ANTI-COAGULANT EFFECT OF AN ERGOT MESYLATE (HYDERGINE): A STUDY IN SPRAGUE-DAWLEY RATS

BY

FAREEHA AMBREEN

DEPARTMENT OF BIOLOGICAL SCIENCES
QUAID-I-AZAM UNIVERSITY
ISLAMABAD

2006

ANTI-COAGULANT EFFECT OF AN ERGOT MESYLATE (HYDERGINE): A STUDY IN SPRAGUE-DAWLEY RATS

A dissertation submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy

IN

BIOLOGY

(ANIMAL PHYSIOLOGY)

BY

FAREEHA AMBREEN

DEPARTMENT OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

2006

CERTIFICATE

This is to certify that this dissertation, submitted by **FAREEHA AMBREEN**, is accepted in its present form by the Department of Biological Sciences. Quaid-i-Azam University, Islamabad as satisfying the requirements for the degree of **Master of Philosophy (Animal Physiology)**.

SUPERVISOR

DR. IRFAN ZIA QURESHI

EXTERNAL EXAMINER

DR. MOHAMMAD ARSHAD

CHAIRPERSON

DR. SAMINA JALALI

Dated: February 13, 2006.

CERTIFICATE

This is to certify that this dissertation, submitted by **FAREEHA AMBREEN**, is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the requirements for the degree of **Master of Philosophy (Animal Physiology)**.

SUPERVISOR _____

DR. IRFAN ZIA QURESHI

EXTERNAL EXAMINER _____

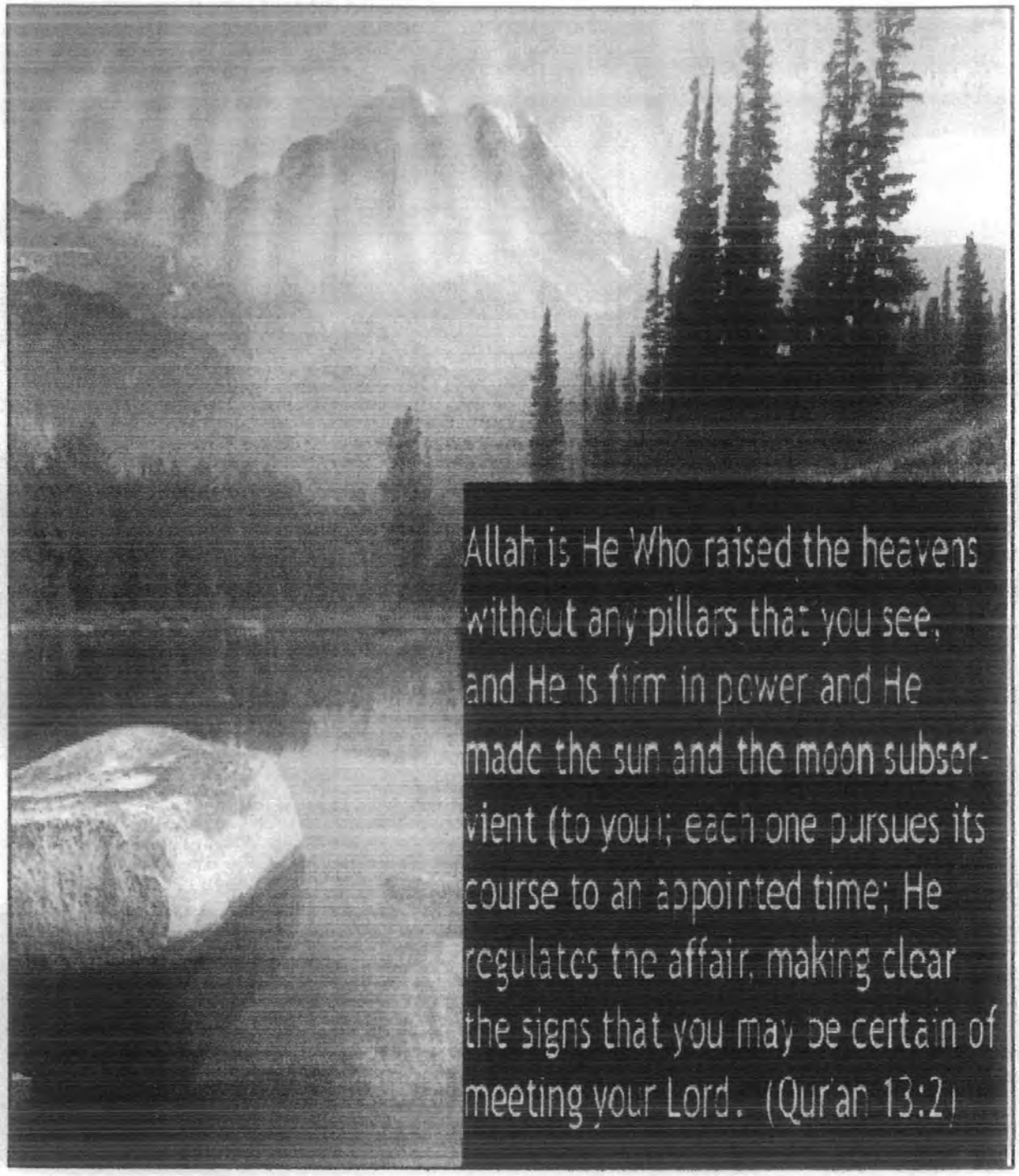
DR. MOHAMMAD ARSHAD

CHAIRPERSON _____

DR. SAMINA JALALI

Dated: February 13, 2006.

IN THE NAME OF ALLAH,
THE MERCIFUL,
THE BENEFICIENT


Allah is He Who raised the heavens
without any pillars that you see,
and He is firm in power and He
made the sun and the moon subser-
vient (to you); each one pursues its
course to an appointed time; He
regulates the affair, making clear
the signs that you may be certain of
meeting your Lord. (Quran 13:2)

DEDICATED TO THE MEMORY

OF LITTLE SCHOLARS WHO

SET OFF IN SEARCH OF FAIRY

OF KNOWLEDGE BUT LOST

THEIR BATTLE OF LIFE IN THE

CATASTROPHE OF

8TH OCTOBER 2005

TABLE OF CONTENTS

TABLE OF CONTENTS FOR TABLES

TABLE OF CONTENTS FOR FIGURES

AKNOWLEDGEMENTS

All praise is to **Allah**, the Lord and creator of the universe, Who showered His blessings upon us and opened new horizons of knowledge for the mankind. Almighty Allah flourished my thoughts and thrived my ambitions in the form of this write up. All respect goes to the Holy Prophet Hazrat Muhammad (PBUH), who enlightened our conscience with the essence of faith in Allah, converging all the kindness upon him.

I feel great pleasure in expressing my heartiest gratitude to my dignified and respectable supervisor Dr. Irfan Zia Qureshi, for his immense encouragement and sincere facilitation for the whole period of my research work, who took me out of my predicament and led me to where I am now, able to present my thesis. It is really a matter of pride for me to work under his supervision.

I am thankful to Dr. Samina Jalali, Professor and Chairperson of Department of Biological Sciences, for providing me a good research environment in the department.

I also express my humble gratitude to Dr. Ayesha Junaid, Hematologist, Shifa College of medicine, Islamabad, for her valuable guidance in setting up the protocol of my experiment. I am also obliged to Dr. Anwar, Incharge OPD and IPD services, Shifa Foundation Hospital, Islamabad, for providing me support in my work regarding blood counts that were performed free of cost due to his chivalrous behavior. Thanks are due to Mr. Yasir and Mr. Hashim Hussian, medical technologists, Mr. Ramzan, phlebotomist, Shifa Foundation Hospital, Islamabad, for providing me technical help in carrying out blood counts.

I am also grateful to Sir Zahid, department of Statistics, Quaid-I-Azam University, Islamabad for his considerate guidance in the statistical analysis of my data. I am thankful to Mr. Tariq Mahmood, laboratory assistant, for his help regarding animal handling during my research work. I want to express my gratitude to all my lab fellows Dr. Fozia, Sumaira, Johar, Sadia, Faiqah, Sima, Saiqa, Salma, Sobia and Madiha for their cooperation, encouragement and help during my research. Special thanks are for my very close friends Faiza, Sobia, Javeria and Mehnaz for their moral support during M. Phil sudies. I extend my thanks to all well-wishers and friends for giving me their pleasant company and support.

Last but not the least, I am grateful and indebted to my benevolent parents, brother and sisters for their prayers, love and care that have brought me to this stage. My sincere thanks are for my elder sister, her husband and my little nieces, Ayesha, Javeria, Hafsa and Aiman, whose lovely company made me relax when I felt exhausted.

May Allah bless us all and bestow upon us the secret of real knowledge to serve the nation sincerely.

FAREEHA AMBREEN

ABSTRACT

Nootropics are cognition enhancers that are used to treat cognitive impairment. Hydergine is one of the nootropics and is an extract of ergot of rye. Physiologically it is a proven vasodilator. In the current study the anticoagulatory effect of hydergine was investigated both *in vivo* and *ex vivo*. For *in vivo* experiments, Sprague-dawley rats were exposed to 1.5mg, 3.0mg and 4.5mg hydergine and 71.4IU/Kg heparin. Same doses were applied with prior fresh plasma exposure in another set of experiments. After 1.5 hour of drug administration, animals were sacrificed and coagulation assays including bleeding time, clotting time, INR, PT, APTT and plasma calcium analysis along with complete blood counts were carried out. For *ex vivo* studies, blood aspired from a group of healthy animals was exposed to 0.1mg, 0.2mg 0.3mg hydergine and to 1IU heparin and fresh plasma solutions taken in separate tubes as parallel controls. Then clotting time, PT, INR, APTT and plasma calcium concentration were analyzed in differently treated blood samples. Means of all the treatment groups were compared using one-way ANOVA, Dunken's and Tukey's tests were applied for intra group comparisons using SPSS software. Results showed that bleeding time and clotting time of animals exposed to high doses of hydergine increased significantly while plasma calcium levels decreased. PT, APTT and INR did not show any significant change. Platelet count, PDW, MPV, PLCR, RBC, RDW, hemoglobin, hematocrit, MCH, MCHC, TLC, and lymphocyte number did not show any variation when compared with the control animals. Lymphocyte percentage was however, elevated in hydergine treated animals. In *ex vivo* experiment CT and APTT were elevated while plasma calcium, PT and INR did not show any significant variation from control. The present findings suggest that hydergine may increase the bleeding efficiency possibly by modulating calcium-signaling action.

