOXAN-DIABETIC RABBITS

Diabetes mellitus can be produced readily in different animals by chemicals or surgical method. In recent years, some genetically diabetic animals have also become available for study. Chemical induction of diabetes has commonly been achieved with β cells

cytotoxic agents. Alloxan and streptozotocin are the most extensively used agents because their diabetogenic dose is usually 4—5 times less than their lethal dose (Gordsky *et al.*, 1982). However, streptozotocin is very costly and it is very difficult to preserve it because it is highly unstable.

Alloxan was first chemical used to induce experimental diabetes mellitus. It was found by Leaibig in mucous excreted during dysentery (Merck Index, 1976). The diabetogenic doses of alloxan vary considerably among species, age and metabolic state of the animal. Some species, for example guinea pig, are completely insensitive to alloxan (Gordsky *et al.*, 1982).

The diabetic condition was induced by injecting alloxan monohydrate 150mg/kg body weight into one of the pineal ear vein of rabbit. Eight days after injecting the alloxan monohydrate, blood glucose level of the surviving rabbits were determined by Gluco–oxidase method. Rabbits with blood glucose level above 300—500mg/dl were considered as diabetic.

CHEMICALS AND DRUGS USED

1. Alloxan-monohydrate NH-CO-NH. CO-CO.H_2O	B.D.H. Laboratories, Poole, England
2. Methanol (CH_3OH)	B.D.H. Chemicals Ltd, Poole, England
3. Acetohexamide (Dimelor)	Eli Lilly and Company Ltd, Basingstoke, England
4. Metformin (Glucophage)	Boots Pharmaceuticals Ltd
5. Blood Glucose Determination Kit	RANDOX Laboratories Ltd, UK
6. Gum tragacanth	A Local Grocery Shop, Bara Dawakhana, D.I.Khan

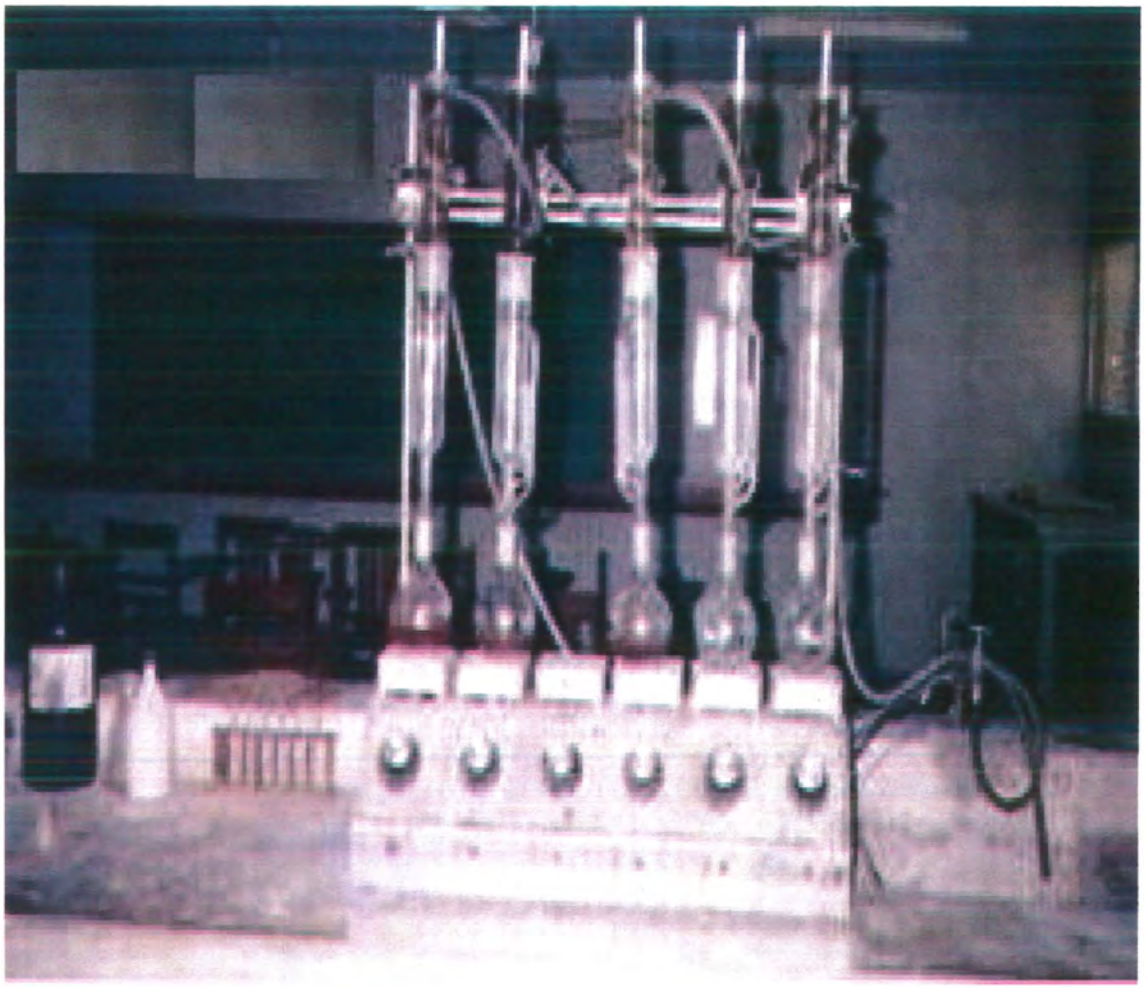

Figure No. 2: Soxhlet's apparatus for methanolic extraction for *Cassia sophera* (seeds)

REAGENTS OF KIT (RANDOX) USED FOR DETERMINATION OF GLUCOSE

(RANDOX Laboratory Ltd, Ardmore, Diamond Road, Crumlin Co, Antrim, UK)

Contents	Initial concentrations of solutions
1. Buffer	
Phosphate Buffer	0.2 mol/l, pH 7.0
Phenol	22 mmol/l
2. GOD-PAP Reagent	
4-aminophenazone	0.77 mmol/l
Glucose oxidase	> 1.5 KU/ l
Peroxidase	> 1.5 KU/ l
3. Standard	
Glucose	5.55 mmol/l (100 mg/dl)

DETERMINATION OF BLOOD GLUCOSE LEVELS

Blood samples were taken by syringes connected to cannulae passed in the ear veins of the experimental rabbits.

SAMPLE

Serum, plasma.

Glucose is stable for 24 hours at +2 to +8°C if the serum or plasma is prepared within 30 minutes after collection.

Blood glucose levels were determined by the glucose oxidase method, which is specific method for glucose as it responds only to glucose. No other physiological constituent is measured. Very high amount of reducing substances like ascorbic acid may interfere by competing with the chromogen, (Ortho-toludine) for the oxygen liberated and thus gives low results. Haemoglobin may interfere by causing premature dissociation of hydrogen peroxide and gives low values (Meyer *et al.*, 1992).

PRINCIPLE OF METHOD

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indictor as shown below:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

PROCEDURE

For serum, three test tubes labeled as B (blank), S (standard) and u (unknown) were set in a rack and various reagents were added to each tube as mentioned below:

	Test Tube: 1 B (Blank)	**Test Tube: 2 S (Standard)**	**Test Tube: 3 u (unknown)**
Standard solution	-	20 µl	-
Test sample	-	-	20 µl
GOD-PAP Reagent	2 ml	2 ml	2 ml

The contents of each tube were mixed thoroughly and kept in a water bath for 10 minutes at 37°C. The absorbance standard and test samples were measured at 500nm against reagent blank within 60 minutes (Barham and Trinder, 1972).

The blood glucose levels were calculated by using the following equation:

$$\text{Glucose concentration (mg/dl)} = A_{\text{sample}} \times \frac{100}{A_{\text{standard}}}$$

GROUPING OF RABBITS

One hundred and twelve rabbits were randomly divided into two major groups.

Group 1: Normal (Non-Diabetic) Rabbits:

There were fifty-six rabbits in Group 1, which were further divided into following seven subgroups of 8 animals each.

Subgroup A_1, served as untreated control as they received 20ml 2% gum tragacanth solution in water only.

Subgroup B_1, C_1 and D_1, were administered 2, 3 and 4g/kg body weight of powdered seeds of *Cassia sophera* orally to each animal by using the feeding polythene tube connected to a 20ml record syringe.

Subgroup A_2 served as untreated control as they received 20ml of 2% gum tragacanth in water.

Subgroups B_2 and C_2 received methanolic and aqueous extract equivalent to 4g/kg body weight dose of the powdered seeds.

Sub group D_2 was administered Acetohexamide 500mg/kg body weight orally.

Group II Diabetic (Alloxan-treated) Rabbits:

Fifty-six rabbits weighing 1000-1500g were made diabetic by injecting 150mg/kg body weight of alloxan monohydrate. The dose/animal was weighed, dissolved in the distilled water and then injected by a tuberculin syringe slowly intravenously (Butt, 1962). The administration of alloxan-monohydrate to the experimental rabbits was carried out very slowly and proper care was taken to avoid death.

Eight days after injecting the alloxan monohydrate, blood glucose levels of the surviving rabbits were determined by glucose oxidase method. Rabbits with blood glucose level of

300-500mg/100ml were considered as diabetic and were employed for further studies and were divided into seven sub groups.

The amount of *Cassia sophera* powder required for each animal on body weight basis was weighed with an electric balance. The same was triturated with 10ml of 2% aqueous gum tragacanth solution and the final volume was made upto 20ml. The drug was administered orally to each animal by using a polythene tube connected to a 20ml syringe.

STATISTICAL ANALYSIS

Data has been expressed as the mean ± SEM and the student's T test was used to find out the *hypoglycaemic* effects and to compare the efficacy of various doses of the plants at different time intervals.

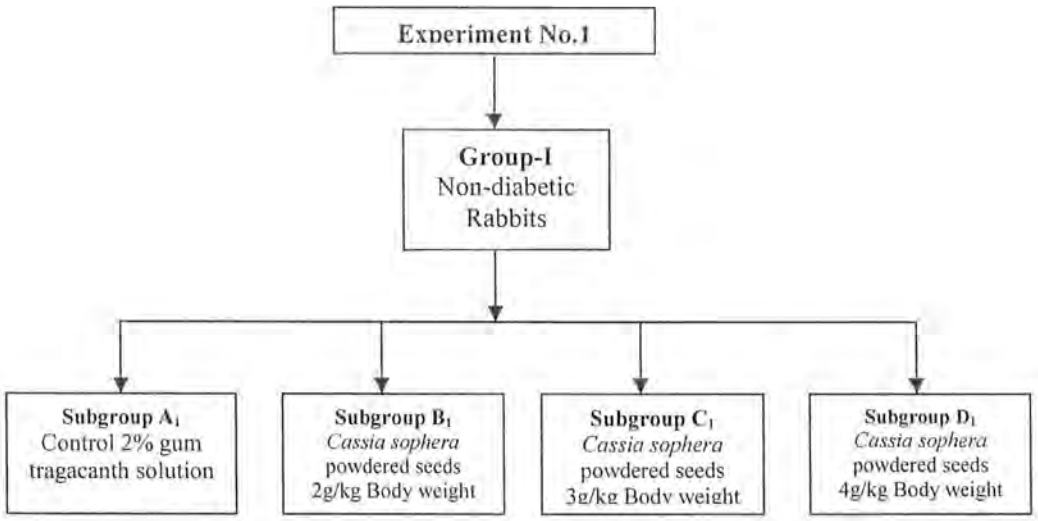

Figure 4(A): The design for the study of antidiabetic activity of *Cassia sophera* powdered seeds. Experiment 1 and Group I, Normal Non diabetic rabbits.

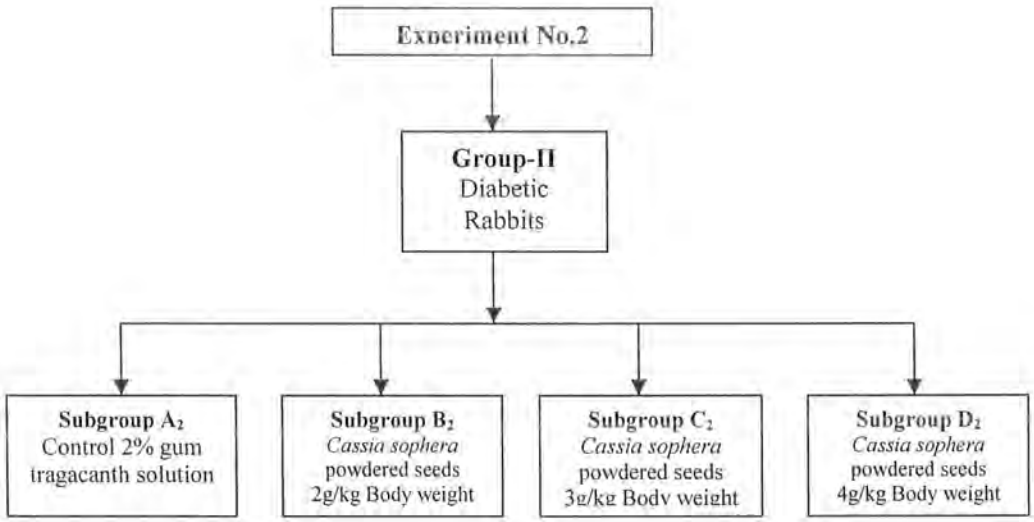

Figure 4(B): The experimental design for the study of antidiabetic activity of *Cassia sophera* powdered seeds. Experiment 2 and Group II, Diabetic rabbits.

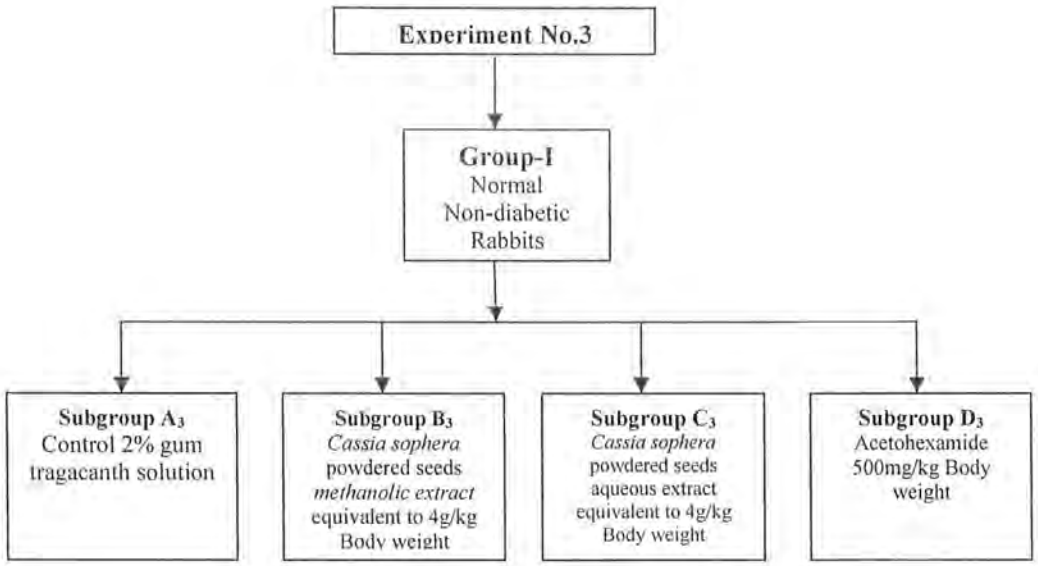

Figure 5(A): The experimental design for the study of antidiabetic activity of *Cassia sophera* powdered seeds. Experiment No 3 and Group I, Normal rabbits.