ABBREVIATIONS

%	Percentage
5-HT	5-hydroxytryptamine
AchE	Acetylcholine Estrase
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
APC	Activated Protein C
APTT	Activated Partial Thromboplastin Time
ATP	Adenosine Triphosphate
BT	Bleeding Time
cAMP	Cyclic Adenosine monophosphate
CBC	Complete Blood Counts
CT	Clotting Time
DIC	Disseminated Intravascular Coagulation
DMAE	Dimethyl Amino Ethanol
DVT	Deep Vein Thrombosis
EDTA	Ethylene Diamine Tetraacetate
fL	Famtolitres
INR	International Normalizing Ratio
IRP	International Reference Preparation
ISI	International Sensitivity Index
IU	International Units
MAO	Monoamine Oxidase
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean corpuscular Volume
MDA	Malonalaldehyde
mg/dl	Milligrams per Deciliter
MPV	Mean Platelet Volume
NGF	Nerve Growth Factor

PAI-1	Plasminogen Activator Inhibitor-1
PDW	Platelet Distribution Width
pg	Picograms
PLCR	Platelet Large Corpuscular Ratio
POMC	Pro-Opiomelanocortin
PRL	Prolactin
PT	Prothrombin Time
PUR	Platelet Uptake Ratio
RBC	Red Blood Cells
RDW	RBC Distribution Width
SCA	Senescent Cell Antigen
SD	Standard Deviation
TF	Tissue Factor
TLC	Total Leukocyte Count
TPA	Tissue Plasminogen Activator
TXA2	Thromboxane A2
TXB2	Thromboxane B2
WHO	World Health Organization
μ	Micron (10^{-6})

INTRODUCTION

INTRODUCTION

Smart Drugs

Smart drugs or cognitive enhancers are also known as "nootropics" derived from two Greek words "noos" or mind and "troops" or bind collectively meaning "binding to the mind". It is a term first used by Dr. Giurgea to describe biochemicals like vitamins, herbs, nutrients that can enhance memory, creativity, alertness, learning, or physical performance. The supposed effect of the cognitive enhancement can be several fold (Andreas, 1993). Nootropics are referred to as the only substances that improve memory in the absence of a cognitive deficit (Kumar *et al.*, 2002). These substances enhance mental performance in a variety of ways, bolstering cognition, lucidity, memory, mood, oxygen and glucose utilization or blood circulation to the brain or a combination of these or other factors (The American heritage dictionary, 2000). They are also useful in the treatment of dementia (Meller *et al.*, 1999). These drugs can overcome natural or induced cognitive impairments (Galeotti *et al.*, 1996), resulting into cognition enhancement that leads to a physiological activation of adoption, in opposition to psycho-stimulants and analeptics (Herrmann and Coper, 1987).

Some of the drugs first came under serious scrutiny as possible treatments for age-related mental decline; among these were vitamin B complex and amino acids such as phenylalanine that were used to treat the psychological trauma induced by drug abuse. Still others, like melatonin and dimethyl amino ethanol (DMAE), are synthetic versions of natural hormones which, when taken as supplements, can optimize physical and emotional well-being and mental performance. Other "smart" substances, such as ginseng ginkgo biloba and piracetsm are among the oldest known herbal medicines and are shown to increase memory and cause a significant decrease in the viscosity of blood (Kessler *et al.*, 2000).

Smart nutrients are classified into three basic groups:

Diet supplements: Supplements as vitamin B-5 and choline are converted to acetylcholine, a major neurotransmitter of brain that figures into memory and learning.

Herbs: Ginseng, *Ginkgo biloba*, and gotu kola, the ancient Chinese medicines are among the most commonly used smart herbs and may sharpen memory and concentration by boosting blood flow in the brain.

Amino acids: Amino acids such as phenylalanine and tyrosine serve as building blocks for bodily proteins and the transmitters that regulate arousal, concentration and energy and hence are leading players in the smart drug diet.

The nootropics can be divided into three generations with reference to their advent. Drugs of brain metabolic improvement and blood expansion and anti-coagulation drugs are the first stage. Acetylcholinestrase (AchE) inhibitor and glutamine receptor inhibitors are second-generation drugs. Anti-inflammation drugs, anti-cholesterol drugs, female hormones, and nerve growth factors that prevent the accumulation of abnormal proteins in brain causing degenerative diseases like Alzheimer's disease are third generation drugs. Moreover, vaccines are also included in the third generation smart drugs. Adjuvant treatment is also considered that involves the elimination of various causes of cell injury but does not increase the cell resistance to the injury. These methods include protection of the nerve cell membrane, supply of nerve transmission material and improvement of brain blood flow. Non-medicinal methods of treatment and prevention of dementia include mental training and exercises. They increase brain circulation caused by various stimuli and thus promote supply of nutrition and oxygen to the nerve cells (Miyanaga, 2005).

Cognitive enhancers are mostly used to treat people with neurological and mental disorders (Eriksson, 1998). Although there are many companies that make smart drinks, smart power bars and diet supplements containing smart chemicals, to date there is little evidence to suggest that such products really work at physiological level to improve

above mentioned ailments. The evidence in support is conflicting and inconclusive (Gerd *et al.*, 1997).

Smart drugs may protect the brain from physical and chemical damage by increasing cerebral circulation or by increasing brain metabolism (Dean, 1998). The known effects of these drugs are increased alertness, increased mental energy, decreased depression and improved memory (Branconnier, 1983). Ginko biloba, a smart drug has been shown to cause a significant decrease in blood viscosity (Erdincler *et al.*, 1996).

Recovery after stroke is accelerated and facilitated by rehabilitation therapy that might be supported by various drugs (Kessler *et al.*, 2000). An antiplatelet drug, clistazol, may be a new therapeutic option for prevention of recurrent strokes in some patients (Fumio *et al.*, 2000). However, it has been suggested that treatment of organic brain syndromes with nootropics should be initiated in an early rather than a late stage (Saletu *et al.*, 1990). Vascular cognitive impairment is considered the second most common form of mental deterioration in the elderly after degenerative dementias. Therapeutic approaches to vascular dementia mainly rely on the identification and treatment of risk factors. A number of drugs have also been tested with the aim of improving or slowing cognitive decline in patients affected by various forms of cerebrovascular diseases (Inzitari *et al.*, 2000).

It has been studied that increased cortisol concentrations in Alzheimer's patients and the improvement of memory induced by certain nootropics is inhibited by elevated steroid levels that are entirely different substances, this indicates that both substances have common site of action. This steroid dependency of nootropics might explain the cause of lower response of limited number of Alzheimer's patients to the therapy with nootropics or cholinomimetics (Mondari *et al.*, 1992).

Hydergine (Co-dergocrine mesylate)

Hydergine is one of the well-identified smart drugs, also known as dihydroergotoxine or ergot mesylate. It is obtained from an ergot, a fungus that grows on rye and a proved

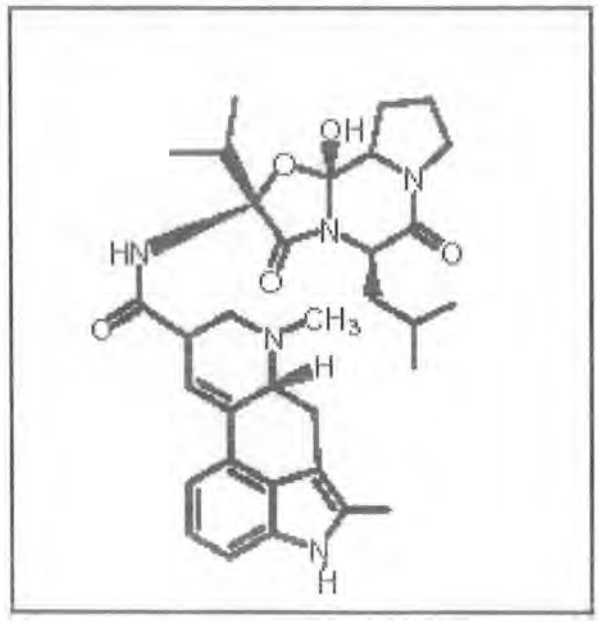
cognitive enhancer. Hydergine was discovered in 1950 at Sandoz laboratories by Albert Hoffman (James *et al.*, 2003). The virtues of the drug were extolled by Durk Pearson and Sandy Shaw (Pearson and Shaw, 1982). Among ergot derived smart drugs bromocriptine and nicergoline are also included in smart drugs in addition to hydergine (Cucinotta *et al.*, 1996).

Hydergine is a peptide alkaloid and consists of an equiproportional mixture of the hydrogenated ergot alkaloids: dihydroergocornine mesylate, dihydroergocristine mesylate and dihydroergokryptine mesylate (Figure 1).

Dihydroergocornine mesylate	(C_{31} H $N_{41}O_3$ .CH_3 O_4 S_3)	Mol. wt.: 659.81
Dihydroergocristine mesylate	(C_{35} H N_{41} O_3.CH_3 O_4 S_3)	Mol. wt.: 707.95
Dihydro- α ergokryptine mesylate	(C_{32} H N_{43} O_3.CH_3 O_4 S_3)	Mol. wt.: 673.84
Dihydro- ⇓ ergokryptine mesylate	(C_{32} H N_{43} O_3.CH_3 O_4 S_3)	Mol. wt.: 673.84

Hydergine is prepared by catalytic hydrogenation of several alkaloids isolated from ergot, *Claviceps purpurea*, a parasitic fungus on the rye plant. The salt mixture is prepared by reaction with methanesulfonic acid (Gennaro, 1995).

Figure1. 2-Bromo-α-ergokryptine (Jan *et al.*, 2000).



Pharmacokinetics

Hydergine is rapidly but incompletely absorbed from the gastrointestinal tract resulting in low concentrations in body fluids. Although the metabolic fate is not completely known, hydergine undergoes first-pass metabolism in the liver and less than fifty percent of the dose reaches systemic circulation unchanged. The elimination from blood is biphasic with half-lives of 1.5-4 hours and 13-15 hours. Biotransformation studies on the individual components of the drug have shown that it is metabolized mainly via oxidation and cleavage of proline in the peptide portion of the molecule. Hydergine is also cleaved at the amide bond, yielding dihydrolysergic acid amide. Several hydroxylation products of dihydro-ergocryptine have also been identified (Eckert *et al.*, 1978; Schran *et al.*, 1988; McEvoy, 1997).

Some basic pharmacological actions of hydergine are modified by 5-HT (5-hydroxytryptamine) receptors, dopamine receptors, and adrenoreceptors. Pharmacological actions may also include depletion of noradrenaline from tissues. Extremely small changes in conformation can account for greater affinity to receptor sites or changes in agonist efficacy. These minor changes can have significant consequences to biological activity. The basic skeleton of the ergot alkaloids selected for autoradiography consists of a D-lysergic acid linked to a tricyclic peptide moiety by a peptide bond. In the ergotamine group, the peptide part consists of 3-amino acids, L -proline, L - hydroxyalanine, and a third amino acid. The third amino acid in ergotamine is L - phenylalanine and L-leucine is the third amino acid in ergosine and ergostine. Ergostine differs from ergosine by the addition of a single methyl group on the proline side chain (Berde and Stürmer, 1978).

The intravenous and oral doses produced similar pharmacological effects, studied with respect to the reduction of plasma prolactin concentrations in spite of the fact that small doses are injected compared to oral doses (Dominiak *et al.*, 1988).

Mechanism of Action of Hydergine In Brain

Exogenously given Hydergine has been detected in the granular layer of the cortex, cerebellum, basal ganglia, and neuronal cells of the reticular formation of cat brain. Animal studies suggest that approximately 60 percent of cerebral hydergine is localized in the synaptic structures (Wadworth and Chrisp, 1992).

Despite the fact that hydergine has been used in the treatment of dementia for many years, its mechanism of action is still not clear. Current studies imply that the major effect of hydergine may be the modulation of synaptic neurotransmission rather than solely increasing blood flow. A prominent feature that accompanies aging is an increase in monoamine oxidase (MAO) levels that results in decreased availability of catecholamines in the synaptic cleft. Increased brain MAO activity in aging can be modified by hydergine treatment in some brain regions (Buyukozturk *et al.*, 1995). An interaction between age and hydergine treatment has been observed in the hypothalamus, hippocampus and cerebellum. The effect of hydergine is more pronounced in the hypothalamus and cerebellum of senile group whereas in hippocampus of the young individuals (Nandy and Schneider, 1978).

Hydergine alters dopaminergic and cholinergic transmission. It has a profile of a selective D_2 agonist and can augment hippocampal cholinergic function while decreasing striatal cholinergic function resulting an increase in energy and memory thus decreasing the symptoms of parkinsonism (Sherkey, 1996).

Some European countries use hydergine for emergencies and accidents that involve shock, hemorrhage, strokes, heart attacks, drowning, electrocution and drug over-dose. Hospitals give hydergine to patients before an operation in order to gain time in case of any ensuing crises. This is because hydergine helps to stabilize brain oxygen levels; if they are too high hydergine lowers them, if they are too low then hydergine improves them. This was graphically illustrated in a cat experiment (Yoshikawa, 1983).

Hydergine has been shown to increase mental abilities, prevents damage to brain cells from hypoxia, and may even be able to reverse existing damage to brain cells. Scientists have analyzed ergot alkaloids since the late 1940s in search of blood-pressure medications. Studies in the elderly population uncovered cognition-enhancing effects of hydergine and it is now a popular treatment for all forms of senility in the U.S., and is used to treat a plethora of problems elsewhere in the world (Gruenward *et al.,* 1998).

Both age and hypertension are risk factors for brain. In the presence of a multiple cerebral infarction as obtained by intra-carotid injection of sodium arachidonate, hydergine is capable in the young and old hypertensive and normotensive rats, of limiting the extent of the edematous reaction to prevent the intracerebral accumulation of calcium ions and invert the fall in cerebral blood flow, all of these effects result in significant improvement in neuromotor behavior (Cahn and Borzeix, 1983).

Hydergine acts in several ways to enhance mental capabilities and to slow down or reverse the aging processes in the brain. A few of the huge number of beneficial effects attributed to hydergine include: increased protein syntheses in the brain; reduced accumulation of lipofuscin in the brain; increased quantities of blood and oxygen delivered to the brain; improvement of memory, learning and intelligence; beneficial improvements in brainwave activity; increased metabolism in brain cells; normalization of blood pressure; and increased production of such neurotransmitters as dopamine and norepinephrine (neurochemical messengers essential to the formation of memory, and also associated with arousal, alertness, elation and pleasure). Hydergine also functions as a powerful antioxidant and thus protects the brain against the damage caused by those infamous rascally free radicals that are unstable and extremely reactive molecules produced by normal metabolism, which cause damage associated with aging, cancer and cardiovascular disease (Emmenegger and Meier, 1968).

Hydergine is known to increase blood supply and oxygen to the brain, enhance brain cell metabolism, protect the brain from free-radical damage during decrease or increase oxygen supply, speeds up the elimination of age pigment (lipofuscin) in the brain,

inhibits free-radical activity, increase intelligence, memory, learning and recall, normalizes systolic blood pressure, lowers abnormally high cholesterol levels in some cases, reduces symptoms of tiredness, dizziness and tinnitus (Amenta *et al.*, 1988).

In old rats, hydergine has been shown to increase metabolism and uptake of glucose in the brain. It also helps to rejuvenate connections between brain cells, protects brain against damage due to oxygen starvation and improves learning capabilities (Jamieson, 1981).

One way that Hydergine may enhance brain functioning is by mimicking the effect of a substance called nerve growth factor (NGF). NGF promotes the growth of dendrites that are long branching fibers by which neurons receive information from other neurons. Scientists studying the effects of learning on the brain have found that it is directly related to dendritic growth. Hydergine seems to work by the same neuro-chemical pathway as NGF to produce neural growth but its prime benefit is through increased blood circulation in the brain, possibly acting via major vessels. Hydergine is used medically to treat senile dementia, much of which is related to brain circulatory decline (Hughes., 1976).

While Hydergine is widely used for the treatment of senility, scientists have also studied its effects, both short term and long term, in normal healthy humans; these studies noted significant improvements in a variety of cognitive function, including alertness, memory, reaction time, abstract reasoning and cognitive processing ability (Rao and Norris, 1971). The mechanism of action by which hydergine causes it actions is unclear but it may improve the cerebral blood flow (Slagle and Mark, 2001).

In a long-term treatment, hydergine caused medical and psychological improvements concerning cardiovascular parameters, cholesterol levels, subjective symptoms and performance in tests of intelligence. These findings show a preventive effect of ergot mesylate against some of the deliberating physical and psychological concomitants of aging (Spiegel *et al.*, 1983).

Hydergine is often used with other medicines, which are thought to be cognition enhancing and life extending, including piracetam, deprenyl, and DMAE. Hydergine when combined with piracetam in experiments with mice, both brain survival time and learning abilities increased, it was noted that the effect of the combination was greater than the sum of the effects of the individual agents and indicated that both drugs affect each other synergistically (Berga, 1986).

The combination of hydergine and nifodipline caused systolic blood pressure drop during a randomized double blind study (Dominiak and Weidinger, 1991).

Effects of Hydergine at Molecular Levels

It is documented that hydergine increases stores of ATP, stabilizes the cAMP content of the nerve cells, improves brain glucose utilization and in turn, cerebral microcirculation (Bertoni, 1994). For humans, a daily dose of 6 - 9mg hydergine has been shown to be safe and is widely used in Europe. Rejuvenation of aging brain synaptic mitochondria may be one more indication for hydergine use (Elwon *et al.*, 1995).

In animal models, it tends to favorably affect the activity of Band-3 protein. Band-3 protein is found on the cell surfaces of many types of cells, including nerve cells. It is well known that nerve cells in the brain die off at a rather predictable rate. Therefore as people become aged cognitive function and memory have a higher propensity to be impaired. Band-3 protein appeared to signal the appropriate timing of apoptosis, the programmed cell death (Barry *et al.*, 2003). In a study, the total mitochondrial volume of old rats was nearly the same as the young rats after treatment with hydergine. Furthermore, the mitochondrial size was altered to a more youthful direction. Recent studies have further suggested that hydergine may reduce Senescent Cell Antigen (SCA), a destructive autoantibody appearing more frequently in elderly cells. SCA damages cells and finally destroys them, (Cover *et al.*, 1996).

It has been observed that 5-HT drugs require functional melanocortin pathways to exert their effects on food intake. Specifically, the anorectic 5-HT drugs activate pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus. There is

evidence that the serotonin 2C receptor (5-HT (2C) R) is expressed on POMC neurons and contributes to this effect. Moreover, 5-HT drug-induced hypophagia is attenuated by pharmacological or genetic blockade of downstream melanocortin 3 and 4 receptors. Activation of the melanocortin system is downstream of 5-HT and is necessary to produce the complete anorectic effect of 5-HT drugs (Heisler *et al.*, 2003).

Effect of Hydergine on Body Mechanisms

Hydergine has prolactin (PRL) inhibitory activity and is useful in the treatment of hyperprolactnimia in anovulatory patients whose basal serum PRL levels are below 100μg/L (Tamura *et al.*, 1989). Moreover, it has also been shown that hydergine inhibits post-partum physiological milk secretion in humans (Elsener *et al.*, 1988).

Hydergine does not exhibit any adrenolytic action on the peripheral blood vessels even in higher local concentrations possible by the intra-arterial route in man. However, vasodilatation is obtained in most of the cases. In those cases that do not react to the vessel, respond more easily to the release of sympathetic tone providing evidence that the drug has a potential effect on neurovascular apparatus (Copeland, 1981).

Following prolonged administration of hydergine in rats there is a dose dependent increase in water and sodium chloride excretion. Blood pressure and heart rate is decreased. Plasma renin activity also shows a fall, however salidiuretic activity of co-dergocrine remains relatively constant (Siegl, 1985). Moreover, it has been suggested that every intensive care patient is a potential organ donor. For preserving organs, an aggressive shock therapy and controlled volume adaptation using the alpha-blocker hydergine is necessary (Kirchner, 1989).