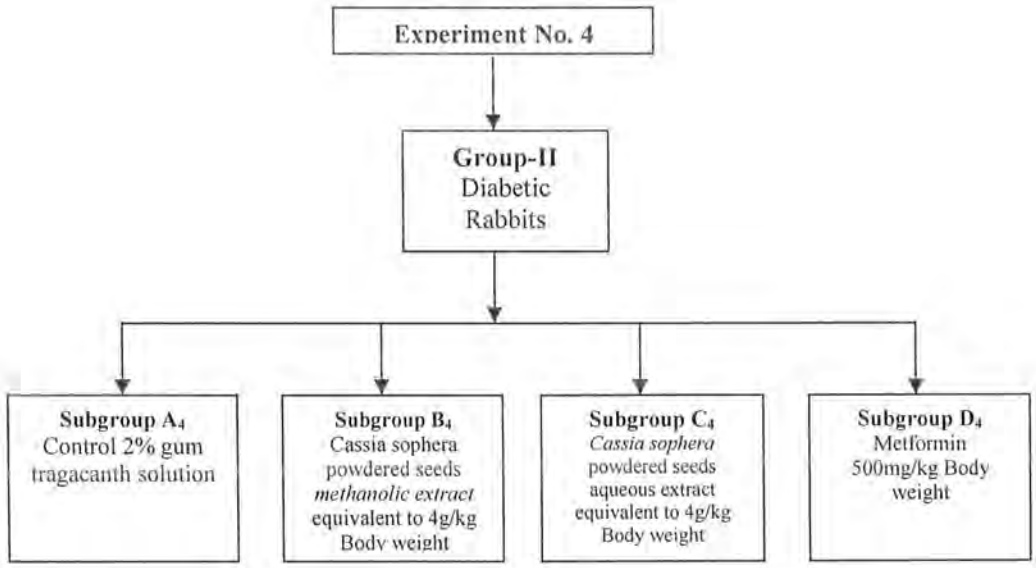

Figure 5(B): The experimental design for the study of antidiabetic activity of *Cassia sophera* powdered seeds. Experiment No 4 and Group II, Diabetic rabbits.

Antidiabetic activity of Cassia sophera

Results

RESULTS

EFFECT OF *CASSIA SOPHERA* POWDERED SEEDS ON BLOOD GLUCOSE LEVELS

EXPERIMENT NO. I

Normal (Non-Diabetic) Rabbits:-

Normal non-diabetic rabbits were divided into four subgroups. In each subgroup, there were eight rabbits and they were given different treatments of *Cassia sophera* powdered seeds.

1. Treatment with 2% gum tragacanth solution in normal non diabetic rabbits (A_1):

The rabbits were treated orally with 2% gum tragacanth solution and at zero hour time interval mean blood glucose level was 95.13±1.48mg/dl. Gum tragacanth did not affect the blood glucose levels, as there was no statistical difference at 2, 4, 8, 12 and 24 hours time intervals. Mean blood glucose levels recorded at these time intervals are given in the Table 1, Figure 6.

2. Treatment with *Cassia sophera* powdered seeds dose, 2g/kg body weight in normal non diabetic rabbits (B_1):

Mean blood glucose levels recorded after two hours of oral administration of powdered seeds of *Cassia sophera* showed slight decrease compared to zero hour. Non-significant decrease was also seen after 4 hours of treatment. Mean blood glucose levels decreased significantly at 8 and 12 hours of interval compared to zero hour interval ($p<0.05$). After 24 hours of treatment blood glucose levels increased nearly equal to that at zero hour. (Table 1, Figure 6)

3. Treatment with *Cassia sophera* powdered seeds dose, 3g/kg body weight in normal non diabetic rabbits (C_1):

Mean blood glucose levels started decreasing from 2 hours time interval onward upto 12 hours time intervals. Highly significant decrease in mean blood glucose levels were

observed at 4, 8 and 12 hours time after treatment ($p<0.001$) compared to zero hour interval. At 24 hours time mean glucose levels rose nearly equal to that at zero hour time. At 8 hours interval ($t_{(14)} = 3.22$; $p<0.05$) and 12 hours interval ($t_{(14)} = 2.62$; $p<0.05$) there was significant decrease in blood glucose levels in 3g/kg body weight compared to that in 2g/kg body weight dose. (Table 1, Figure 6)

4. Treatment with *Cassia sophera* powdered seeds dose, 4g/kg body weight in normal non diabetic rabbits (D_1):

There was sharp decrease in mean blood glucose levels at 2 hours time after treatment, but this decrease was not significant compared to that at zero hours. Highly significant decrease in mean blood glucose levels were seen at 4 hours, 8 hours and 12 hours intervals ($p<0.001$) compared to that at zero hour. (Table 1, Figure 6)

Comparison among subgroups B_1, C_1 and D_1

The above results showed that 4g/kg body weight dose is more effective than other doses used. Hence, in later experiments only 4g/kg body weight was used for all practical purposes. This is obvious from the results that in 4g/kg body weight dose after 2 hours intervals there was significant decrease in blood glucose levels ($t_{(14)} = 2.45$; $p<0.05$) compared to that of 3g/kg body weight dose. In similar comparisons between 4g/kg vs 3g/kg body weight at 4 hours intervals ($t_{(14)} = 6.62$; $p<0.001$), 8 hours interval ($t_{(14)} = 2.65$; $p<0.05$), 12 hours interval ($t_{(14)} = 6.62$; $p<0.001$) there was highly significant decrease in blood glucose levels with 4g/kg body weight dose. At 24 hours interval mean blood glucose levels increase but there were non-significantly less than that in 3g/kg body weight dose. (Table 1, Figure 6)

Table 1: Mean blood glucose levels mg/dl of normal (non-diabetic) rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral treatment with 20ml 2% gum tragacanth and *Cassia sophera* powdered seeds 2, 3 and 4g/kg body weight.

	20ml	*Cassia sophera* powdered seeds g/kg body weight		
Time interval	2% gum tragacanth	2	3	4
(Hours)	Subgroups A_1	B_1	C_1	D_1
0	95.13 ± 1.48	97.63 ± 1.97	99.31 ± 1.76	92.50 ± 2.3
2	95.00 ± 1.16	95.69 ± 1.39	96.00 ± 2.13	88.33 ± 1.0 ^{h*}
4	94.44 ± 1.91	94.25 ± 1.54	87.75 ± 1.98 ^{a***}	83.33 ± 1.4 ^{a***} ^{h***}
8	95.00 ± 1.44	92.63 ± 0.97 ^{a*}	83.19 ± 1.96 ^{a***} ^{g*}	76.66 ± 0.5 ^{a***} ^{h*}
12	94.00 ± 1.61	89.13 ± 0.90 ^{a*}	84.56 ± 0.91 ^{a***}	72.50 ± 0.9 ^{a***} ^{h***}
24	94.63 ± 1.57	97.19 ± 1.79	97.44 ± 1.89	91.25 ± 1.9

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$

a = 0 hour vs all time intervals

= 2 hours vs 4 hours

c = 4 hours vs 8 hours

d = 8 hours vs 12 hours

e = 12 hours vs 24 hours

= Gum tragacanth vs with all time intervals

g = Subgroup B_1 vs subgroup C_1 and subgroup D_1

h = Subgroup C_1 vs subgroup D_1

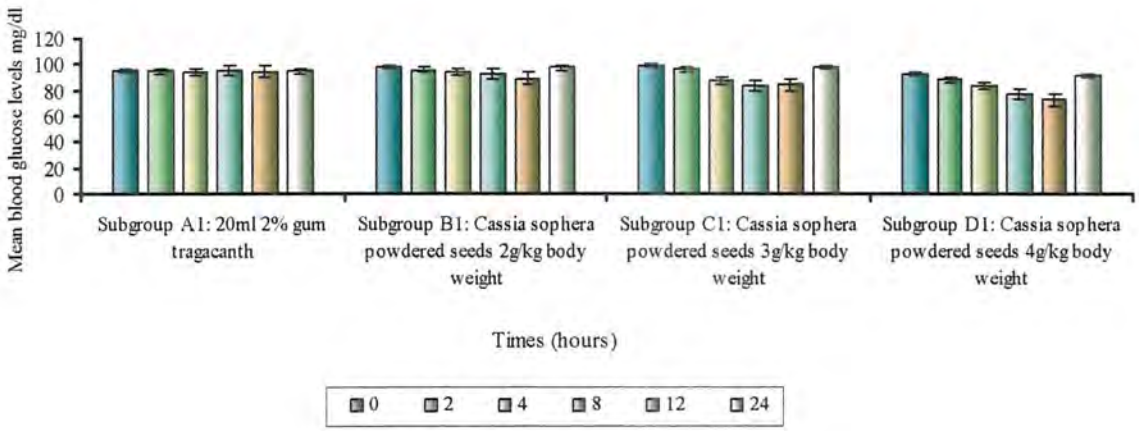

Figure 6: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval after oral treatment of 20ml 2% gum tragacanth, *Cassia sophera* powdered seeds 2, 3 and 4 g/kg body weight in normal (non-diabetic) rabbits.

EXPERIMENT NO. 2

In this experiment, diabetic rabbits (Alloxan-induced diabetes) were used. Diabetic rabbits were divided into four groups, each group containing 8 rabbits. This experiment was designed on the same pattern as that of experiment No.1, i.e. three doses of *Cassia sophera*, 2g/kg body weight, 3g/kg body weight and 4g/kg body weight were used for oral treatment. In the control group 2% gum tragacanth was administered. It was intended to see which dose of *Cassia sophera* would be more effective in decreasing mean blood glucose levels in diabetic rabbits.

1. Treatment with 2% gum tragacanth solution in diabetic rabbits (A_2):

The mean blood glucose levels in these rabbits did not show appreciable change compared to at 0 hour records at 2, 4, 8, 12 and 24 hours after treatment. Slight changes in mean blood glucose levels were not statistically different from zero hour records. (Table 2, Figure 7)

2. Treatment with *Cassia sophera* powdered seeds dose 2g/kg body weight in diabetic rabbits (B_2):

Decrease in mean blood glucose levels at 2 and 4 hours time interval were not significantly different from that at zero hour time. There was significant decrease in blood glucose level at 8 hours time ($p<0.05$) and 12 hours time ($p<0.05$) compared to that at zero hour. Mean blood glucose levels increased at 24 hours time interval which was not significantly different from zero hour ($p>0.05$). (Table 2, Figure 7)

3. Treatment with *Cassia sophera* powdered seeds dose 3g/kg body weight in diabetic rabbits (C_2):

Mean blood glucose levels gradually decreased after 2, 4 and 8 hours of treatment, but these were not significantly different from that of zero hour ($p>0.05$). Highly significant decrease in blood glucose levels compared to zero hour levels was observed at 12 hours time interval ($p<0.001$). There was significant increase in blood glucose levels at 24 hours intervals compared to 12 hours time ($t_{(14)} = 3.07$; $p<0.01$), but this increase was not significantly different from that at zero hours ($p>0.05$). (Table 2, Figure 7)

4. Treatment with *Cassia sophera* powdered seeds dose 4g/kg body weight in diabetic rabbits (D_2):

Mean blood glucose levels started decreasing from 2 hours interval upto 12 hours interval. At 2 hours and 4 hours interval mean blood glucose levels were not significantly different from that of zero hour. Compared to zero hour levels, significant decrease was observed at 8 hours ($p<0.05$) and highly significant decrease at 12 hours interval ($p<0.001$).

Comparison among subgroups B_2, C_2 and D_2

At 24 hours interval mean blood glucose levels increased nearly equal to that at zero hours time. At 8 hours time interval 2g/kg body weight vs 3g/kg body weight shows non-significant increase in mean blood glucose levels in the later case. However, at this time interval (8 hours) 3g/kg body weight vs 4g/kg body weight shows highly significant decrease in later group ($t_{(14)} = 14.38$; $p<0.001$). Both 3g/kg body weight ($t_{(14)} = 2.28$; $p<0.05$) and 4g/kg body weight groups ($t_{(14)} = 2.21$; $p<0.05$) show significant decrease at 12 hour time interval compared to 8 hour blood glucose levels. (Table 2, Figure 7)

Table 2: Mean blood glucose levels mg/dl of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral treatment with 20ml 2% gum tragacanth and *Cassia sophera* powdered seeds 2, 3 and 4 g/kg body weight.