The effects of hydergine on the secretion of pancreatic juice in the dog has been investigated in preparations of the isolated, blood-perfused pancreas, and compared with those of secretin and dopamine. Hydergine (30-300μg) injected intra-arterially caused dose-dependent increase in the secretion of pancreatic juice after a delay of a few minutes. The secretory activity of 300μg of hydergine was approximately equal to that of 0.05 units of secretin and 2μg of dopamine. Secretory responses to hydergine were

inhibited by treatment with sulpiride, which is a selective dopamine D2 antagonist with antipsychotic and antidepressant activity, but phentolamine, propranolol, atropine or metiamide did not affect the secretory activity of hydergine. The concentration of bicarbonate in the pancreatic juice induced by hydergine increased in a dose-dependent manner. However, protein concentration was scarcely changed. These secretory actions were analogous to those of dopamine. From these results, it is concluded that hydergine mainly stimulated pancreatic secretion by acting on dopaminergic receptors of the dog pancreas (Iwatsuki *et al.*, 1983).

Intravenous administration of 10μg/kg co-dergocrine depresses pressor responses to a psychological stimulus or raising the forequarters in conscious dogs, and produces marked fall in blood pressure and heart rate in anaesthetized, baroreceptor-denervated dogs. Thus the cardiovascular responses to acute administration of low doses of co-dergocrine were due to stimulation of prejunctional dopamine receptors. Heart rate increases evoked by stimulating the accelerans nerve of ganglion-blocked cats are inhibited in a dose-dependent pattern by co-dergocrine from a dose of 1μg/kg intravenous therapy. The drug also depresses pressor responses to stimulation of the lumbar sympathetic outflow in pithed rats (Clark *et al.*, 1985).

Effect of Hydergine on Blood Coagulation

With the understanding of molecular mechanisms of hematological disorders, it has become possible to target therapy precisely to the underlying defect. Targeted therapy can increase safety and potency, while causing fewer side effects than standard treatments. Smart drugs and gene therapy have shown great promise in a wide range of malignant hematological and coagulation disorders (Ross *et al.*, 2002; Andreas *et al.*, 2000).

Effects of hydergine on local platelet accumulation in the carotid artery has been studied by means of the platelet uptake ratio and on the systemic platelet vascular wall interactions calculated from platelet half life. The results indicate that hydergine decreases *in vivo* platelet residence time to artherosclerotic lesions of the carotid artery.

Hydergine may therefore be of benefit in prevention of transient ischemic attacks and artherosclerosis (Steurer *et al.*, 1989).

Treatment with hydergine decreases platelet activity to a significant extent as shown by a number of platelet function parameters, such as thromboxane B2 (TXB2), beta thromboglubulin, platelet factor 4, malonalaldehyde (MDA) and ADP-induced aggregation. The findings of a placebo study in humans suggest that hydergine might be able to decrease platelet activity and improve interaction with the walls of a blood vessel to a significant degree (Sinzinger, 1985).

Hydergine like extract of ergot of rye, Nicergoline, showed protein kinase C mediated alpha secretase amyloid precursor protein processing in human neuroblastoma cell culture (Cedazo-Minguez *et al.*, 1999).

In a placebo controlled double blind randomised study, the effect of hydergine on local platelet accumulation in the carotid artery region was carried out by means of the platelet uptake ratio (PUR) and on the systemic platelet-vascular wall interaction as calculated from platelet half-life. Co-dergocrine treatment resulted in a significant decrease in platelet deposition, PUR and platelet half-life decreased on day 5 of therapy to statistically significant level in the paired comparison. No relevant effects on ADP-induced platelet aggregation, platelet-release reaction, platelet aggregate ratio, Thromboxane B2 (TXB2) plasma levels and thrombin-induced MDA-formation could be detected. These results indicate that co-dergocrine decreased *in vivo* platelet residence time to atherosclerotic lesions of the carotid artery. Co-dergocrine may therefore be of benefit in prevention of mural thrombus formation and prevention of transient ischemic attacks, but also of atherosclerosis in man (Steurer *et al.*, 1989). However, no animal studies have yet been carried out objectively in this regard.

Hydergine is mostly used to treat hypertension. It has been shown that hypertension may cause activation of blood platelets *in vivo*. One of the possible mechanisms could be adrenergic activation of platelets by catecholamines. Specific binding of the alpha 2-adrenoceptor blocker, 3H-yohimbine, to platelets in order to elucidate the role of alpha 2-

adrenoceptors of platelets in hypertensive animals was investigated. Particularly, competitive inhibition of 3H-yohimbine binding to platelets by hydergine, and plasma catecholamine levels were investigated in stress induced hypertensive and normotensive monkeys. It was demonstrated that 3H-yohimbine binds to platelets of rhesus monkeys with high affinity and specificity. The binding was found to be saturable and reversible. Additionally, it was shown that hydergine inhibits specific binding of 3H-yohimbine to platelets of hypertensive monkeys more potently than to those from normotensive animals. The obtained data suggested that the total number of available free alpha 2-adrenoceptors were reduced on the platelets of hypertensive monkeys. The decreased adrenaline level in the plasma of hypertensive animals confirmed the latter (Pham *et al.*, 1988).

Side Effects of Hydergine

Hydergine has been well tolerated in patients with age-related cognitive decline with few participants in controlled studies developing adverse reactions to therapy. Gastrointestinal disturbances like nausea, gastric upset, anorexia, abdominal pain, and vomiting were the most frequently reported adverse effects. Dizziness, headache, nasal stuffiness, precordial discomfort, drowsiness, sleep disorders, and restlessness occurred in less than 3 percent of participants in non-blind studies (Wadworth and Chrisp, 1992).

Hydergine is reported to be non-teratogenic in animals; no information is available on the teratogenicity of the drug in humans (Schardein, 1976; Bechter and Schön, 1988). It has been suggested that pregnant women should consult their doctor before taking hydergine. Hydergine is negative in the dominant lethal assay in mice (Roberts and Rand, 1978). Hydergine induced chromosomal aberrations in human lymphocytes *in vitro*. It also induced chromosomal aberrations in mouse bone marrow cells *in vitro* but was considered negative in an *in vivo* chromosomal aberrations assay in guinea pigs (Matter, 1976; Robert and Rand, 1977a,b; 1978). Hydergine was negative in *in vivo* micronucleus assays in mice and guinea pigs (Matter, 1976).

Hydergine was also well tolerated in healthy elderly patients and no side effects or drug-related changes were seen in standard laboratory test values after hydergine administration for over 5 years (Huber *et al.*, 1986). In 6- to 14-year old children, nasal stuffiness was the most frequently reported side effect following hydergine treatment for 12 weeks to assess the drug's effectiveness in improving cognitive function and behavior in children with learning difficulties (Tareen *et al.*, 1988). It is contraindicated for individuals with acute or chronic psychosis, or those with a known sensitivity to the medication. Over dosage can paradoxically, cause an amnestic effect (Yoshikawa, 1983).

Hemostasis

The process of blood clotting and subsequent dissolution of the clot, following repair of the injured tissue, is termed as hemostasis. Whenever a vessel is severed or ruptured, hemostasis is achieved by several different mechanisms, including vascular spasm, formation of platelet plug, blood coagulation and eventual growth of fibrous tissues into the clot to close the hole in the vessel permanently (Guyton and Hall, 2006).

Coagulation or blood clotting involves the formation of a blood clot known as thrombus that prevents further blood loss from damaged tissues, blood vessels or organs. This is a complicated process with a cellular system comprising of platelets that circulate in the blood and serve to form a platelet plug over damaged vessels (Beutler and Williams, 2001).

Thus hemostasis initially involves vascular constriction that limits the flow of blood from the area of injury, and then platelets become activated by thrombin and aggregate at the site of injury, forming a temporary, loose platelet plug. The protein fibrinogen is primarily responsible for stimulating platelet clumping. Platelets clump by binding to collagen that becomes exposed following rupture of the endothelial lining of vessels. Upon activation, platelets release adenosine-5'-diphosphate (ADP) and Thromboxane A_2 (activates additional platelets), serotonin phospholipids and lipoproteins. In addition to induced secretion, activated platelets change their shape to accommodate the formation of the plug. To ensure stability of the initially loose platelet plug, a fibrin mesh, also called

the clot, forms and entraps the plug. If the plug contains only platelets it is termed a white thrombus; if red blood cells are present it is called a red thrombus. Finally, the clot must be dissolved in order for normal blood flow to resume, following tissue repair. The dissolution of the clot occurs through the action of plasmin (Kenneth, 2003).

Role of Platelets in Blood Clotting

Primary function of the platelets is to stick to the injured blood vessel called platelet adherence, attach to other platelets to enlarge the forming plug called platelet aggregation and to provide support as molecules on the surface of platelets are required for many of the reactions involved in the coagulation cascade (Beutler and Williams, 2001).

When a break in a blood vessel occurs, certain molecules are exposed that normally are not in direct contact with the blood flow. These substances that are primarily collagen and von Willebrand factor allow the platelets to adhere to the broken surface. Once a platelet adheres to the surface, it releases chemicals that attract additional platelets to the damaged area, referred to as platelet aggregation. These two processes are the first responses to stop bleeding. The protein-based system called coagulation cascade serves to stabilize the clot that has formed and further seals up the wound (Rodak, 2002).

The third role of the platelet is to support the coagulation cascade. This support is provided, in part, by one of the components of the outside of a platelet, called phospholipids, which are required for many of the reactions in the clotting cascade.

The goal of the cascade is to form fibrin, which will form a mesh within the platelet aggregate to stabilize the clot. All of the factors have an inactive and an active form. Once activated, the factor will serve to activate the next factor in the sequence until fibrin is formed (Figure 2).

The Clotting Factors

Blood clotting also involves a second system based upon the actions of multiple proteins called clotting factors (Table 1) that act in concert to produce a fibrin clot. The platelets

and the clotting factors work in concert to form a clot; disorders in either system can lead to disorders that cause either too much or too little clotting (Colman, 2001).