	20ml	*Cassia sophera* powdered seeds g/kg body weight.		
Time interval (Hours)	2% gum tragacanth Subgroups A_2	2 B_2	3 C_2	4 D_2
0	343.54 ± 9.0	345.00 ± 7.94	362.97 ± 7.31	373.13 ± 16.74
2	339.91 ± 8.19	340.09 ± 8.53	358.97 ± 7.31	367.84 ± 15.77
4	339.63 ± 8.25	334.47 ± 7.09	352.44± 7.69	357.91 ± 13.58
8	339.41 ± 8.52	320.03 ± 8.16 ^{a*}	344.58 ± 7.81	325.38 ± 5.58 ^{a*h***}
12	335.71 ± 8.15	315.00 ± 6.32 ^{a*}	308.91 ± 7.79 ^{a***d*}	300.09 ± 5.85 ^{a***d*}
24	338.79 ± 9.19	338.91 ± 9.00	356.84 ± 6.53 ^{e**}	370.00 ± 15.61

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$
a = 0 hour vs all time intervals
b = 2 hours vs 4 hours
c = 4 hours vs 8 hours
d = 8 hours vs 12 hours
e = 12 hours vs 24 hours
= Gum tragacanth vs with all time intervals
g = Subgroup B_2 vs subgroup C_2 and subgroup D_2
h = Subgroup C_2 vs subgroup D_2

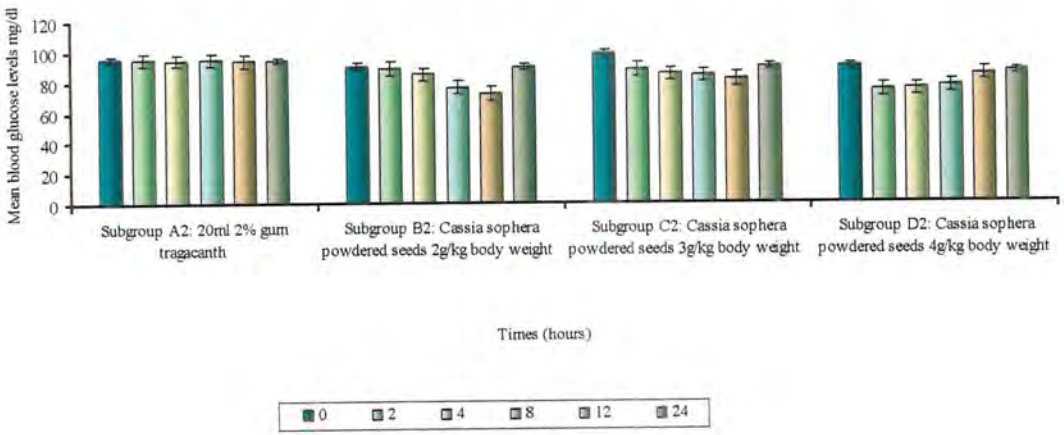

Figure 7: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval after oral treatment of 20ml 2% gum tragacanth, *Cassia sophera* powdered seeds 2, 3 and 4 g/kg body weight in diabetic rabbits.

EXPERIMENT NO. 3

Basic design of experiment was the same as in previous two experiments. In this experiment, one group was of control (A_3) and another group methanolic extract of *Cassia sophera* seeds (B_3), aqueous extract of *Cassia sophera* seeds (C_3) and the medicine Acetohexamide (D_3) was used. These treatments were given to normal non-diabetic rabbits.

1. Treatment with 2% gum tragacanth solution in normal non-diabetic rabbits (A_3):

This group was maintain as control and as in previous experiments mean blood glucose levels were recorded at 2, 4, 8, 12 and 24 hours after treatment. In this group no appreciable changes in mean blood glucose levels was observed during these time intervals. (Table 3, Figure 8)

2. Treatment with methanolic extract of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight in normal non diabetic rabbits (B_3):

Mean blood glucose levels starting decreasing from 2 hours time upto 12 hours time interval. Highly significant decrease in blood glucose levels was seen at 8 hours and 12 hours after treatment ($p<0.001$) compared to that at zero hour. Lowest mean blood glucose level were observed at 12 hours time. There was significant decrease in blood glucose level at 8 hours time compared to that at 4 hours ($t_{(14)} = 4.14$; $p<0.01$). After 24 hours of treatment there was highly significant increase in blood glucose levels compared to that after 12 hours time ($t_{(14)} = 7.18$; $p<0.001$). (Table 3, Figure 8)

3. Treatment with aqueous extract of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight in normal non diabetic rabbits (C_3):

Non-significant decrease in blood glucose levels was recorded at 2 hours after treatment. Significant decrease in blood glucose levels compared to that at zero hour, started from 4 hours to 12 hours after treatment ($p<0.05$). After 24 hour of treatment blood glucose levels increased near to zero hour levels. There was significant increase ($p<0.05$) in blood glucose levels at 24 hours compared to that at 12 hours time. (Table 3, Figure 8)

4. Treatment with medicine Acetohexamide in normal non diabetic rabbits (D_3):

Highly significant decrease in mean blood glucose levels was observed at 4 and 8 hours after treatment ($p<0.001$) compared to that at zero hour. An increase in blood glucose level at 12 hours and 24 hours time was significantly different from zero hour ($p<0.05$). There was significantly increase in blood glucose levels at 12 hours time interval compared to that at 8 hours time ($t_{(14)} = 3.41$; $p<0.01$). (Table 3, Figure 8)

Comparison among subgroups B_3, C_3 and D_3

The result of this experiment indicates that methanolic extract of *Cassia sophera* seeds seems to be better (in terms of efficacy) than the aqueous extract in producing low levels of blood glucose. This has been observed at 8 hours interval ($t_{(14)} = 3.20$; $p<0.05$) and 12 hours intervals ($t_{(14)} = 4.98$; $p<0.05$) after treatment compared to that during these intervals with aqueous extracts. Compared to aqueous extracts at 4 hours interval ($t_{(14)} = 5.72$; $p<0.001$) and 8 hours interval ($t_{(14)} = 2.66$; $p<0.05$) blood glucose levels were significantly low with Acetohexamide treatment.

Table 3: Mean blood glucose levels mg/dl of normal (non-diabetic) rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral treatment with 20ml 2% gum tragacanth, methanolic and aqueous extracts of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight and Acetohexamide 500mg/kg body weight.

	20ml	*Cassia sophera* powdered seeds extracts equivalent to 4g/kg body weight		500mg/kg body weight
Time interval (Hours)	2% gum tragacanth Subgroups A_3	Methanolic B_3	Aqueous C_3	Acetohexamide D_3
0	95.13 ± 1.48	90.13 ± 2.54	98.31 ± 1.19	89.75 ± 1.24
2	95.00 ± 1.16	89.06 ± 1.21	87.88 ± 0.98	74.38 ± 1.46
4	94.44 ± 1.91	85.00 ± 0.96	84.94± 0.80 ^{a*}	74.81 ± 0.91 ^{a***h*}
8	95.00 ± 1.44	76.38 ± 1.12 ^{a***c**}	83.88 ± 1.22 ^{a*g*}	76.69 ± 1.48 ^{a***h***}
12	94.00 ± 1.61	72.56 ± 0.96 ^{a***}	81.63 ± 0.86 ^{a*g*}	84.69 ± 0.86 ^{a*d**}
24	94.63 ± 1.57	89.81 ± 1.44 ^{e**}	89.94 ± 1.36 ^{e*}	86.00 ± 0.88 ^{a*}

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$

a = 0 hour vs all time intervals

b = 2 hours vs 4 hours

c = 4 hours vs 8 hours

d = 8 hours vs 12 hours

e = 12 hours vs 24 hours

f = Gum tragacanth vs with all time intervals

g = Subgroup B_3 vs subgroup C_3 and subgroup D_3

h = Subgroup C_3 vs subgroup D_3

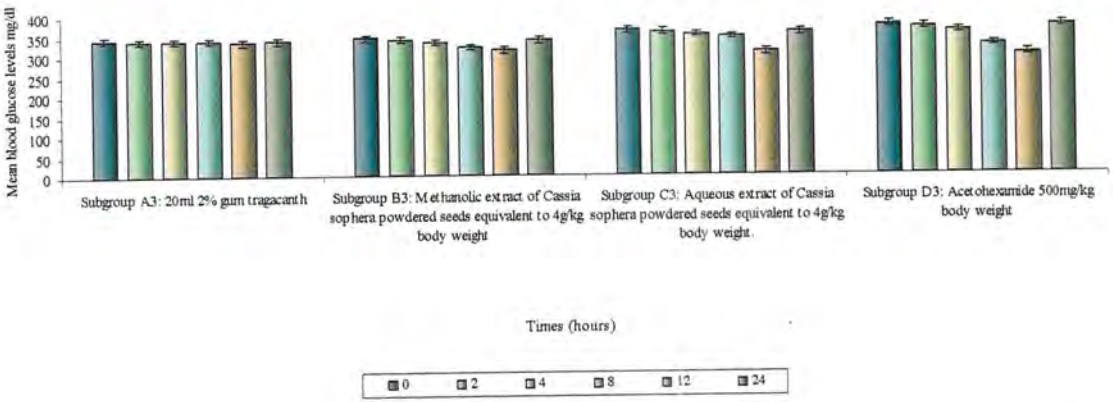

Figure 8: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval after oral treatment of 20ml 2% gum tragacanth, methanolic and aqueous extracts of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight and Acetohexamide 500mg/kg body weight in normal (non-diabetic) rabbits.

EXPERIMENT NO. 4

The experimental design was the same as with experiment No.3 i.e. methanolic and aqueous extracts of *Cassia sophera* and the medicine metformin was orally administered in this experiment. All rabbits used here were diabetic. (Table 4, Figure 9)

1. Treatment with 20ml 2% gum tragacanth in diabetic rabbits (A_4):

Mean blood glucose levels recorded after 0, 2, 4, 8, 12 and 24 hours of treatment showed no appreciable changes in these levels. This group was maintained as a control in this experiment. (Table 4, Figure 9)

2. Treatment with methanolic extract of *Cassia sophera* seeds in diabetic rabbits (B_4)

Two hours after administration of methanolic extracts of *Cassia sophera* seeds there was significant decrease in blood glucose levels compared to that at zero hour ($p<0.05$). Highly significant decrease in blood glucose levels at 4 hours interval ($p<0.001$) compared to that at zero hour was observed. Decrease in these levels at 4 hours interval was also significant compared to that at 2 hours interval ($t_{(14)} = 3.0$; $p<0.05$). Highly significant decrease in blood glucose levels continued up to 8 hours and 12 hours ($p<0.001$) compared to that at zero hour. There was highly significant increase in blood glucose levels at 24 hours time compared to that at 12 hours time ($t_{(14)} = 4.11$; $p<0.001$). But this increase was not significantly different compared to that at zero hour time. Systematic decrease in blood glucose levels was observed after 2, 4, 8 and 12 hours time intervals with methanolic extract of *Cassia sophera* seeds. (Table 4, Figure 9)

3. Treatment with aqueous extract of *Cassia sophera* seeds in diabetic rabbits (C_4):

Aqueous extract of *Cassia sophera* seeds did cause decrease in blood glucose levels after 2 and 4 hours of treatment but these were not significantly different from that at zero hour time but significant decrease was seen at 8 hour interval ($p<0.05$) and highly significant decrease in blood glucose levels was seen at 12 hours time ($p<0.001$). There was highly significant increase in blood glucose levels at 24 hours interval compared to that at 12

hours interval ($t_{(14)} = 5.87$; $p<0.001$), but these levels were also significantly higher than that at zero hour interval ($t_{(14)} = 3.31$; $p<0.001$). (Table 4, Figure 9)

4. Treatment with Metformin in diabetic rabbits (D_4):

Metformin treatment showed fluctuations in blood glucose level, i.e. increase and decrease. There was significant decrease in blood glucose levels at 2 hours ($p<0.05$) and 4 hours interval ($p<0.05$) but highly significant increase in blood glucose levels compared to 12 hours levels was observed at 8 hours time interval ($t_{(14)} = 4.57$; $p<0.001$). The increase in blood glucose levels at 24 hours intervals was not significantly different from 12 hours and zero hour intervals. (Table 4, Figure 9)

Comparison between methanolic extract of *Cassia sophera* and Metformin

Methanolic extract showed better results than metformin in developing *hypoglycaemia*. At 4 hours interval there was highly significant decrease in blood glucose levels with methanolic extract compared to that with metformin ($t_{(14)} = 3.34$; $p<0.001$). Similarly, highly significant decrease in blood glucose level with methanolic extract at 8 hours intervals ($t_{(14)} = 6.11$; $p<0.001$) and 12 hours interval ($t_{(14)} = 2.90$; $p<0.05$) compared to that of metformin was observed.

Table 4: Mean blood glucose levels mg/dl of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after after oral treatment with 20ml 2% gum tragacanth, methanolic and aqueous extracts of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight and Metformin 500mg/kg body weight.

	20ml	*Cassia sophera* powdered seeds extracts equivalent to 4g/kg body weight		500mg/kg body weight
Time interval (Hours)	2% gum tragacanth Subgroups A_4	Methanolic B_4	Aqueous C_4	Metformin D_4
0	343.54 ± 9.0	365.34 ± 5.16	332.97 ±7.89	360.59 ±7.13
2	339.91 ± 8.19	343.22 ± 5.57 ^{a*}	320.13 ±8.02	336.94 ±3.02 ^{a*}
4	339.63 ± 8.25	315.38 ± 3.71 ^{a***f***g***}	319.50 ±2.59	330.00 ±1.79 ^{a*}
8	339.41 ± 8.52	311.41 ± 2.99 ^{a***g***}	313.00 ±2.90 ^{a*}	352.00 ±3.65 ^{d***}
12	335.71 ± 8.15	304.78 ± 7.15 ^{a***g*}	301.13 ± 5.61 ^{a***}	335.38 ±3.38
24	338.79 ± 9.19	358.41 ± 5.96 ^{e***}	368.63 ± 5.88 ^{e***}	350.56 ±4.80

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$

a = 0 hour vs all time intervals

b = 2 hours vs 4 hours

c = 4 hours vs 8 hours

d = 8 hours vs 12 hours

e = 12 hours vs 24 hours

f = Gum tragacanth vs with all time intervals

g = Subgroup B_4 vs subgroup C_4 and subgroup D_4

h = Subgroup C_4 vs subgroup D_4

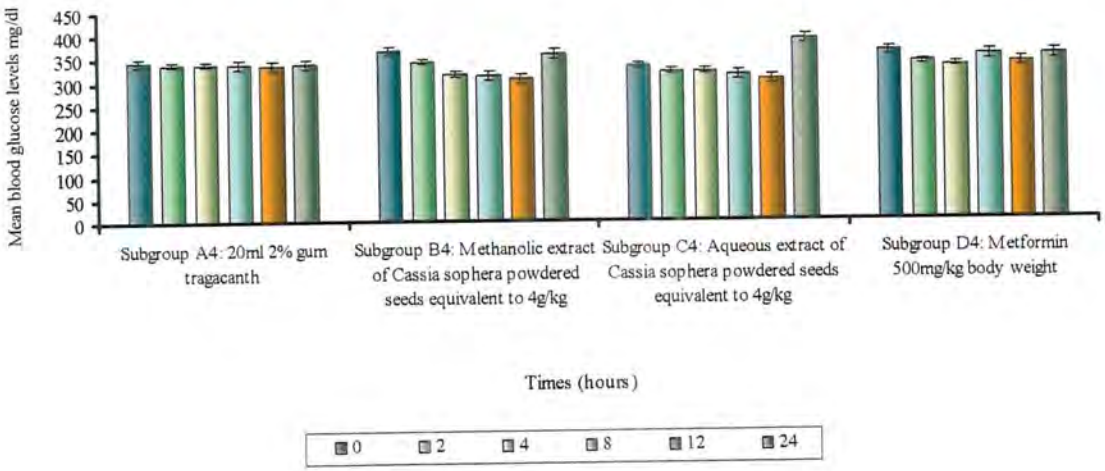

Figure 9: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval after oral treatment of 20ml 2% gum tragacanth, methanolic and aqueous extracts of *Cassia sophera* powdered seeds equivalent to 4g/kg body weight and Metformin 500mg/kg body weight in diabetic rabbits.

Anti-diabetic activity of Caralluma tuberculata and Achillea santolina

Experiments No. 5 to 8 have been designed to evaluate the hypoglycaemic effects of two plants Caralluma tuberculata and Achillea santolina in normal and in alloxan-induced diabetic rabbits. Crude extracts of both plants and the carbon tetrachloride (CCl_4) fraction in capsule and in cooking oil dosage form are used. The hypoglycaemic effects are compared with Metformin.

MATERIAL AND METHODS

MATERIAL AND METHODS

In this study, two plants were used i.e. (1). *Caralluma tuberculata* and (2). *Achillea santolina.*

PLANT MATERIAL USED

CARALLUMA TUBERCULATA

Caralluma tuberculata whole 15kg was purchased from local market in January 2004, Dera Ismail Khan, Pakistan

Botanical Name: *Caralluma tuberculata*

Family Name: Asclepiadaceae

Common Names: Charungli/Chung, Aputag, Marmat

Caralluma plants are widely distributed in the Mediterranean region. It is largely grown plant in Pakistan, India and South East of Egypt. In Pakistan, it is found in N.W.F.P (Presently Khyber Pakhtunkhwa), Punjab, Waziristan and Baluchistan (Ali and Nasir, 1983). The aerial parts of *Caralluma tuberculata* are shown in Figure 10.

Caralluma tuberculata is a perennial herb, stem succulent, erect, thick angular (4`-angled), toothed, up to 15cm tall, branches 8-13 mm broad, having grooves. The *Caralluma* is almost leafless but in some cases, minute, spine like leaves in the angle of stem is present. The plant has few flowered fascicles at the upper node, 8-9mm in the diameter, purple, sepal ovate-lanceolate; 5-lobed, deeply divided lobes lanceolate; glasbrous, stigma 5 angled, conical, follicles, 8-10.5cm, glabrous, gradually tapering towards the tip, tip rounded, seeds flat, winged with a tuft of hair (Jaffri, 1996 and Baquar, 1989). *Caralluma* is used as vegetable and is reputed to be a cure for diabetes and effective in rheumatism. Juicy stem is bitter, tonic, febrifuge, carminative, used for rheumatism (Nadharni, 1954). The previous studies on toxicity of plant shows its use by Saudi folk medicine practitioners for the treatment of snake, scorpion bite and is also

known to have the hypotensive effect (Al-Yahya and Al-Meshal, 1978). *Caralluma tuberculata* has been introduced in Indian glossary as *Caralluma tuberculata* and has been used for the treatment of blood diseases; this effect of plant is attributed to the presence of glycoside. It is also used in the folk medicine in the treatment of leprosy.

Nikaido *et al.*, in (1967) reported that *Caralluma tuberculata* gave a mixture of glycoside, which was hydrolysed under mild conditions to give Cymarose, sarmentose, Oleandrose or digitoxose as the sugar components. The aglycon portion was separated into several acids and a crystalline neutral fraction containing Beucerin. Mossa *et al.*, (1983) working on phytochemical and biological screening of Saudi medicinal plants reported medicinally active compounds from *Caralluma pencillata*, whole plant. These chemical constituents include volatile basis alkaloids, cardiac glycosides, flavonoids, saponins, sterols/triterpenes and tannins.

Momin (1987) reported the chemical composition of the *Caralluma tuberculata* benth as shown in the following table.

Composition of 100gms Fresh Edible Portion

Moisture %	95.50	Mn (mgs)	8.21
Total position %	0.17	Mg (mgs)	11.58
Total Lipids %	0.23	Ca (mgs)	145.20
Total Carbohydrates %	2.52	K (mgs)	144.57
Crude Fibers %	0.59	Na (mgs)	17.38
Ash %	1.58	Zn (mgs)	4.42
PO4 (mgs)	9.80	Fe (mgs)	24.68

Usmanghani *et al.*, (1987) reported the ethyl acetate extract of medicinal plant *Caralluma tuberculata* yielded two crystalline compounds, which were identified through spectroscopy as β-sitosterol and lupeol. Hayashi *et al.*, 1988 reported for pregnane glycoside AI, AII, BI, BII from *Caralluma tuberculata.* Ahmad *et al.*, (1988) isolated two new pregnane glycoside caratuberoside and caratubersides as a white crystalline

compound from the *Caralluma tuberculata.* Further work on *Caralluma tuberculata* by Ghazala *et al.,* (1990) resulted in the isolation of three flavones glycosides.

Trustoma *et al.,* (1990) reported ten pregnane glycosides from *Caralluma tuberculata.* The *Caralluma tuberculata* furnished a pregnane type compound, caratuberside A.2. Khalil (1994) reported two new pregnane ester a glycones from 75% ethanolic extract of *Caralluma.* Ghazala *et al.,* (1994) reported that ethanolic extract of whole plant of *Caralluma tuberculata* yielded other two new pregnane glycoside. Halim and Khalil (1996) isolated and identified two-pregnane ester glycoside from alcoholic extract of aerial parts of *Caralluma.*

Caralluma species have been used by humans since time immemorial that bitter principal of the *Caralluma tuberculata* have febrifuge activity and could be used as tonic. Juicy stem of this plant possess carminative activity. Reports are also available about anthelmintic and antitumour activity of the plant (Chopra *et al.,* 1956).

Figure No. 1: *Caralluma tuberculata*

ACHELLIA SANTOLINA

The aerial parts of *Achillea santolina* 10kg were collected from Mastung (Baluchistan), Pakistan.

Botanical Name: *Achillea santolina*

Family: *Compositae*

Common Name: *Zawal*

Achillea is a large genus, of perennial herbs, all natives of the temperate regions of the northern hemisphere. A large number of them are cultivated in gardens for their ornamental foliage. Two medicinal species *A. millefoliun* and *A. santolina* are found in India. They are used in folk medicine due to its various healing properties. The aerial parts of *Achillea santolina* are shown in the Figure 11.

Achillea santolina is also called *Achillea leptophylla*. It is found in Khozdar, Quetta, Mastung and Kurram etc. *Achillea santolina* normally grows on occasionally inundated clay soils. This is a small perineal villous herb, growing to 0.3 liters leaves alternate usually radiate. It has pinkish flower with an aromatic smell. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects.

The plant prefers light (sandy), medium (loamy) and heavy (clay) soils, requires well-drained soil and can grow in nutritionally poor soil. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires dry or moist soil. The plant can tolerate maritime exposure.

It is used for its alleged antidiabetic activity, which is also mentioned in Ayurvedic and Islamic system of medicines. *Achillea santolina* is used to relieve pain or dryness of the navel, stomach pain or gas. It is also used to relieve the symptoms of the common cold.

Phytopharmacological literature review indicates that *Achillea santolina* specie have not been fully investigated for its pharmacological effects, only a limited number of research

reports are available on the preliminary screening. Rehman and Zaman (1989) have mentioned that aqueous decoction of leaves and young twigs of *Achillea santolina* a popular remedy for the treatment of various skin lesions. This plant is claimed to be remedy for cancer in its early stages and preliminary pharmacological tests of aqueous extracts on mice have shown some anti-cancer activity (Kiritikar and Basu, 1988).

Reports are available about the use of both in *Caralluma tuberculata* and *Achillea santolina* in folk medicine for the treatment of diabetes (Nadharni, 1954 and Volkovic, 1975).

Figure 11: The aerial parts of *Achillea santolina* plant.

Preparation of crude extracts of *Caralluma tuberculata* and *Achillea santolina*:

The aerial parts of *Caralluma tuberculata* and *Achillea santolina* were washed in order to remove soil, sand, and other foreign material and were dried in a shadow. The dried material of *Caralluma tuberculata* was powdered in a grinder and passed through a mesh. The ground plant material was put in distilled water and kept in a closed glass container at room temperature for several days, followed by squeezing in a muslin cloth. This procedure was repeated thrice.

The same procedure was used for crude extraction of *Achillea santolina.* After the preparation of crude extracts of *Caralluma tuberculata* and *Achillea santolina,* gave green syrupy residues.

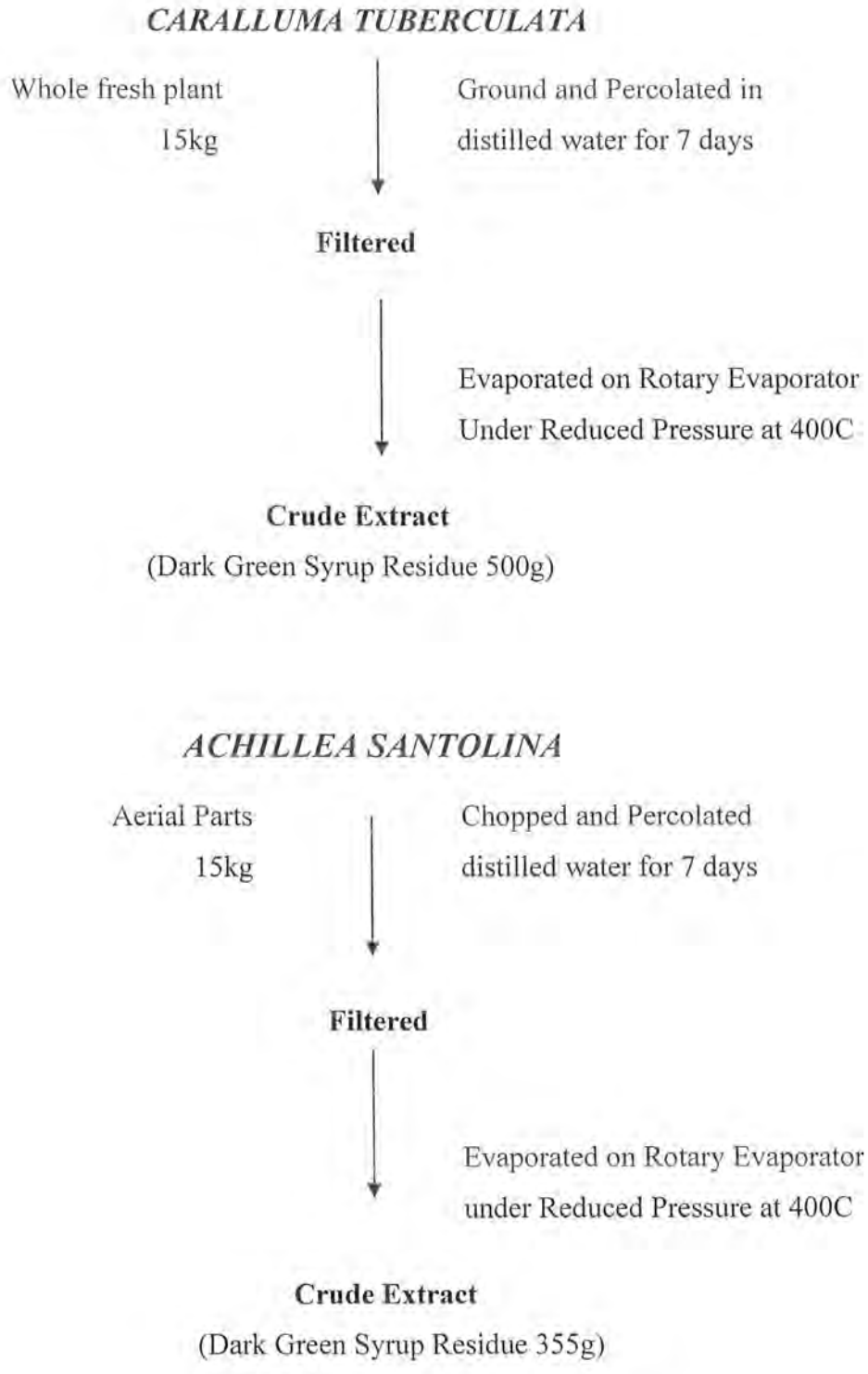

Figure 12: Crude extract preparation of *Caralluma tuberculata* and *Achillea santolina*

Fractionation of *Caralluma tuberculata* and *Achillea santolina* by carbon tetrachloride:

Fractionation of crude extract of *Caralluma tuberculata* was carried out by suspending the crude extract in water, then partitioned first with carbon tetrachloride (CCl_4) by vigorous shaking in a separating funnel. As a result, two layers, i.e. CCl_4 layers and aqueous layer were obtained. The CCl_4 layer was separated this procedure was repeated thrice. The combined CCl_4 fraction was evaporated under reduced pressure on rotary evaporator. After this water was evaporated on rotary evaporator under vacuum. The resultant residue was then treated with methanol, the methanol soluble fraction was separated from the insoluble (water soluble) material and evaporated on rotary evaporator. The same procedure was used for carbon tetrachloride (CCl_4) fractionation of crude extract of *Achillea santolina.*

ANIMALS USED

Normal (Non-diabetic) Rabbits

The rabbits weighing 1000-1500mg were kept under observation for one-week before experimentation in the animal house at Gomal Medical College, Dera Ismail Khan, Pakistan. The animals were kept on a balanced feed comprising of green fodder and animals had free access to water. It was based on four subgroups with six normal rabbits in each subgroup. Blood glucose levels in the rabbits were recorded at the similar time intervals as were observed in previous experiments. Subgroups A_5, B_5 and C_5 rabbits were administered with crude extracts of *Caralluma tuberculata*, *Achillea santolina* and Metformin (glucophage) 500mg. Subgroup D_5 rabbits were orally administered with 20ml 2% gum tragacanth solution respectively.

GROUPING OF ANIMALS

To study antidiabetic activity, eighty-four rabbits were divided randomly into two main groups.