The Clotting Cascade

The intrinsic cascade is initiated when contact is made between blood and exposed endothelial cell surfaces. The extrinsic pathway is initiated upon vascular injury that leads to exposure of tissue factor (TF), a subendothelial cell-surface glycoprotein that binds to phospholipid. The extrinsic and intrinsic pathways converge at the activation of factor X to Xa. Factor Xa has a role in the further activation of factor VII to VIIa. Active factor Xa hydrolyzes and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fribrinogen to fibrin and to activate factor XIII to XIIIa. Factor XIIIa cross-links fibrin polymers solidifying the clot (Dahlback, 2000) (Figure 2).

Figure 1. **The Clotting Cascades** (Dahlback, 2000).

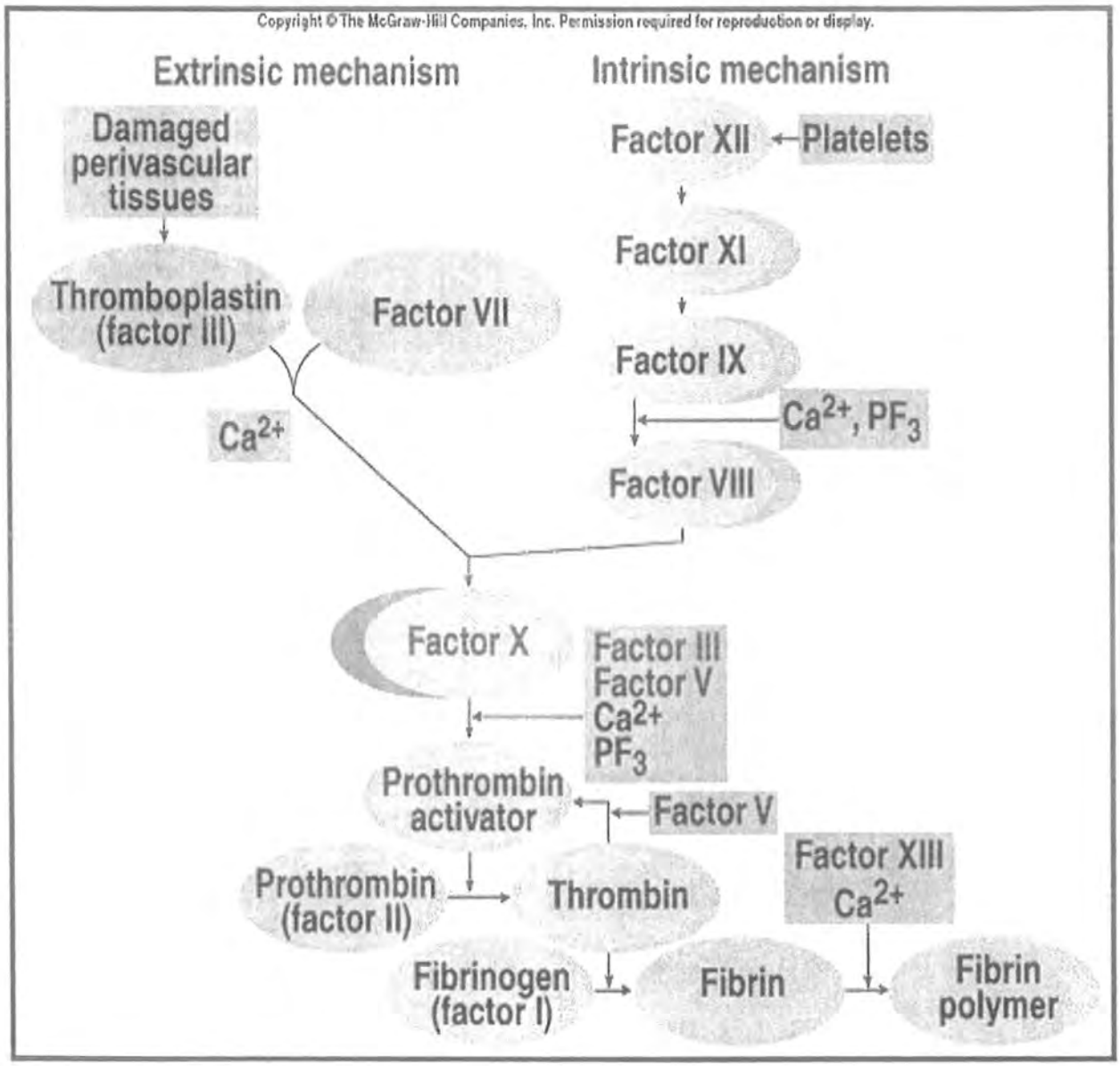


Table 1. Primary Clotting factors and Their Functional Classification (Sarah and Machin, 2004).

Factors	Functional Status	Activities
I	Fibrinogen	Cleaved by thrombin to form fibrin clot
II	Prothrombin	Activated on surface of activated platelets by prothrombinase complex
III	Tissue factor	Sub endothelial cell-surface glycoprotein that acts as a cofactor for factor VII
IV	Calcium	Promote all reactions
V	Proaccelerin	Activated by thrombin; factor Va is a cofactor in the activation of prothrombin by factor Xa
VI (Va)	Accelerin	This is Va, redundant to Factor V
VII	Proconvertin	Activated by thrombin in presence of Ca^2
VIII	Antihemophilic factor A	Activated by thrombin; factor VIIIa is a cofactor in the activation of factor X by factor Ixa
IX	Christmas factor	Activated by factor XIa in presence of Ca^{2+}
X	Staurt-power factor	Activated on surface of activated platelets by tenase complex and by factor VIIa in presence of tissue factor and Ca^{2+}
XI	Plasma thromboplastin antecedent	Activated by factor XIIa
XII	Hageman factor	Binds to exposed collagen at site of vessel wall injury, activated by high-MW kininogen and kallikrein
XIII	Protransglutaminase	Activated by thrombin in presence of Ca^{2+}; stabilizes fibrin clot by covalent cross-linking.
Prekallikrein	Fletcher factor	Activation of factor IX
High molecular weight kininogen	Contact activation cofactor; Fitzgerald,	Involved in intrinsic pathway for activation of Ixa
von Willebrand factor	Regulatory proteins	Associated with subendothelial connective tissue; serves as a brigde between platelet glycoprotein GPIb/IX and collagen
Protein C	Regulatory proteins	Activated to protein Ca by thrombin bound to thrombomodulin; then degrades factors VIIIa and Va
Protein S	Regulatory proteins	Acts as a cofactor of protein C; both proteins contain *gla* residues
Thrombomodulin	Regulatory proteins	Protein on the surface of endothelial cells; binds thrombin, which then activates protein C
Antithrombin III	Regulatory protein	Most important coagulation inhibitor, controls activities of thrombin, and factors IXa, Xa, XIa and XIIa

Disorders Related to blood Coagulation

Disorders related to blood coagulation may be of two major types i.e., those caused due to inability of blood to coagulate leading to excessive blood loss called bleeding disorders and those called clotting disorders where arterial blood clots called embolus or atherosclerotic plaques are formed that move in the bloodstream from their point of origin to a new location and cause arterial blockage (Hampton and Preston, 1997).

Bleeding disorders

There are various conditions where excessive bleeding is caused due to damage or cut of the blood vessels. These may include thrombocytopenia where number of platelets reduces drastically, genetic conditions where one or more of the clotting factors are not made, of these the most well known is haemophilia A where factor VIII is lacking. Deficiency of vitamin K can cause bleeding problems, as this vitamin is required to make certain clotting factors. Liver disorders can sometimes cause bleeding problems as most of the clotting factors are manufactured in liver (Hampton and Preston, 1997).

Clotting disorders

Sometimes a blood clot forms within a blood vessel that has not been injured or cut. These conditions may involve a blood clot, which forms within a coronary artery, or in an artery within the brain leading to common cause of heart attack and stroke. The platelets become sticky and clump next to patches of atheroma (fatty material) in blood vessels and activate the clotting mechanism. Sluggish blood flow can also make the blood clot more readily than usual. This is a factor in deep vein thrombosis (DVT), which is a blood clot that sometimes forms in a leg vein. Certain genetic conditions can make the blood clot more easily than usual. Certain medicines can affect the blood clotting mechanism, or increase the amount of some clotting factors, which may result in the blood clotting more readily. Liver disorders can sometimes cause clotting problems as liver synthesizes some of the chemicals involved in preventing and dissolving clots (Hampton and Preston, 1997).

Blood Clotting Tests

Blood clotting tests are used to diagnose and assess bleeding problems, and to monitor people who take anticoagulant medicines. There are a number of different tests. The ones chosen depend on the circumstances, and what is the suspected problem. They include the following.

i. **Blood Count**

This is a routine blood test, which can count the number of red cells, white cells, and platelets per ml of blood. It will detect a low level of platelets (Bernard *et al.*, 2001).

ii. **Bleeding Time**

In this test a tiny cut is made in the earlobe, or forearm, and the time taken for the bleeding to stop is measured. It is normally 3-8 minutes in humans.

iii. **General Blood Clotting Tests**

A blood sample is taken into a bottle that contains an anticoagulant that is a chemical that prevents the blood from clotting. It is then analyzed for clotting factors, like the prothrombin time (PT) and the activated partial thromboplastin time (APTT). These tests measure the time it takes for a blood clot to form after certain activating chemicals are added to the blood sample. If the time taken is longer than for a normal blood sample, then one or more clotting factors are absent or low. There are other similar tests where different chemicals are added to the blood sample, the aim being to identify which clotting factors are low or absent (Shannon and Jeffery, 2005a).

The prothrombin time is also called the international normalized ratio (INR). This test is used to monitor blood clotting in people who take medicines to prevent blood clotting like warfarin. The dose of medicine should be enough to delay clotting a little, but not too much which may cause bleeding problems (Gunnar and Ingela, 2004).

iv. **Specific Blood Clotting Factors**

The amount of various clotting factors and anti-clotting factors in the blood can be measured by various techniques. One or more of these tests may be done if a general blood-clotting test identifies a problem with clotting. For example, the amount of factor VIII can be measured in a blood sample and the level is very low or absent in people with haemophilia A.

v. **Platelet Aggregation Test**

This measures the rate and extent platelets form clumps (aggregate) after a chemical is added which stimulates aggregation. It tests the function of the platelets.

vi. **Tests to Check if Blood Clots too Easily**

If there are unexplained blood clots in a normal blood vessel, then the possible causes should be traced. For example, a blood test to check for factor V Leiden. This is an abnormal variation of factor V, which tends to make the blood clot more readily than normal.

vii. **Other tests**

Various conditions such as vitamin deficiencies, leukaemia, liver disorders, or infections may effect clotting. So, in some cases other tests may be needed to find the cause of abnormal levels of platelets or clotting factors (Hampton and Preston, 1997).