Group I. Normal (Non-Diabetic) Rabbits:
Group II. Diabetic (Alloxanized) Rabbits:

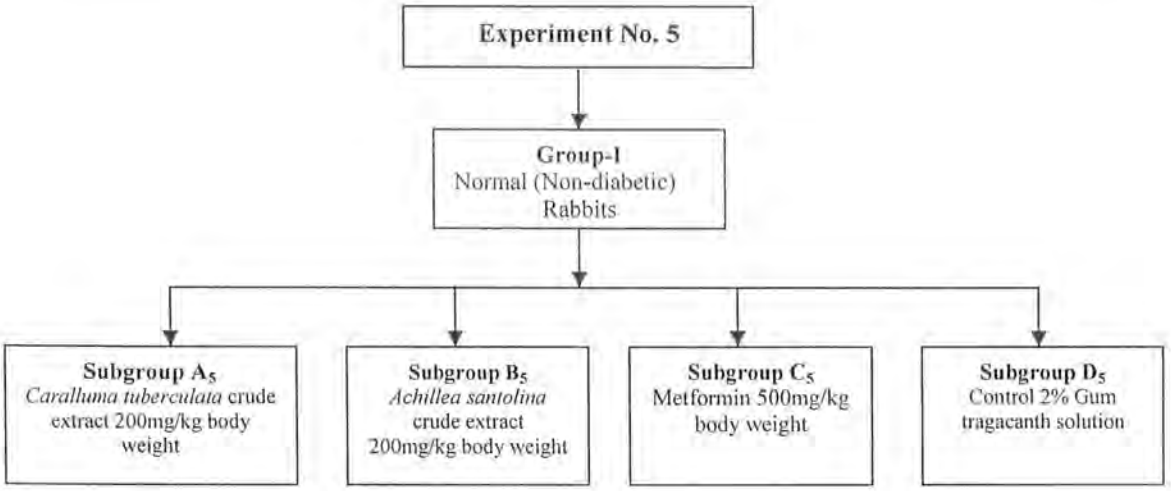

Figure: 13(A) The experimental design for the study of antidiabetic activity of *Caralluma tuberculata and Achillea santolina* crude extracts 200mg/kg body weight in normal rabbits. Experiment No 5.

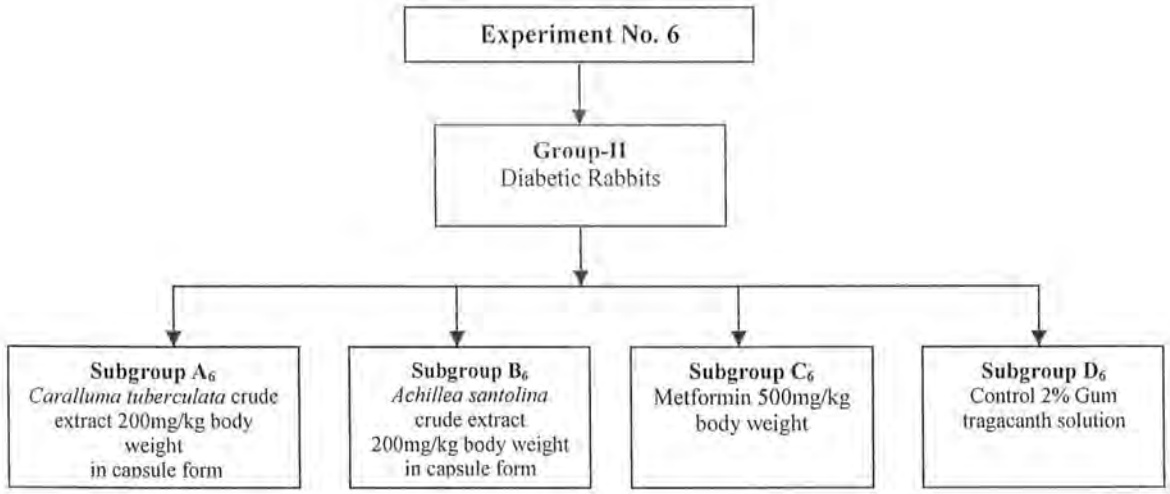

Figure: 13(B) The experimental design for the study of antidiabetic activity of *Caralluma tuberculata and Achillea santolina* crude extracts 200mg/kg body weight in diabetic rabbits. Experiment No 6.

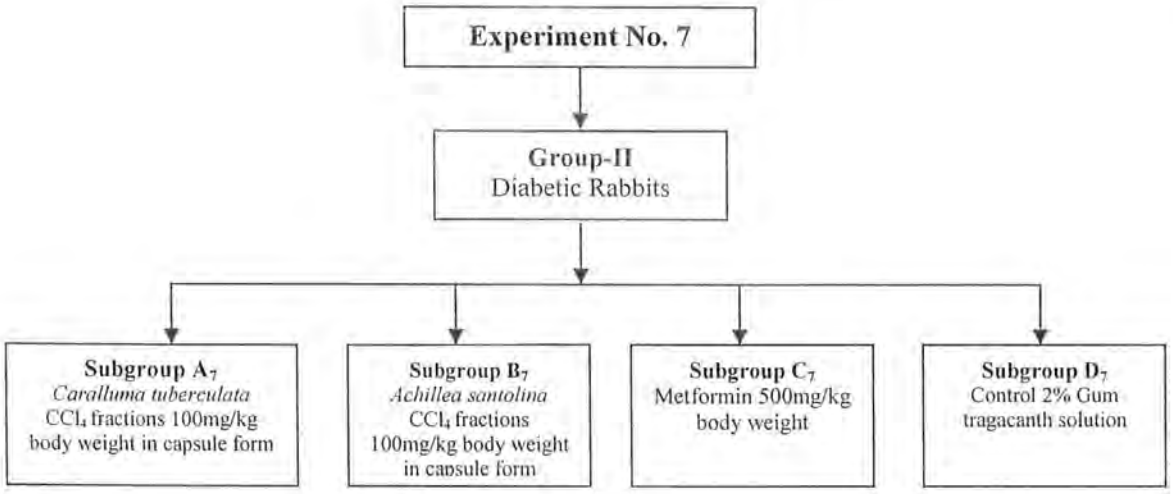

Figure: 14(A) The experimental design for the study of antidiabetic activity of *Caralluma tuberculata and Achillea santolina* CCl_4 fractions 100mg/kg body weight in capsule form. Experiment No 7.

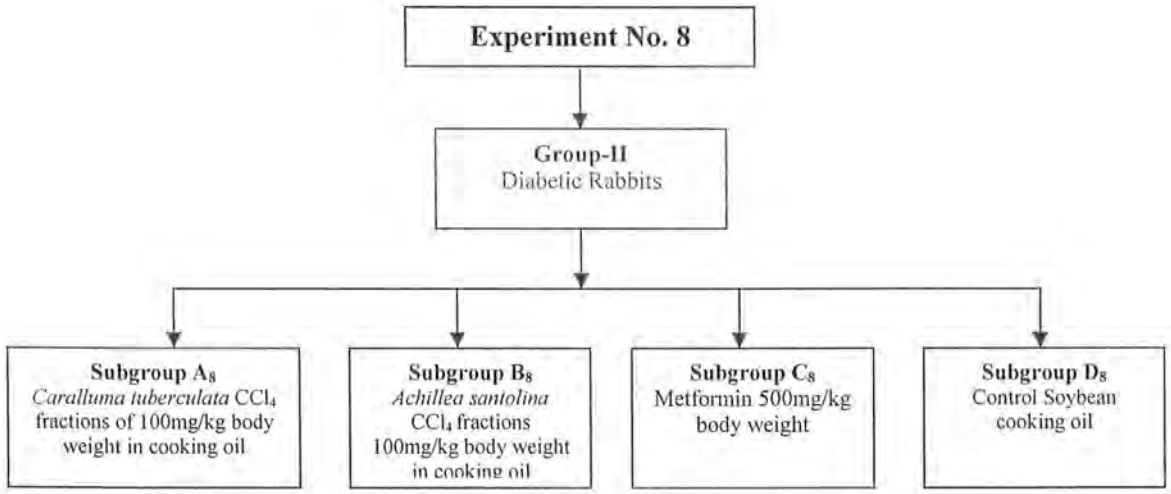

Figure: 14(B) The experimental design for the study of antidiabetic activity of *Caralluma tuberculata and Achillea santolina* CCl_4 fractions 100mg/kg body weight in cooking oil. Experiment No. 8.

Antidiabetic study of Caralluma tuberculata and Achillea santolina

RESULTS

EXPERIMENT NO. 5

In this experiment, the *hypoglycaemic* activity of crude extracts of *Caralluma tuberculata* and *Achillea santolina* were studied in group I, normal non diabetic rabbits.

It was based on four subgroups with 8 normal rabbits in each subgroup. Blood glucose levels in the rabbits were recorded at the similar time interval as were observed in the previous experiments. Subgroups A_5, B_5 and C_5 rabbits were administered with crude extracts of *Caralluma tuberculata* and *Achillea santolina* and Metformin 500mg. Subgroup D_5 rabbits were orally administered with 20ml 2% gum tragacanth solution.

Subgroup A_5: Treated with crude extract of *Caralluma tuberculata* 200mg/kg body weight in normal (non-diabetic) rabbits.

Subgroup B_5: Treated with crude extract of *Achillea santolina* 200mg/kg body weight in normal (non-diabetic) rabbits.

Subgroup C_5: Treated with Metformin 500mg/kg body weight in normal (non-diabetic) rabbits.

Subgroup D_5: Serving as control receiving 20ml 2% gum tragacanth solution in normal (non-diabetic) rabbits.

1. Treatment with crude extract of *Caralluma tuberculata* in normal rabbits (A_5)

Highly significant decrease in mean blood glucose levels were observed at 2 hours ($p<0.001$), 4 hours ($p<0.001$) 8 hours ($p<0.001$) and 12 hours interval after treatment compared to zero hour level. Highly significant increase in blood glucose levels was seen at 24 hours interval compared to that at 12 hours interval ($t_{(14)} = 10.84$; $p<0.001$). (Table 5, Figure 15)

2. Treatment with crude extract of *Achillea santolina* in normal rabbits (B_5):

Significant decrease in mean blood glucose levels was seen at 2 hours interval after treatment ($p<0.05$), but highly significant mean blood glucose levels, compared to that at zero hour, was at 4 hours ($p<0.001$), 8 hours ($p<0.001$) and at 12 hours interval. There was non-significant increase in blood glucose levels compared to that at 8 hours interval ($t_{(14)} = 1.67$; $p=0.20$). There was highly significant increase in blood glucose level at 24 hours interval compared to that at 12 hours interval ($t_{(14)} = 10.84$; $p<0.001$).

Comparison of Crude extracts of *Caralluma tuberculata* and *Achillea santolina*

Treatment with *Caralluma tuberculata* (A_5) and *Achillea santolina* (B_5) show significant decrease in blood glucose levels during different time intervals. There was, however, sharp difference in mean blood glucose level in *Caralluma tuberculata* (A_5) and *Achillea santolina* (B_5). In *Caralluma tuberculata* (A_5) at 2 hours interval ($t_{(14)} = 3.31$; $p<0.05$), 4 hours ($t_{(14)} = 2.89$; $p<0.05$) and 12 hours time ($t_{(14)} = 5.35$; $p<0.05$) mean blood glucose levels were significantly low compared to that in *Achillea santolina* (B_5). These comparisons indicate that *Caralluma tuberculata* is better in producing *hypoglycaemia* than *Achillea santolina*. (Table 5, Figure 15)

3. Treatment with Metformin 500mg/kg body weight in normal rabbits (C_5):

Treatment with Metformin (glucophage) 500mg/kg body weight showed significant decrease from zero hour after 2 hours interval ($p<0.05$) and highly significant decrease at 4 hours ($p<0.001$) and 8 hours interval ($p<0.001$) but significant decrease in blood glucose level at 12 hours level ($p<0.05$) compared to that at zero hour. There was significant increase in blood glucose levels at 24 hours interval ($t_{(14)} = 3.66$; $p<0.05$) compared to that at 12 hours interval.

Comparison among *Caralluma tuberculata, Achillea santolina* and Metformin

The effect of Metformin (glucophage) on mean blood glucose levels was more or less intermediate between that of crude extract of *Caralluma tuberculata* and *Achillea santolina*, with glucophage blood glucose levels decreased after 2 hours interval and 4 hours interval but this started increasing from 8 hours to 24 hours interval after treatment. This increase is significantly higher compared to that at 12 hours interval ($t_{(14)} = 3.66$; $p<0.01$). The results of this experiment indicates that treatment with *Caralluma tuberculata* gives better results than oral administration with *Achillea santolina* in terms of *hypoglycaemia* produced by the former plant. Also at 24 hours interval decrease in blood glucose levels is significantly low with *Caralluma tuberculata* than with *Achillea santolina* ($t_{(14)} = 2.30$; $p<0.05$). (Table 5, Figure 15)

4. Treatment with 20ml 2% gum tragacanth suspension in normal rabbits (D_5):

Oral administration of 20ml 2% gum tragacanth suspension did not produce any appreciable decrease in blood glucose levels. More or less consistency in blood glucose levels was observed. (Table 5, Figure 15)

Table 5: Mean blood glucose level mg/dl of normal (non-diabetic) rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral administration of crude extracts of *Caralluma tuberculata* and *Achillea santolina* 200mg/kg body weight, Metformin (glucophage) 500mg/kg body weight and 20ml 2% gum tragacanth.

	Crude extract 200mg/kg body weight		500mg/kg Body weight	20ml
Time interval	*Caralluma*	*Achillea santolina*	Metformin	2%gum tragacanth
Hours	*tuberculata*			
Subgroups	A_5	B_5	C_5	D_5
0	104.33 ±1.33	107.00 ± 1.78	92.16±0.83	94.17±0.60
2	79.67 ± 2.96 ^{a***g*}	97.17 ± 2.32 ^{a*}	82.00±0.96 ^{a*}	94.00±0.58
4	73.17 ± 2.64 ^{a***g*}	88.00 ± 2.51 ^{a***}	72.50±1.31 ^{a***}	94.33±0.81
8	80.33 ± 2.23 ^{a***}	83.50 ± 1.08 ^{a***}	79.66±1.76 ^{a***}	93.50±0.67
12	72.50 ± 0.88 ^{a***g*}	89.00 ± 2.20 ^{a***}	87.16±0.75 ^{a*}	91.67±0.50
24	99.50 ± 1.61 ^{e***h*}	102.33 ± 1.64 ^{e***}	92.00±0.57 ^{e*}	
92.50±1.05				

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$
a = 0 hour vs all time intervals
b = 2 hours vs 4 hours
c = 4 hours vs 8 hours
d = 8 hours vs 12 hours
e = 12 hours vs 24 hours
f = Gum tragacanth vs with all time intervals
g = Subgroup C_5 vs subgroup B_5 and subgroup A_5
h = Subgroup B_5 vs subgroup A_5

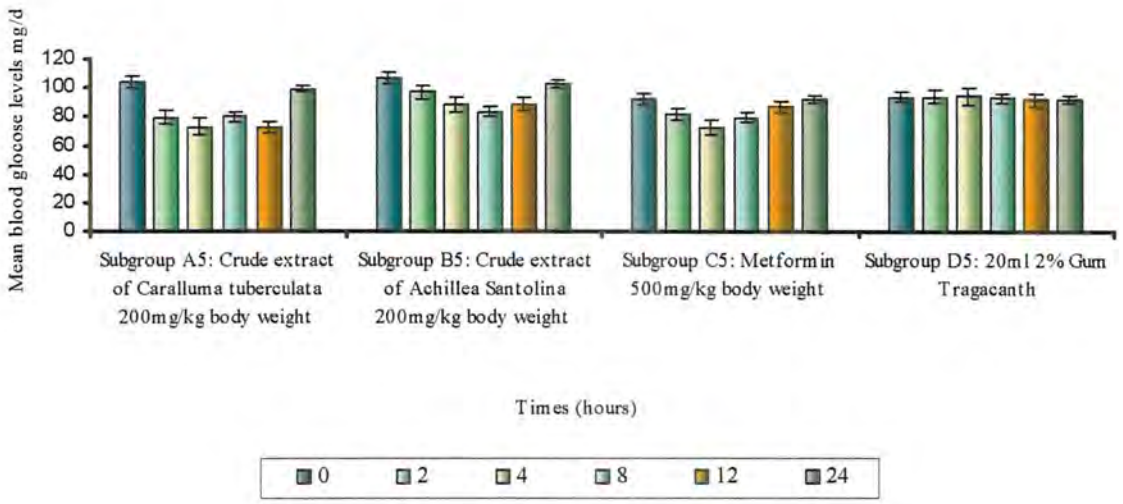

Figure 15: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval crude extracts of *Caralluma tuberculata* and *Achillea santolina* 200mg/kg body weight, Metformin 500mg/kg body weight and 20ml 2% gum tragacanth in normal (non-diabetic) rabbits.