Thrombolytic Agents

Heparin is used for its thrombolytic properties to stop the coagulation pathway process after the primary intervention has failed. The intravenous dosage is 5-10 U/kg/hour, although subcutaneous injections of 100 U/kg every 4-6 hours may be used. Heparin treatment may initially aggravate the bleeding, so, careful observation and support is

Blood Clotting Tests

Blood clotting tests are used to diagnose and assess bleeding problems, and to monitor people who take anticoagulant medicines. There are a number of different tests. The ones chosen depend on the circumstances, and what is the suspected problem. They include the following.

i. **Blood Count**

This is a routine blood test, which can count the number of red cells, white cells, and platelets per ml of blood. It will detect a low level of platelets (Bernard *et al.*, 2001).

ii. **Bleeding Time**

In this test a tiny cut is made in the earlobe, or forearm, and the time taken for the bleeding to stop is measured. It is normally 3-8 minutes in humans.

iii. **General Blood Clotting Tests**

A blood sample is taken into a bottle that contains an anticoagulant that is a chemical that prevents the blood from clotting. It is then analyzed for clotting factors, like the prothrombin time (PT) and the activated partial thromboplastin time (APTT). These tests measure the time it takes for a blood clot to form after certain activating chemicals are added to the blood sample. If the time taken is longer than for a normal blood sample, then one or more clotting factors are absent or low. There are other similar tests where different chemicals are added to the blood sample, the aim being to identify which clotting factors are low or absent (Shannon and Jeffery, 2005a).

The prothrombin time is also called the international normalized ratio (INR). This test is used to monitor blood clotting in people who take medicines to prevent blood clotting like warfarin. The dose of medicine should be enough to delay clotting a little, but not too much which may cause bleeding problems (Gunnar and Ingela, 2004).

required. Repeated anti-Xa chromogenic heparin assays may be necessary to control heparin dosage (Rodak, 2002).

Antithrombin concentrate may also be given with heparin in typical dose of 3000units to average size patient. Likewise, if protein C levels are down, protein C concentrate is indicated. If hemorrhage continues, antifibrinolytic therapy may be used. In this case however, the fibrinolytic process must be carefully monitored with plasminogen, Tissue plasminogen activator (TPA) and plasminogen activator inhibitor-1 (PAI-1) levels to prevent the propagation of widespread microthrombi (Rodak, 2002).

Replacement of Hemastatic Components

Vitamin K may correct the bleeding disorders associated with des-γ-carboxyl prothrombin and factors VII, IX, and X, although its therapeutic effect is less effective than in uncomplicated vitamin K deficiency. In some severe liver diseases fresh frozen plasma and virus-free solvent/ detergent-treated plasma are the only products that provide all the coagulation factors in hemostatic concentrations (Horowiz *et al.*, 1994). Fresh frozen plasma provides most of the necessary coagulation factors and replaces blood volume lost during acute disseminated intravascular coagulation (DIC) hemorrhage. Its effects are best monitored with repeated prothrombin test and activated partial thromboplastin test. Individual factors concentrates such as factor VIII or IX concentrates, prothrombin complex concentrates, or activated factor VII are effective only if plasma expansion must be avoided (Rodak, 2002).

Platelet concentrate is necessary if thrombocytopenia is severe. Effectiveness is monitored with platelet counts and computation of corrected platelet count platelet count increments. Red cells are used as necessary to respond to the resulting anemia (Rodak, 2002).

Objectives of the Study

The current study was carried out to investigate:

- The anticoagulatory properties of hydergine since animal studies regarding the effect as per see do not exist. Human placebo studies provided a clue for the investigations.
- The effects of hydergine on extrinsic and intrinsic pathway of coagulation.

MATERIAL AND METHODS

MATERIAL AND METHODS

Animals and Maintenance

Sprague-Dawley rats weighing 175-353 grams were purchased from National Institute of Health Islamabad and acclimatized for fifteen days before the experimentation. They were kept at standard laboratory conditions; temperature 24°C± 2°C, 12-hour light-dark cycle and fed normal rat chow and water *ad libitum.*

A-Experiments *in vivo*

To investigate the anticoagulatory effect of hydergine *in vivo,* rats were divided into eleven groups each containing five animals. Experimental procedure was as under:

i) Administration of Hydergine

In the first set of experiments, three groups of animals received intraperitoneal injections of hydergine at 1.5 mg, 3mg and 4.5mg doses. Positive and negative control groups of rats were injected with 1ml of 0.85% saline and 71.4 IU/Kg body weight heparin sodium leurquin (Glaxo) respectively.

ii) Administration of Hydergine With Fresh Plasma

In the second set of experiments, animals were pretreated with exogenous clotting factors in the form of fresh plasma, prepared through centrifugation of the cardiac blood aspired from healthy rats, at 1258*g* (2500rpm) for 10 minutes (Eppendorf centrifuge 5810R). Fresh plasma was injected intraperitoneally. One hour after the administration of fresh plasma three groups of rats were injected with 1.5mg, 3mg and 4.5mg of hydergine respectively while parallel control groups of rats were given fresh plasma alone, fresh plasma and 0.85% saline and fresh plasma along with 71.4 IU/kg heparin sodium leurquin. Both saline and heparin were injected one hour after administration of fresh

plasma as was done for hydergine treated groups. Animals were weighed prior to drug administration.

Drug Administration

Appropriate doses of hydergine were prepared by dissolving hydergine powder (1.5mg hydergine tablets, Novartis) in 1ml sterile injectable water. Clotting time (CT) and bleeding time (BT) of all the animals were estimated before and after plasma introduction as well as after the drug administration, by standard hematological procedures. Animals were kept under strict observation after injecting the drug.

Estimation of Bleeding Time (BT)

Bleeding time was estimated by cutting the tail approximately 2cm from the tip with a sharp cut. Time in seconds was counted the moment first drop of blood emerged from the tail until the time when no bloodstain appeared on the filter paper.

Estimation of Clotting Time (CT)

The clotting time was estimated using 75µl non-heparinized hematocrit capillary tubes. Blood was drawn into the capillaries from the cut end of the tail. Capillaries were then broken into small pieces at 10-second interval until formation of fibrin in the clot. Time was recorded in seconds.

Blood Collection From the Animal

CT and BT of the animals were estimated again 1.5 hours after injecting the drug. Animals were killed afterward with an overdose (100mg/kg) of sodium pentobarbital. Blood was collected directly from the heart in syringes pretreated with 3.2% trisodium citrate (0.1ml/ml of blood) as an anticoagulant of choice. 3ml of aspired blood was collected in the EDTA.K_3 coated vacutainers.1ml blood was separated from each sample for plasma preparation to conduct calcium analysis and another 1ml blood was separated

for carrying out prothrombin time (PT) and activated partial thromboplastin time (APTT) analyses.

Preparation of Plasma

Blood was centrifuged as above and plasma was separated from the pellet and stored at –20^{O}C until further analysis. Plasma for PT and APTT was not separated where the test was to be carried out within 4 hours of separation. In either case it was separated and kept at 4^{O}C and analysis was carried out within 24 hours of separation.

Blood Counts Through Hematology Analyzer

Blood collected in the vacutainers was immediately processed for the complete blood counts that were performed on hematology analyzer (Sysmax KX-21). Blood counts provided estimation of red blood cells, total leukocyte count, platelet count, hemoglobin, hematocrit, lymphocyte percentage, lymphocyte number, platelet distribution width, mean corpuscular volume, mean corpuscular hemoglobin concentration, RBC distribution width, mean platelet volume and platelet large corpuscular ratio.

Estimation of Activated Partial Thromboplastin Time (APTT)

APTT was determined with a Diagnostic Alexin^{TM} kit (Sigma, USA). The kit reagent consisted of purified rabbit brain cephalin in elagic acid (0.1mmol/L) with buffer, stabilizer and preservative. This is a general procedure for the detection of coagulation abnormalities in the intrinsic pathway. The assay was developed from the plasma recalcification time by the addition of a surface activator to a suspension of rabbit brain cephalin. Following the manufacturers' instructions for APTT, the reagent, 0.020mol/L calcium chloride (2.22g/l) and citrated plasma were incubated at 37^{O}C for at least 15 minutes in a heating water bath (Julabo, SW22).

To carry out the test, 100µl of plasma was incubated at 37^{O}C in a torpedo tube for 60-180 sec and 100µl reagent was then added to the plasma, the mixture was gently swirled for 5 sec and incubated again at the same temperature for 180 sec. Finally 100µl of calcium

chloride was added. Timer was switched on and time was noted in seconds until coagulation of plasma.

Estimation of Prothrombin Time (PT)

Prothrombin time was estimated using calcium reagent kit (Sigma diagnostics Thrombomax®). The reagent contained lyophilized extract of rabbit brain with buffer, stabilizer and calcium chloride. The extract contained thromboplastin that in the presence of calcium ions accelerates the blood clotting by the activation of extrinsic pathway of coagulation. The reagent was reconstituted with the addition of 4ml deionized water. It was left for approximately 30 minutes at room temperature with occasional swirling. The citrated plasma was kept at $37^{O}C$ for at least 15 minutes in the water bath. 50µl of plasma was added in a torpedo tube and incubated at $37^{O}C$ for 60-180 seconds, 100µl reagent was then added to the plasma and time was noted in seconds until coagulation of the plasma.

Estimation of International Normalized Ratio (INR)

The international committee for standardization in hematology and the International committee on thrombosis and hemostasis have agreed on recommendations for reporting of prothrombin time results as an International normalized ratio (INR), based upon the international sensitivity index (ISI) of thromboplastin reagents (WHO experts committee on biological standardization, 1983; International committee for the standardization of the hematology and international committee on thrombosis and hemostasis, 1985).