EXPERIMENT NO. 6

This experiment was started with diabetic rabbits. Experiment design was the same as with experiment No. 5. The rabbits were administered with crude extracts of *Caralluma tuberculata* and *Achillea santolina* in capsule form. In each of the four subgroups, eight rabbits were used.

Subgroup A_6: Treated with crude extract of *Caralluma tuberculata* 200mg/kg body weight.
Subgroup B_6: Treated with crude extract of *Achillea santolina* 200mg/kg body weight.
Subgroup C_6: Treated with Metformin (glucophage) 500mg/kg body weight.
Subgroup D_6: Received only 20ml 2% gum tragacanth suspension.

1. **Treatment with crude extract of *Caralluma tuberculata* 200mg/kg body weight in capsule form in diabetic rabbits (A_6):**

Mean blood glucose levels at zero time interval were recorded and afterwards at different time intervals. Changes in blood glucose levels were recorded. The highest mean blood glucose levels were recorded at zero hour intervals. After 2 hours interval highly significant decrease in mean blood glucose levels was observed as compared to zero hour level ($p<0.001$). Highly significant decrease in blood glucose levels at 4 hours interval ($p<0.001$), 8 hours intervals ($p<0.001$), 12 hours intervals ($p<0.001$) and 24 hours interval significant decrease ($p<0.05$) compared to that at zero hour was observed. Compared to 2 hours interval, there was no significant decrease in blood glucose levels at 4 hours interval ($t_{(14)} = 1.76$; $p=0.2$) and 8 hours interval ($t_{(14)} = 1.97$; $p=0.2$). There was significant decrease in blood glucose levels at 12 hours interval compared to that 8 hours interval ($t_{(14)} = 2.59$; $p<0.05$) and highly significant increase at 24 hours interval compared to that at 12 hours interval ($t_{(14)} = 9.10$; $p<0.001$). (Table 6, Figure 16)

2. **Treatment with crude extract of *Achillea santolina* 200mg/kg body weight in capsule form in diabetic rabbits (B_6):**

Crude extract of *Achillea santolina* was orally administered to diabetic rabbits. Highest mean blood glucose levels were recorded at zero hour interval. Compared to zero hour

blood glucose levels significant decrease was seen at 4 hours interval ($p<0.05$) and 8 hours interval ($p<0.05$), but there was non-significant increase at 12 hours interval and 24 hours interval. Compared to 2 hours interval levels, there was significant decrease in blood glucose levels at 4 hours interval ($t_{(14)} = 2.58$; $p<0.05$) and at 8 hours interval compared to that at 4 hours interval ($t_{(14)} = 2.47$; $p<0.05$). Non-significant increase in blood glucose levels compared to 8 hours level was seen at 12 hours interval ($t_{(14)} = 1.35$; $p=0.2$). Non-significant increase in blood glucose levels at 24 hours interval compared to that at 12 hours interval was observed ($t_{(14)} = 1.49$; $p=0.2$). (Table 6, Figure 16)

3. Treatment with Metformin (glucophage) 500mg/kg body weight in diabetic rabbits (C_6):

Glucophage with 500mg/kg body weight dose was orally administered to rabbits. After 2 hours of treatment significant decrease ($p<0.05$) in blood glucose levels was recorded compared to zero hour levels. In comparison 2 hours vs 4 hours ($t_{(14)} = 2.77$; $p<0.05$); 4 hours vs 8 hours ($t_{(14)} = 2.60$; $p<0.05$) and 12 hours vs 8 hours ($t_{(14)} = 3.41$; $p<0.01$) after treatment significant decrease in mean blood glucose levels were observed. Significant increase in blood glucose levels after 24 hours of treatment compared to 12 hours level ($t_{(14)} = 2.25$; $p<0.05$) was recorded. (Table 6, Figure 16)

4. Treatment of rabbits with 20ml 2% gum tragacanth suspension (D_6):

The rabbits were treated with 2% Gum tragacanth and was maintained as control. There was no appreciable decrease or increase in mean blood glucose level in this case.

Comparison of *Caralluma tuberculata, Achillea santolina* and Metformin

In the crude form, performance of *Achillea santolina* in developing *hypoglycaemia* was seems to be better than *Caralluma tuberculata*. This is obvious from comparison of blood glucose at different time intervals. There is significantly low mean blood glucose levels in treatment with *Achillea santolina* compared to that with *Caralluma tuberculata* at 2 hours interval ($t_{(14)} = 2.72$; $p<0.05$), 4 hours interval ($t_{(14)} = 6.92$; $p<0.001$), 8 hours interval ($t_{(14)} = 5.63$; $p<0.001$) and 12 hours interval ($t_{(14)} = 6.70$; $p<0.001$).

Treatment with Metformin (glucophage) appears to be less effective compared to that with *Caralluma tuberculata* and *Achillea santolina.* Compared to treatment with Metformin (glucophage), *Caralluma tuberculata* and *Achillea santolina* show highly significantly low mean blood glucose level at 2 hours interval ($t_{(14)}$=9.06; p<0.001), 4 hours interval ($t_{(14)}$=15.05; p<0.001), 8 hours interval ($t_{(14)}$=10.56; p<0.001), 12 hours interval ($t_{(14)}$=16.66; p<0.001) and 24 hours interval after treatment ($t_{(14)}$ = 7.12; p<0.001).

Comparison between *Achillea santolina* and Metformin

Similar results of treatment with *Achillea santolina* compared to that with Metformin (glucophage) were observed. *Achillea santolina* developed significantly high *hypoglycaemia* compared to that with Metformin (glucophage) at 2 hours interval ($t_{(14)}$ = 8.67; p<0.001); 4 hours interval ($t_{(14)}$ = 13.12; p<0.001); 8 hours interval ($t_{(14)}$ = 11.88; p<0.001); 12 hours interval ($t_{(14)}$ = 8.25; p<0.001) and 24 hours interval ($t_{(14)}$ = 8.29; p<0.001). (Table 6, Figure16)

Table 6: Mean blood glucose level mg/dl of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral administration of crude extracts of *Caralluma tuberculata* and *Achillea santolina* 200mg/kg body weight in capsule forms. Metformin (glucophage) 500mg/kg body weight and 20ml 2% gum tragacanth.

	Crude extracts 200mg/kg body weight		500mg/kg Body weight	20ml
Time interval Hours	*Caralluma tuberculata*	*Achillea santolina*	Metformin	2%gum tragacanth
Subgroups	A_6	B_6	C_6	D_6
0	318.16 ± 2.05	271.50 ± 4.46	335.16 ± 3.04	309.00 ± 1.62
2	268.00 ± 2.61 ^{a***g*}	246.00 ± 5.40 ^{a*g***}	318.50 ± 2.96 ^{a*}	309.66 ± 1.78
4	263.00 ± 1.24 ^{a***g***}	220.50 ± 4.90 $^{a*, g***}$	306.50 ± 1.65	309.00 ± 0.89
8	269.33 ± 1.97 ^{a***g***}	233.17 ± 4.45 ^{a*c*g***}	322.66 ± 3.08 ^{c*}	309.16 ± 3.25
12	260.33 ± 1.50 ^{a***d*g***}	255.17 ± 6.20 ^{g***}	327.00 ± 2.50 ^{d*}	312.00 ± 2.85
24	287.83 ± 3.52 ^{a*e***g***}	271.50 ± 4.72 ^{g***}	340.80 ± 3.63 ^{e*}	310.33 ± 2.50

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$
a = 0 hour vs all time intervals
b = 2 hours vs 4 hours
c = 4 hours vs 8 hours
d = 8 hours vs 12 hours
e = 12 hours vs 24 hours
f = Gum tragacanth vs with all time intervals
g = Subgroup C_6 vs subgroup A_6 and subgroup B_6
h =Subgroup B_6 vs subgroup A_6

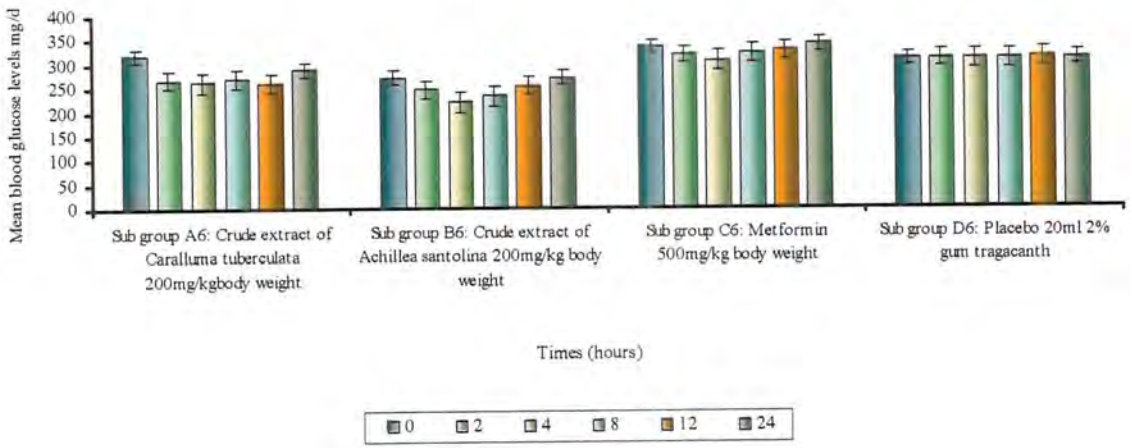

Figure 16: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval crude extracts of *Caralluma tuberculata* and *Achillea santolina* 200mg/kg body weight, Metformin 500mg/kg body weight and Placebo 20ml 2% gum tragacanth in diabetic rabbits.

EXPERIMENT NO. 7

This experiment was started with diabetic rabbits. The design of this experiment was the same as with that of normal rabbits in Experiment No 5. In one subgroup of rabbits, *Caralluma tuberculata* was orally administered with carbon tetrachloride fraction (100mg/kg body weight) in capsule form (A_7); *Achillea santolina* with carbon tetrachloride fraction (100mg/kg body weight) in capsule form (B_7); Metformin (glucophage) with a dose of 500mg/kg body weight (C_7) and fourth subgroup received 20ml of 2% gum tragacanth suspension (Placebo D_7).

Subgroup A_7: Treatment with *Caralluma tuberculata* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in capsule form in diabetic rabbits.

Subgroup B_7: Treatment with *Achillea santolina* carbon tetrachloride fraction 100mg/kg body weight in capsule form in diabetic rabbits.

Subgroup C_7: Treatment with Metformin 500mg/kg body weight in diabetic rabbits.

Subgroup D_7: Treatment with 20ml 2% gum tragacanth suspension in diabetic rabbits.

1. **Treatment with *Caralluma tuberculata* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in capsule form in diabetic rabbits (A_7):**

At zero hour, blood glucose levels were very high (Table 7). At 2 hours interval after oral administration of *Caralluma tuberculata* with carbon tetrachloride in capsule, blood glucose levels dropped markedly. This decrease was highly significant ($t_{(14)} = 3.87$; $p<0.001$) compared to that at zero hour interval. After 4 hours interval, blood glucose levels further decreased but non-significantly compared to that 2 hours interval. Increase in mean blood glucose levels were observed at 8 hours interval while increase was significantly higher than at 4 hours interval ($t_{(14)} = 3.17$; $p<0.01$). Similarly, increase after 24 hours interval in mean blood glucose levels was significantly higher than at 12 hours time ($t_{(14)} = 8.10$; $p<0.01$). (Table 7, Figure 17)

2. **Treatment with *Achillea santolina* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in capsule form in diabetic rabbits (B_7):**

The highest mean blood glucose levels were observed at zero hour intervals. But significant decrease in these levels was observed after 2 hours interval ($t_{(14)} = 9.89$; $p<0.01$). Significant

decrease in blood glucose levels was also observed at 4 hours interval compared to 2 hours interval ($t_{(14)}$ = 3.03; p<0.01). There was not much difference in mean blood glucose levels at 12 hours interval compared to that at 4 hours interval. Significant increase at 24 hours interval compared to that at 12 hours interval was observed ($t_{(14)}$ = 3.48; p<0.01). (Table 7, Figure 17)

3. Treatment with Metformin 500mg/kg body weight in diabetic rabbits (C_7):

Administration of Metformin (glucophage) resulted in significant decrease in blood glucose levels at 2 hours intervals vs zero hours interval ($t_{(14)}$ = 2.77; p<0.01) and 4 hours vs 2 hours interval ($t_{(14)}$ = 2.60; p<0.01). No appreciable difference in levels between 8 hours and 12 hours interval was noticed. Significant increase in blood glucose levels was at 24 hours interval compared to that at 12 hours time ($t_{(14)}$ = 3.48; p<0.01). (Table 7, Figure 17)

Comparison between *Caralluma tuberculata* and *Achillea santolina*

Differences in mean blood glucose levels between treatments were also calculated. Comparison between *Caralluma tuberculata* vs *Achillea santolina* treatment shows highly significant decrease in blood glucose levels due to *caralluma* treatment at 2 hours interval ($t_{(14)}$ = 17.68; p<0.001); 4 hours interval ($t_{(14)}$ = 14.01; p<0.001); 8 hours time ($t_{(14)}$ = 8.37; p<0.001); 12 hours time ($t_{(14)}$ = 10.34; p<0.001) but at 24 hours time ($t_{(14)}$ = 1.98; p=0.2) the difference between these levels was not statistically significant. (Table 7, Figure 17)

Comparison between *Caralluma tuberculata* and Metformin

Comparison between *Caralluma tuberculata* vs Metformin (glucophage) treatment show that *caralluma* produces highly significantly low blood glucose levels at 2 hours interval ($t_{(14)}$=36.04; p<0.001); 4 hours interval ($t_{(14)}$=22.73; p<0.001); 8 hours intervals ($t_{(14)}$=7.24; p<0.001); 12 hours intervals ($t_{(14)}$ = 23.64; p<0.001) and 24 hours intervals ($t_{(14)}$=11.45; p<0.001). (Table 7, Figure 17)

Comparison between *Achillea santolina* and Metformin

Similarly, comparison between *Achillea* and Metformin (glucophage) treatment shows that *Achillea* treatment produces significantly low mean blood glucose levels quite late compared to Metformin (glucophage) treatment. Due to *Achillea* treatment vs Metformin (glucophage) at

8 hours interval. The former plant produces significantly low mean blood glucose levels ($t_{(14)}$ = 2.13; $p<0.05$). Significantly, low mean blood glucose levels at 12 hours interval ($t_{(14)}$ = 2.81; $p<0.05$) and 24 hours intervals ($t_{(14)}$ = 10.50; $p<0.001$) was produced due to *Achillea* treatment than with Metformin (glucophage). (Table 7, Figure 17)

4. <u>Treatment with 20ml 2% gum tragacanth suspension in diabetic rabbits (D_7):</u>

Starting from zero hour intervals to 24 hours interval, there was no appreciable change in mean blood glucose levels. Compared to control subgroup (D_7) treatment given with *Caralluma* and *Achillea* shows highly significant decrease in mean blood glucose levels in different time interval. (Table 7, Figure 17)

Table 7: Mean Blood glucose levels mg/dl of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral administration of carbon tetrachloride (CCl_4) fractions of *Caralluma tuberculata* and *Achillea santolina* 100mg/kg body weight in capsule forms, Metformin (glucophage) 500mg/kg body weight and 20ml 2% gum tragacanth.

	Crude extracts 100mg/kg body weight		500mg/kg Body weight	20ml
Time interval Hours	*Caralluma tuberculata*	*Achillea santolina*	Metformin	2%gum tragacanth
Subgroups	A_7	B_7	C_7	D_7
0	309.50 ± 1.38	326.00 ± 2.60	335.16 ± 3.04	309.00 ± 1.62
2	194.16 ± 2.49^{a***h***g***}	278.00 ± 2.25^{a**}	318.50 ± 2.96^{a**}	309.66 ± 1.78
4	184.16 ± 3.73^{a***h***g***}	265.00 ± 2.04^{a**}	306.50 ± 1.65^{a**}	309.00 ± 0.89
8	207.67 ± 3.68^{a**c**h***g***}	256.66 ± 2.17^{a**g*}	322.66 ± 3.08^{a*}	309.16 ± 3.25
12	206.67 ± 2.59^{a***h***g***}	253.33 ± 1.92^{a**g**}	327.00 ± 2.50	312.00 ± 2.85
24	257.33 ± 3.66^{a***e**g***}	270.66 ± 3.05^{a**e**g***}	340.80 ± 3.63^{e**}	310.33 ± 2.50

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$

a = 0 hour vs all time intervals

b = 2 hours vs 4 hours

c = 4 hours vs 8 hours

d = 8 hours vs 12 hours

e = 12 hours vs 24 hours

f = Gum tragacanth vs with all time intervals

g = Subgroup A_7 vs subgroup B_7 and subgroup C_7

h = Subgroup A_7 vs subgroup B_7

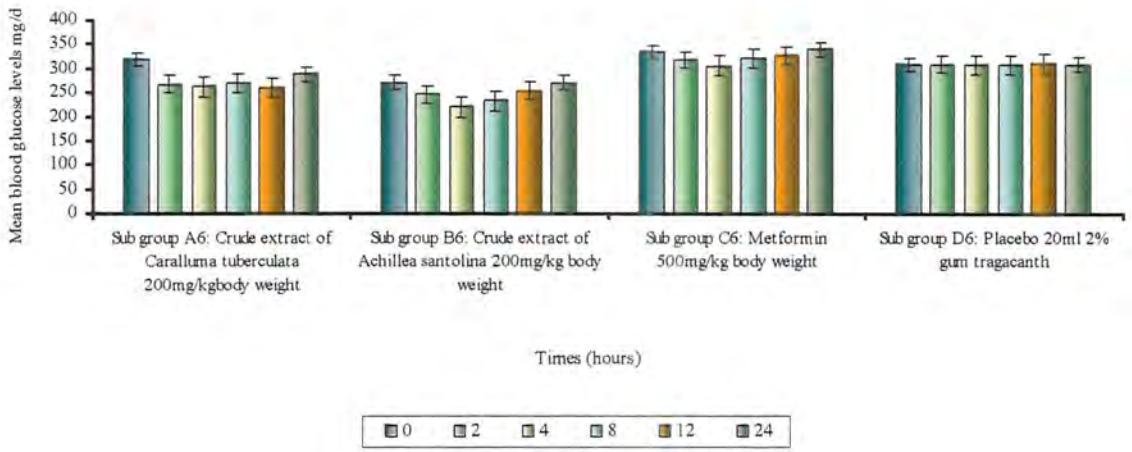

Figure 16: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours interval crude extracts of *Caralluma tuberculata* and *Achillea santolina* 200mg/kg body weight, Metformin 500mg/kg body weight and Placebo 20ml 2% gum tragacanth in diabetic rabbits.

EXPERIMENT NO. 8

In this experiment *Caralluma tuberculata* (A_8) and *Achillea santolina* (B_8) with 100mg/kg body weight of carbon tetrachloride (CCl_4) fraction in cooking oil was orally administered to diabetic rabbits. Treatment of rabbits with Metformin (glucophage) 500mg/kg body weight (C_8) and one subgroup of control (D_8) was orally administered with 20ml of soybean cooking oil. The design of this experiment was the same as with experiment No. 7.

Subgroup A_8: Treatment with *Caralluma tuberculata* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in cooking oil in diabetic rabbits.

Subgroup B_8: Treatment with *Achillea santolina* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in cooking oil in diabetic rabbits.

Subgroup C_8: Treatment with Metformin 500mg/kg body weight in diabetic rabbits.

Subgroup D_8: Treatment with 20ml soybean cooking oil in diabetic rabbits.

1. Treatment with *Caralluma tuberculata* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in cooking oil in diabetic rabbits (A_8):

Caralluma tuberculata was orally administered. The highest mean blood glucose levels were at zero hour. There was highly significant decrease in mean blood glucose levels after 2 hours interval compared to that at zero hour ($p<0.001$). There was highly significant decrease in comparison with that at zero hour after 4 hours, 8 hours, 12 hours and 24 hours intervals ($p<0.001$).

There was significant difference in blood glucose levels at 4 hours interval compared to that at 2 hours interval ($t_{(14)} = 2.25$; $p<0.05$). No significant increase in comparison 4 hours vs 8 hours ($t_{(14)} = 0.33$; $p=0.2$); 8 hours vs 12 hours ($t_{(14)}=0.8$; $p=0.2$) and 12 hours vs 24 hours ($t_{(14)}=1.10$; $p=0.2$) was observed. (Table 8, Figure 18)

2. Treatment with *Achillea santolina* carbon tetrachloride (CCl_4) fraction 100mg/kg body weight in cooking oil in diabetic rabbits (B_8):

The highest blood glucose levels were at zero hour intervals after 2 hours of treatment, there was highly significant decrease in mean blood glucose levels ($t_{(14)} = 27.63$; $p<0.001$). Similarly, in

comparison at zero hour vs 8 hours; 0 hour vs 12 hours and zero hour vs 24 hours the decrease in mean blood glucose levels was highly significant ($p<0.001$). There was significant increase at 4 hours interval compared to 2 hours interval ($t_{(14)} = 2.16$; $p<0.05$) but at 8 hours interval increase in mean blood glucose levels was not significant compared to that at 4 hours interval ($t_{(14)} = 1.005$; $p=0.2$). However, in 8 hours vs 12 hours ($t_{(14}=2.78$; $p<0.05$) and 24 hours vs 12 hours interval ($t_{(14)}=7.01$; $p<0.001$) the increase in blood glucose levels were statistically significant. (Table 8, Figure 18)

3. Treatment with Metformin (glucophage) 500mg/kg body weight in diabetic rabbits (C_8):

Administration of Metformin (glucophage) resulted in significant decrease in blood glucose levels at 2 hours and 4 hours intervals vs zero hours interval ($t_{(14)} = 2.77$; $p<0.01$) and 4 hours vs 2 hours interval ($t_{(14)} = 2.60$; $p<0.01$). No appreciable difference in levels between 8 hours and 12 hours interval was noticed. Significant increase in blood glucose levels was at 24 hours interval compared to that at 12 hours time ($t_{(14)} = 3.48$; $p<0.01$). (Table 8, Figure 18)

4. Treatment with 20ml Soybean cooking oil in diabetic rabbits (D_8):

There was no appreciable difference in mean blood glucose levels during different time intervals. This experiment was maintained as control against treatment groups. (Table 8, Figure 18)

Comparison of *Caralluma tuberculata, Achillea santolina* and Metformin (glucophage)

This experiment indicates that *Caralluma tuberculata* produces more *hypoglycaemic* effect than with *Achillea santolina* comparisons were carried out at different hours after treatment. At 2 hours intervals after treatment with *Caralluma tuberculata* vs *Achillea santolina,* there was highly significant decrease in blood glucose levels with *Caralluma tuberculata* than with *Achillea santolina* ($t_{(14)} = 2.92$; $p<0.001$). The same significant trend for decrease in blood glucose levels with *Caralluma tuberculata* was observed at 4 hours interval compared to *Achillea santolina* ($t_{(14)} = 19.29$; $p<0.001$). The blood glucose levels at 8 hours interval was highly significantly different in *Caralluma* vs *Achillea* comparison ($t_{(14)} = 14.41$; $p<0.001$). Highly significant increase in blood glucose levels with *Achillea santolina* treatment compared to *Caralluma tuberculata* treatment was observed at 12 hours interval ($t_{(14)} = 16.76$; $p<0.001$) and at 24 hours interval ($t_{(14)} = 21.26$; $p<0.001$). (Table 8, Figure 18)

Comparison of *Caralluma tuberculata* and *Achillea santolina* filled in capsule and in cooking oil dosage forms

In Experiments No 7 and 8, oral treatments with *Caralluma tuberculata* and *Achillea santolina* in capsule form and cooking oil respectively was also compared. It was also analyzed to see treatment given in what way produce more *hypoglycaemic* effect.

These two experiments show that *Caralluma tuberculata* administered in cooking oil leads to higher reduction in blood glucose levels than that administered in capsule form. This has been seen in oral administration of *Caralluma* in capsule form vs in cooling oil that at 2 hours interval ($t_{(14)}$ = 12.25; $p<0.001$), 4 hours interval ($t_{(14)}$ = 5.48; $p<0.001$) 8 hours interval ($t_{(14)}$ = 8.00; $p<0.001$), 12 hours interval ($t_{(14)}$ = 9.02; $p<0.001$) and 24 hours interval ($t_{(14)}$ = 13.44; $p<0.001$). There is highly significant decrease in blood glucose levels due to administration of *Caralluma tuberculata* in cooking oil than in capsule form. Similarly, *Achillea santolina* administered in cooling oil shows highly significant decrease in mean blood glucose levels than when given in capsule form at 4 hours interval ($t_{(14)}$ = 8.69; $p<0.001$), 8 hours interval ($t_{(14)}$ = 8.04; $p<0.001$) and 12 hours interval ($t_{(14)}$ = 2.65; $p<0.001$). However, non-significant difference in mean blood glucose level was observed at 2 hours interval ($t_{(14)}$ = 1.68; $p=0.2$) and there was no difference in blood glucose levels at 24 hours interval in capsule vs cooking oil treatment. (Tables 7 and 8, Figures 17 and 18)

Table 8: Blood Glucose level mg/dl of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hours intervals after oral administration of carbon tetrachloride (CCl_4) fractions of *Caralluma tuberculata* and *Achillea santolina* 100mg/kg body weight in cooking oil, Metformin 500mg/kg body weight and control 20ml Soybean cooking oil.