Thromboplastin reagents are assigned an ISI value by the calibration against an international reference preparation (IRP) with a defined ISI. Each IRP has been sequentially calibrated against the first IRP that by definition had an ISI of 1.0 using the manual tilt method (Tripodi *et al.*, 1995). The ISI value assigned to the commercial thromboplastin reagents defines a relative sensitivity compared to the IRP preparation. Thromboplastin with lower ISI values has a broader range between normal range and coumadin plasma P, and is considered more sensitive.

The INR was calculated from the tested plasma PT result, the mean normal PT and the thromboplastin ISI using the following equation:

$$INR = R^{ISI}$$

Where ISI = Lot specific International Sensitivity Index for reagent/ instrument system.

$$R = \frac{\text{PT of treated animal}}{\text{Mean PT of the control rat group}}$$

The ISI value of the kit used was 1.08 for mechanical assay method.

Calcium Analysis

Plasma calcium levels were analyzed using Calcium AS FS* reagent kit (Dia Sys Diagnostic System, Germany). The reagent consisted of phosphate buffer, 8-hydroxyquinoline-5-sulfonic acid, arsenazo III and detergents. The principle of the reagent is that calcium with arsenazo III at neutral pH yields a blue colored complex, whose intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline-5-sulfonic acid.

To carry out the test 10 µl citrated plasma was added to 1000 µl of reagent in the cuvette, the mixture was incubated in the water bath at 37^{O}C for 5 minutes and absorbance was noted at 635nm using a visible light spectrophotometer (Schmidzu graphic printer PR-1 spectrophotometer). Absorbance of standard (10mg/dl concentration) provided with the kit was observed first and put into the formula for estimation of calcium (mg/dl) in plasma samples.

$$\text{Calcium [mg/dl]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ Standard / Cal}} \times \text{Conc. Standard / Cal [mg/dl]}$$

Where ΔA was the absorbance noted through the spectrophotometer.

B- Study for the Effects of Hydergine *ex vivo*

Anticoagulatory effect of hydergine was further tested *ex vivo* in another set of experiments.

Estimation of Clotting Time *ex vivo*

Five healthy animals were dissected and blood was aspired in a 10ml syringe without any anticoagulant. At least 7ml blood was taken. The aspirated blood was added to the tubes that contained 0.1mg/ml, 0.2mg/ml and 0.3mg/ml hydergine respectively. Hydergine ampules containing 0.3mg/ml (Novartis) were used for this purpose. Controls were set in the tubes containing 1IU/ml heparin sodium leurquin, 1ml 0.85% saline and an empty tube having no fluid but pure blood. In another tube 1ml fresh plasma was taken. Time in seconds for coagulation was recorded for each tube separately.

Estimation of PT And APTT and Calcium Analysis *ex vivo*

For this purpose another group of five healthy animals was dissected and blood was collected in 10ml syringes pretreated with anticoagulant (0.1ml of 3.2% Trisodium citrate/ml of blood). Experimental tubes contained 0.1mg/ml, 0.2mg/ml and 0.3mg/ml hydergine respectively whereas tubes containing 1 IU/ml heparin sodium leurquin, 1ml 0.85% saline, 1ml fresh plasma and an empty tube having no additional fluid other than pure blood served as controls. 1ml citrated blood was added to each tube. Blood collected in the tubes was then centrifuged in microcentrifuge immediately at 559*g* (2500rpm) for 10 minutes in a microcentrifuge (Eppendorf centrifuge 5417C). The prepared plasma was then used to carry out PT and APTT immediately while 50µl of the plasma was separated to carry out spectrophotometeric calcium analysis at 635nm wavelength.

Statistical Analysis

The results were compared through one-way analysis of variance (ANOVA) using SPSS software. All the groups were compared with saline treated group that was considered the control group. Post hoc analysis applied by Tukey's and Dunken's analyses further revealed the variation of different parameters among differently treated groups of

animals. A probability p value less than 0.05 was considered as significant. Sigma stat software was used to apply paired T-test to verify the variation in bleeding time and clotting time of the animals before and after drug introduction. Wilcoxan signed rank test was applied in the same software in case of the groups where normality test failed

RESULTS

RESULTS

A-General Observations of *in vivo* Experiments

Rats exposed to hydergine appeared lethargic compared to controls. Hydergine administration resulted into diarrhea, which is a known side effect of the drug but on the other hand confirmed its penetration.

The tails of the treated animals that were cut for the estimation of CT and BT before drug administration started bleeding again some time after drug introduction in the animal groups that were exposed to high doses of hydergine. Moreover, it was directly observed that some animals that were administered high doses of hydergine showed hemorrhages in the eyes.

The rats that were treated with fresh plasma did not bleed even when their tails were cut about 3cm from the tips after one hour of plasma administration. It was also observed that the blood of hydergine treated animals that oozed out in the thoracic cavity during cardiac puncture did not clot as early as that of the control animals.

In addition plasma of some of the animals that were treated with high doses of hydergine did not clot at all when mixed with the reagent (rabbit brain cephalin and $CaCl_2$) for APTT determination. Similar were the observations in heparin treated rats.

It was also noticed even though all the animals were treated with the same dose of anesthetic the hearts of the hydergine treated groups continued to beat for a longer time period as compared to controls and plasma treated groups of rats.

A-1. Blood Coagulation Parameters of *in vivo* Experiments

The animals that were administered different doses of hydergine, heparin and hydergine with fresh plasma showed an over all trend of increased bleeding time compared to the control groups while a decreased bleeding time was the out come in the animals injected with fresh plasma. Different parameters related to bleeding showed following results.

Clotting Time (CT)

Clotting time of the animals treated with different doses of hydergine differed significantly from controls (P=0.00). Post hoc analysis verified that the clotting time of the rats treated with 1.5mg, 3.0mg and 4.5mg hydergine increased significantly than the control groups. Whereas animals treated with fresh plasma, fresh plasma with normal saline, fresh plasma with 1.5mg, fresh plasma with 3.0mg and fresh plasma with 4.5mg hydergine and fresh plasma with heparin showed a significant decrease in clotting time to that of 3.0mg hydergine treated group. Similarly, the clotting time of the animals treated with 4.5mg hydergine was significantly greater than the animals treated with heparin, fresh plasma and fresh plasma with normal saline. Paired t-test showed that there occurred a significant increase in clotting time of the animals exposed to hydergine and heparin ($P<0.05$) but 1.5mg hydergine treated and fresh plasma without hydergine treated groups did not show any difference in the clotting time after exposure to the drug (Table 2). Animals exposed to high doses of hydergine had in general significantly prolonged clotting time while those administered with fresh plasma had reduced clotting time (Figure 3).

Bleeding Time (BT)

Animals treated with different doses of hydergine showed no significant difference in the bleeding time from the control although there was significant difference in bleeding time among certain groups (P=0.00). Post hoc analysis within treatments demonstrated that animals treated with 1.5mg hydergine had significantly prolonged bleeding time than those treated with fresh plasma, fresh plasma and normal saline and fresh plasma along with heparin. Animals treated with fresh plasma and fresh plasma with 3mg hydergine showed a significant increase in bleeding time compared to fresh plasma and 4.5mg hydergine treated group of animals. Paired t-test demonstrated that the bleeding time of the animals treated with different doses of hydergine increased significantly ($P<0.05$) after drug introduction (Table 2). The animal groups administered with moderate concentration of hydergine had prolonged bleeding time while those injected with fresh plasma had reduced bleeding time (Figure 4)

Prothrombin Time (PT)

Prothrombin time for any of the groups did not differ significantly from controls (P=0.207). Post hoc analysis showed that the prothrombin time of neither hydergine treated group nor groups treated with both fresh plasma and hydergine differed significantly when comparison were made amongst them (Table 3).

International Normalized ratio (INR)

International normalized ratio of the animal groups treated with different doses of hydergine was non-significantly different from control animals (P=0.263). Post hoc ANOVA established insignificant differences among differently treated groups (Table 3).

Activated Partial Thromboplastin Time (APTT)

APTT of hydergine and fresh plasma with hydergine treated groups was not significantly different from the control group (P=0.002). Post hoc analysis showed that APTT in rats treated with 1.5mg hydergine was significantly higher from those treated with 3.0mg hydergine, heparin and fresh plasma. Rats in the rest of the groups showed no significant difference (Table 3).

Calcium Analysis

Plasma calcium concentration in the animals treated with different doses of hydergine, fresh plasma with hydergine and heparin differed significantly from the control group (P=0.00). Post hoc analysis showed that the animals treated with heparin, fresh plasma with 1.5mg hydergine, fresh plasma with 4.5mg hydergine and fresh plasma with heparin had significantly lower plasma calcium concentration as compared to the control group. Moreover, animals treated with 1.5mg hydergine, showed a significant increase in plasma calcium levels compared to the groups treated with fresh plasma and 4.5mg hydergine (Table3).

Overall comparison showed that different doses of hydergine caused somewhat parallel change in PT and APTT while plasma calcium levels was maximum in the animals exposed to moderate doses of the drug. The remaining treatment groups responded

parallel to heparin treated groups other than those administered with fresh plasma (Figure 5).

A-2. Complete Blood Counts of *in vivo* Experiments

The complete blood counts (CBC) of the animals treated with hydergine, fresh plasma and hydergine and heparin established that the drug specially affected those parameters that were related to platelet activities.

Platelet Count

The platelet count of the animals treated with different doses of hydergine, fresh plasma and heparin turned significantly different from the control group (P=0.001). Post hoc analysis established that the animals treated with fresh plasma and heparin had significantly reduced platelet number compared to the groups that were treated with normal saline, different doses of hydergine, fresh plasma, and fresh plasma with 3.0mg hydergine while, animals treated with fresh plasma and 4.5mg hydergine showed no significant difference in the platelet number when compared with fresh plasma and heparin treated group (Table 4).

Different doses of hydergine did not cause any noticeable change in platelet number. However, fresh plasma resulted in an increase in platelet count while heparin reduced platelet number even in case where animals were priorly exposed fresh plasma (Figure 6).