	CCl_4 100mg/kg body weight		500mg/kg Body weight	20ml
Time interval Hours	*Caralluma tuberculata*	*Achillea santolina*	Metformin	Soybean cooking oil
Subgroups	A_8	B_8	C_8	D_8
0	287.55 ± 1.96	336.50 ± 2.12	335.16 ± 3.04	301.00 ± 2.08
2	142.33 ±1.74 ^{a***} ^{h*} ^{g***}	233.16 ± 1.62 ^{a***} ^{g*}	318.50 ± 2.96 ^{a*}	301.00 ± 2.35
4	151.33 ± 2.26 ^{a***} ^{h***} ^{g***}	226.16 ± 1.62 ^{a***} ^{g*}	306.50 ± 1.65 ^{a*}	302.66 ± 3.48
8	153.16 ± 3.13 ^{a***} ^{h**} ^{g***}	230.00 ± 2.20 ^{a***} ^{g*}	322.66 ± 3.08 ^{a*}	301.16 ± 1.58
12	158.00 ± 2.80 ^{a***} ^{h***} ^{g***}	242.33 ± 2.23 ^{a***} ^{d*} ^{g**}	327.00 ± 2.50	303.16 ± 2.24
24	164.66 ± 3.23 ^{a***} ^{h***} ^{g**}	270.33 ± 1.74 ^{a***} ^{e***} ^{g***}	340.80 ± 3.63 ^{e**}	303.83 ± 2.10

Mean ± SEM, $p<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$

a = 0 hour vs all time intervals

b = 2 hours vs 4 hours

c = 4 hours vs 8 hours

d = 8 hours vs 12 hours

e = 12 hours vs 24 hours

f = Soybean cooking oil vs all time intervals

g = Subgroup C_8 vs subgroup B_8 and subgroup A_8

h = Subgroup B_8 vs subgroup A_8

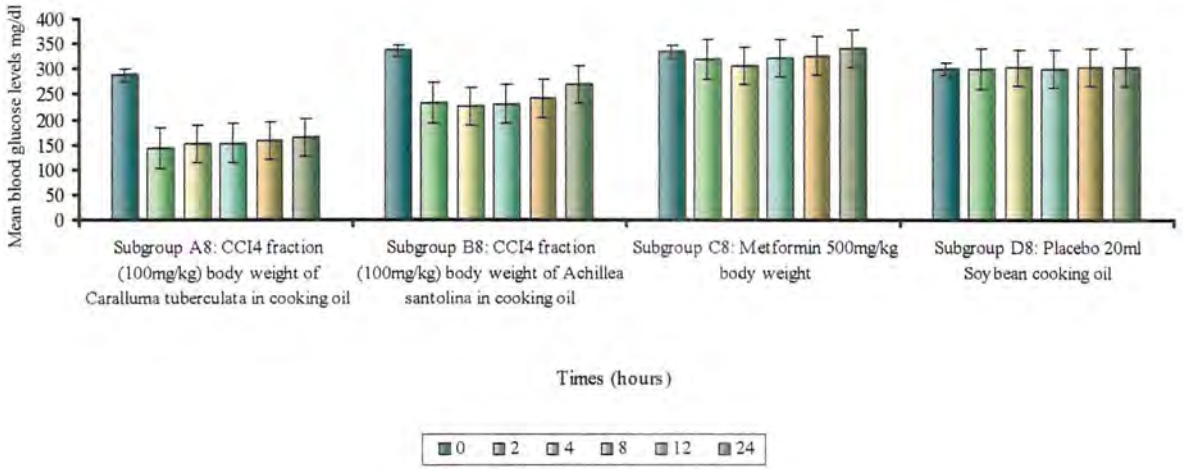

Figure 18: Mean blood glucose levels mg/dl at 0, 2, 4, 8, 12 and 24 hours of CCl_4 fraction of *Caralluma tuberculata* and *Achillea santolina* 100mg/kg body weight in cooking oil, Metformin 500mg/kg body weight and placebo 20ml 2% gum tragacanth in soybean cooking oil in diabetic rabbits.

DISCUSSION

In the present study, three plants *Cassia sophera*, *Caralluma tuberculata* and *Achillea santolina* were used for experimentation. The seeds and fruits of these plants were used in different forms to find out in what form, like crude extract, methanolic extract and carbon tetrachloride fractions (CCl_4) in capsule and in cooking oil to find out which of these plants will be more effective in developing hypoglycaemia. Different treatment doses, i.e. 2g/kg body weight, 3g/kg body weight and 4g/kg body weight were tried with *Cassia sophera,* which indicated that 4g/kg body weight dose is the one that developed better hypoglycaemic effect. Methanolic extracts of *Cassia sophera* reduced blood glucose levels after 2 hours of treatment and continued upto 12 hours, after treatment compared to aqueous extract where the turning point started from 8 hours after treatment and continued upto 12 hours of treatment. Methanolic extract of *Cassia sophera* powdered seeds was highly effective than aqueous extracts.

Crude extracts of *Caralluma tuberculata* and *Achillea santolina* with 200mg/kg body weight dose were administered in capsule form to diabetic rabbits. *Caralluma tuberculata* developed highly significant reduction in blood glucose after 2 hours of treatment, which continued upto 12 hours of time. *Achillea santolina* did produce just significant *hypoglycaemia* after 2 hours treatment which continued until 8 hours after treatment. Metformin (glucophage) 500mg/kg was also used simultaneously in one group, which developed *hypoglycaemia* comparable to that of *Achillea santolina.*

When carbon tetrachloride (CCl_4) fractions of *Caralluma tuberculata* and *Achillea santolina* (100mg/kg body weight) in capsule form were administered, the best results in developing *hypoglycaemia* were obtained from *Caralluma tuberculata.* This showed highly significant reduction in blood glucose levels from 2 hours after treatment until 24 hours. No doubt, *Achillea santolina* produced highly significant reduction in blood glucose level but that was observed from 8 hours after treatment to 24 hours. However, the comparison at different time intervals between *Caralluma tuberculata* and *Achillea santolina* clearly showed that highly significant *hypoglycaemia* was developed with

Caralluma tuberculata after 2 hours, 4 hours, 8 hours and 12 hours of treatment than with *Achillea santolina*. Glucophage as such was not that effective as were these two plants.

Carbon tetrachloride (CCl_4) fractions of *Caralluma tuberculata* and *Achillea santolina* (100mg/kg body weight) in cooking oil were also administered to diabetic rabbits. Although both these plants developed highly significant *hypoglycaemia* 2 hours after treatment, but effect with *Caralluma tuberculata* continued until 24 hours after treatment. However, with *Achillea santolina hypoglycaemia* was developed from 2 hours after treatment to 12 hours time. Comparison between *Caralluma tuberculata* and *Achillea santolina* at different time intervals indicated that from 2 hours, after treatment to 24 hours time *Caralluma tuberculata* developed significantly high *hypoglycaemia* than with *Achillea santolina*. Placebo and diabetic control groups did not show appreciable difference in blood glucose levels at different time intervals.

Ethanol extracts from *Cassia klenii* leaf has active fraction, which is very promising to develop standardized phytomedicines for diabetes mellitus. Different plants have been used individually or in formulation for treatment of diabetes and its completion. One of the major problem with this herbal formulation is that the active ingredients are not well defined it is important to find out active components in molecular interaction, which will help to analyze therapeutic efficacy of the product and also investigate mechanism of action of some of these plants using model systems. (Babu *et al.*, 2003).

Neveen Helmy Abu El-Soud *et al.*, (2007) orally administered alkaloid extract of fenugreek-dried seeds (*Trigonella foenum–graceum*) for twenty-one days to streptozotocin *hyperglycaemic* rats. They observed significant reduction of blood glucose and increase in serum insulin. The herbal preparation also resulted in significant decrease in serum lipids and helps to recover the pathological effects of diabetes on liver and kidney of streptozotocin induced diabetic rats. Mehmet *et al.*, 2006 orally administered water extract of aerial parts of *Artemisia herba-alba* and *Teucrium polium* to diabetic rabbits. Blood glucose levels were estimated before and 2, 4, 6 and 8 hours after

administration of extracts. A *herba-alba* produced significant effect in normal diabetic rabbits but *Teucrium polium* had insignificant effects.

Production of *hypoglycaemic* response in normal animals has also been reported for many medicinal plants like *Momordica charantia, Blighia spida* and *Allium cepa* etc (Hessal *et al.*, 1954 and Akhtar *et al.*, 1981). Doses of *Cassia sophera* have more prolonged duration of action as compared to the Acetohexamide, which has been reported to produce significant *hypoglycaemic* effect in human patients for about 6—8 hours (Katzung, 1994).

Alloxan, a selective beta cytotoxic drug has been demonstrated to produce in the treated animals all the clinical signs of human diabetes i.e. *hyperglycaemia, Glycosuria, Polydipsia, Polyuria* and *Polyphagia* loss in body weight and acidosis (Reurup, 1970). It has been observed that the single intravenous injection of 150mg/kg of alloxan to rabbits destroys their pancreatic β cells (Butt, 1962; Laurence and Bacharach, 1984) therefore; this dose of alloxan already known to kill the β cells of the rabbits was selected for the present experiment. Thus, administration of alloxan was observed to increase the blood glucose levels of the rabbits to about 3—4 times of their initial normal levels. However, sulphonylurease have been demonstrated not to decrease the blood glucose levels of alloxan-induced diabetic animals and it is only insulin that can lower the Blood glucose levels of alloxan induced diabetic animals (Selye, 1976).

Furthermore, drug like biguanides have been reported to produce *hypoglycaemia* by enhancing glycolysis and decreasing the gluconeogenesis in the liver. Also, these drugs decrease the intestinal glucose absorption and increase the uptake of glucose in muscles (Larner and Haynes, 1975). However, biguanides including metformin and phenformin do not produce *hypoglycaemia* in the normal subjects because in them the increase in peripheral glucose utilization is compensated by an increase in hepatic glucose output. Keeping all these in mind and the data presented it may be suggested that the three plants used here might contain some *hypoglycaemic* principles, which act either by stimulating the release of insulin and or themselves posses some insulin-like action (Larner, 1985).

Although the definite phytochemical analysis of these three plants has not yet been carried out but because of their *hypoglycaemic* effects both in normal and diabetic rabbits showed that they have some alkaloids in them and their effects are like plants alkaloids like vindoline and leurosine isolated from *Vinca rosea* reported to lower the blood glucose levels in normal animal only. Similarly, *Charantia* from *Momordica charantia* (*Karela*) possess *hypoglycaemic* activity (Satyavati *et al.*, 1976). Thus on the same analogy, it may be hypothesized that presently used plants contain some alkaloids and some other types of *hypoglycaemic* components, which act together to produce *hypoglycaemia* in normal rabbits by some indirect mechanism. On the contrary, in the alloxan diabetic rabbits, the plant-powdered seeds do not seem to stimulate the release of insulin, as the alloxan treatment causes permanent destruction of β cells (Laurence and Bacharach, 1984). If this is true then it would mean that the drug should decrease the blood glucose levels in both normal and diabetic animals to the same extent. However, this was not the case as 4g/kg dose of *Cassia sophera* plant produced a highly significant ($p<0.001$) decrease in blood glucose of normal rabbits at 8 and 12 hours while in the diabetic rabbits the decrease was only significantly ($p<0.05$) lower than zero hour at 8 and 12 hours after drug administration. It may, therefore, be suggested that in the normal rabbits this substance exerts not only a direct insulin-like effect but also acts indirectly by stimulating the releasing of insulin and/or facilitating its release from the pancreatic β cells and thus utilization of glucose by the body cells.

Baker (1982) has described that blood glucose undergoes Phosphorylation before it is utilized by the cells, which is magnesium dependent process. Therefore, it is also possible that in normal rabbits the powdered plant might have produced *hypoglycaemic* effect by facilitating glucose uptake by the cells and increasing the rate of Phosphorylation of glucose. It has also been suggested that alloxan exert its selective beta cytotoxicity by causing complexes with biological metals present in the β cells and thereby producing their deficiency.

In addition, plant drug might also initiate the release of insulin by providing the trace minerals like manganese, chromium and zinc etc, which are known to stimulate (induce) certain enzymatic processes as proposed by Donsbach (1982) who has used the term

"*Hypoglycaemic* elements" for such minerals in the biological matter. In conclusion, all the data discussed so far, it is conceivable that in these plants have more than one *hypoglycaemic* principles, both organic and inorganic, which produce significant fall in blood glucose levels in normal as well as diabetic rabbits by producing an organotropic effect on the β cells which results in increased release of insulin from the pancreatic β cells in the rabbits. In addition, the plant drug might also possess some orally active insulin-like substance such as that reported to be present in *Memoridica charantia* fruit by Akhtar *et al.*, (1981).

Production of *hypoglycaemia* through some other entirely different mechanism cannot be excluded, *hypoglycaemia* by different mechanism, for example, somatostatin, anterior pituitary and sex hormones, corticosteroids, prostaglandins (Oliver, 1980). The lowering of the blood glucose by nicotinic acid rich plants like *Trigonella foenum-graceum* have also been reported (Shani *et al.*, 1974). Therefore, comprehensive chemical and pharmacological investigations are further needed to elucidate the exact mechanism of *hypoglycaemic* effect of these plants

Some authors have used observational techniques to get information regarding acute toxicity and changes in behavioral pattern in treated animals (Laurence and Bacharach. 1984). During present study all the treated animals did not show any toxic effects and changes in behavioral patterns of the plants under study which were used in different forms. Rabbits were used as mammalian model, which indicated that the use of these plants in different forms are also safe. Therefore, the present plants are safe for use by humans is also recommended.

In conclusion, in this study no doubt powder form, aqueous extraction and alcohol extraction were used as was followed by other authors. But in this study, carbon tetrachloride fractions in capsule and cooking oil forms were used to find which of them show better efficacy than other additive (supplements). The reported information regarding effects of medicinal plants in developing *hypoglycaemia* were observed at maximum of 6 hours after administration of dose. In this study, strategy was adopted to evaluate effect after two hours of administration of dose and again after 4, 8, 12 and 24

hours of time. It was aimed that how long the dose effect of these plants sustains the control of blood glucose levels.

All these plants, *Cassia sophera*, *Caralluma tuberculata* and *Achillea santolina* studied showed significant effect in controlling blood glucose levels but *Caralluma tuberculata* turned out to be the best of all these plants in developing *hypoglycaemia*. It is suggested that use of *Caralluma tuberculata* may be preferred to control diabetes mellitus disorders particularly when taken with cooking oil.

The three local plants studied to do have significant effect on controlling blood glucose levels and their use should be cost effective as well. Although *Caralluma tuberculata* is preferred for use. But this is subject to availability of plant in some localities. Otherwise, *Achillea santolina* is recommended as second option.

Conclusion

CONCLUSION

In conclusion, the present results provide convincing evidence that the indigenous medicinal plants *Cassia sophera*, *Caralluma tuberculata* and *Achillea santolina* have *hypoglycaemic* activity in mammalian model rabbits. It is also evident that *Caralluma tuberculata* comparatively produces better *hypoglycaemia* than the other two plants. It can also be used for its antihyperglycaemic effects without any adverse effect because of its extensive use as vegetable since time immemorial. In the present study, also neither behavioral changes nor acute toxic effects were noticed in the treated animals. This is of no surprise since alternative treatments have been most widely used by traditional healers because they consider that herbal drugs have least adverse effects.

However, the efficacy of herbal medications most commonly used as alternative therapy need to be further evaluated by well designed, controlled clinical studies. Because various non-standardized forms of the herbs have often been the testing material. Moreover, preparations of standardized medicinal herbs are urgently needed for further studies and therapy. Conventional herbal drugs for diabetes mellitus can be considered for potentional adverse herbal drugs interaction also.

FUTURE DIRECTIONS

These plants do have *hypoglycaemic* effects in mammalian model i.e. rabbit.

- To study the *hypoglycaemic* effects of these three medicinal plants in patients. suffering from type 2 diabetes mellitus.
- To study the effects of these plants in patients taking oral *hypoglycaemic* agents.
- To evaluate and correlate the *hypoglycaemic* effects in type 2 diabetes patient as a single agent and along with *hypoglycaemic* agent.
- To reduce dose of oral *hypoglycaemic* agent when given in combination with the plant drug.
- To monitor the adverse effects (if any) of these plants in patients from type 2 diabetes mellitus.
- To investigate the possible toxicity produced by these plants extracts.

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