Platelet Distribution Width (PDW)

Animals treated with different doses of the drug did not show any significant difference in platelet distribution width from those of controls (P=0.520). Post hoc analysis demonstrated that the animals treated with fresh plasma and heparin had significantly greater platelet distribution width compared with other groups as well the control but excepting the group treated with fresh plasma and 4.5mg hydergine (Table 4).

Mean Platelet Volume (MPV)

Mean platelet volume of the animals treated with different doses of hydergine did not differ significantly from the control (P=0.006). Post hoc ANOVA showed that only one group of animals that was treated with fresh plasma and heparin had significantly higher mean platelet volume than all other groups while remaining groups did not show any significant difference amongst them (Table 4).

Platelet Large Corpuscular Ratio (PLCR)

All groups of the treated animals showed a significant difference in platelet large corpuscular ratio as compared to the control group (P=0.003). Post hoc analysis confirmed that fresh plasma and heparin treated groups had significantly greater PLCR than all other groups including control. PLCR in the group treated with fresh plasma and 4.5mg hydergine remained non-significantly different from all other groups (Table 4).

Rats treated with different doses of hydergine showed a parallel variation in PDW, MPV and PLCR. Animals exposed to high concentration of hydergine with prior fresh plasma administration had maximum elevation in the parameters, which are related to platelet morphology; similar was the observation in heparin treated group (Figure 7).

Red Blood Cells (RBC)

No significant difference was observed in the number of red blood cells of the treated animals from the control group (P=0.312). Post hoc analysis further verified that t RBC number did not differ significantly among differently treated groups (Table 5).

RBC Distribution Width (RDW)

RBC distribution width of the treated animals was significantly different from the control groups (P=0.000). Post hoc analysis showed that RDW of all groups decreased significantly from the control excepting 3.0mg hydergine and 4.5mg hydergine and fresh plasma treated animals. Moreover, 1.5mg hydergine treated animals showed significant drop in RDW than 3.0mg hydergine and fresh plasma with 3.0mg hydergine treated animals (Table 5).

Mean Corpuscular Volume (MCV)

The animals treated with different doses of hydergine, fresh plasma with hydergine and heparin had their mean corpuscular volume non-significantly different from those of control animals (P=0.095). Post hoc analysis further revealed that none of the groups differed significantly from the other (Table 5).

Generally, no significant effect of different doses of hydergine and heparin was observed on RBC number and cell morphology related to cell volume and distribution width (Figure 8).

Hemoglobin Concentration, Hematocrit, MCH and MCHC

ANOVA did not show any significant difference in hemoglobin concentration, hematocrit, MCH and MCHC of the treated groups as compared to control rats. P values for hemoglobin, hematocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were 0.309, 0.361, 0.087 and 0.252 respectively. Post hoc analysis confirmed non-significant difference (Table 6) (Figure 9).

Total Leukocyte Count (TLC)

Mean TLC of animals treated with different concentrations of the drug was significantly different when compared with the control (P=0.014). Post hoc analysis revealed that the group that was treated with fresh plasma and normal saline had significantly higher mean TLC values than the animals treated with fresh plasma with 3.0mg and fresh plasma plus 4.5mg hydergine (Table 7).

Lymphocyte Number

Lymphocyte counts in animals under different treatments of hydergine were not significantly different from the controls (P=0.475). Post hoc analysis also revealed that none of the groups had significantly different lymphocyte number from any other group (Table 7).

Fresh plasma caused a rapid increase in both leukocyte and lymphocyte number but no particular change in the leukocyte number was observed as result of exposure to different doses of hydergine (Figure 10).

Lymphocyte Percentage

The percentage of lymphocytes of hydergine treated animals was significantly different from the control (P=0.001). Post hoc analysis illustrated that the lymphocyte percentage of the animals exposed to fresh plasma with 4.5mg hydergine was significantly elevated from those treated with fresh plasma only, 4.5mg hydergine treatment group and the control group (Table 7).

Higher doses of hydergine caused elevation of lymphocyte percentage in case of prior exposure to fresh plasma (Figure 11).

B-Results of *ex vivo* Experiments

To verify the *in vivo* experiments, a set of experiments was run *ex vivo* in test tubes to analyze the effects of different concentrations of hydergine on certain blood coagulation parameters outside the animal body. Following results were observed in *ex vivo* part of experiment.

Clotting Time (CT)

Clotting time of the blood samples treated with different concentrations of hydergine turned significantly different from control (P=0.001). The blood samples treated with heparin and 0.3mg/ml hydergine did not clot at all or clotted very late. Post hoc analysis showed that blood samples treated with fresh plasma had significantly decreased clotting time from those treated with different doses of hydergine and heparin. Heparin treated samples also showed a significant difference in clotting time from the empty control group (Table 8).

Activated Partial Thromboplastin Time (APTT)

The APTT of blood samples treated with different concentrations of hydergine showed a nearly significant difference from the control (P=0.011). Post hoc analysis revealed that only 0.3mg/ml hydergine sample had significantly higher APTT from the sample that was treated with fresh plasma. None of the other treatments caused significantly different effect on APTT than any other group (Table 8). Hydergine caused a parallel effect on CT and APTT. Samples exposed to heparin did not clot at all. Low doses of hydergine caused prolongation of CT and APTT (Figure 12). In addition, hemolysis was also observed in the blood sample containing 0.1 mg hydergine and heparin.

Plasma Calcium Concentration

Plasma calcium concentration of the blood samples of treated groups differed significantly from the control (P=0.000). Post hoc analysis also verified these findings as the sample that was in empty tube had significantly greater calcium concentration than those that were treated with 0.2 mg/ml hydergine, 0.3mg/ml hydergine and heparin. The samples treated with fresh plasma had significantly greater calcium concentration than those treated with 0.1mg/ml, 0.2 mg/ml, 0.3mg/ml hydergine and heparin (Table 8).

Prothrombin Time (PT)

The Prothrombin time of the blood samples treated with hydergine did not vary significantly from the control sample (P=0.094). Also, no significant difference was observed amongst various groups (Table 8).

There was no net effect of any hydergine concentration on the PT while calcium levels were reduced in the hydergine and heparin treated samples (Figure 13).

International Normalized Ratio (INR)

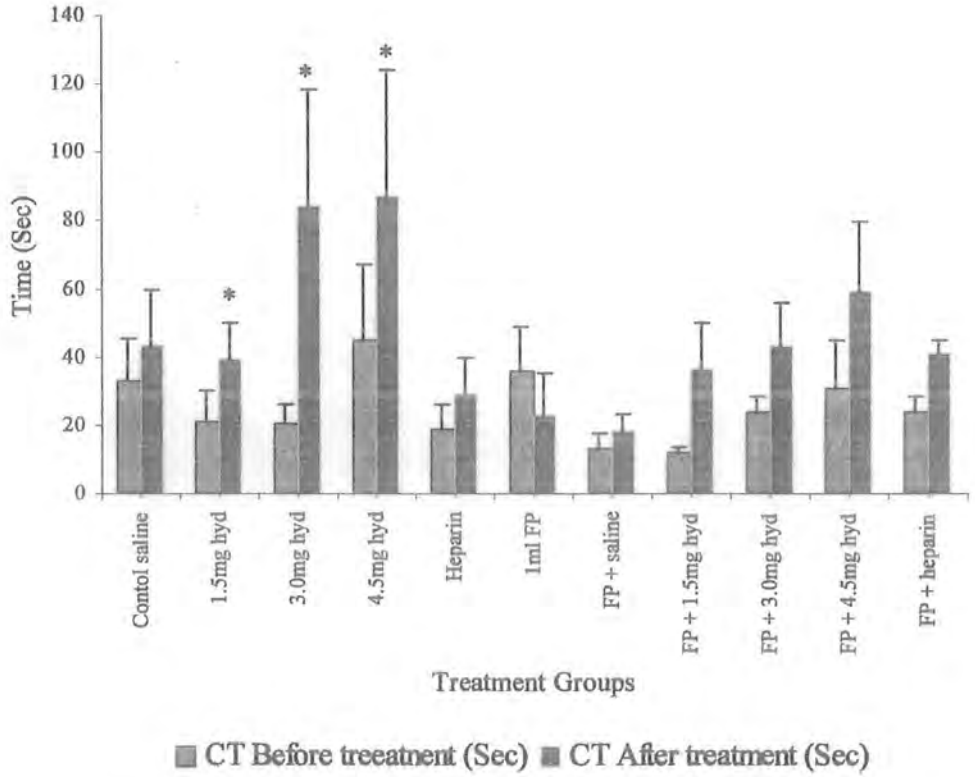
The INR of the blood samples did not show any significant difference from the control (P=0.091). Post hoc analysis further verified the same results (Table 8).

Table 2 **Bleeding time and clotting time of animals before and after intraperitoneal injection of different doses of hydergine, fresh plasma and fresh plasma with hydergine and difference of means.**
(Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N = 5)	Clotting time (Seconds)			Bleeding Time (Seconds)		
	Before treatment	After treatment	Difference	Before treatment	After treatment	Difference
Control (1ml 0.85% saline)	33.0 ± 12.54	43.0 ± 17.53	10.0 ± 7.90	12.4 ± 2.50	16.40 ± 3.50	4.0± 3.08
1.5mg hydergine	21.0 ± 8.94	39.0 ± 10.80	18.0± 4.47*	17.2 ± 16.85	34.20 ± 15.10^{+}	26.20± 17.88
3.0mg hydergine	20.4±5.77	84.0 ± 34.53^{+}	63.60± 31.42*▪	40.4 ± 9.26	53.0 ± 8.36^{+}	12.60± 2.50
4.5mg hydergine	44.8± 22.64	87.0 ± 37.18^{+}	42.20± 20.20*	17.4 ± 3.28	44.20 ± 21.11^{+}	24.80± 17.39
71.4 IU/kg heparin	19.0 ± 7.41	29.0 ± 10.8^{+}	10.0± 5.00▲‡	12.0 ± 7.58	24.0 ± 6.51	12.0± 2.73
1ml Fresh plasma	35.60 ± 13.93	23.0 ± 12.54	-12.60± 2.50▲‡	35.0 ± 22.63	21.40 ± 22.07^{+}	-13.60± 4.72▪
Fresh plasma + 1ml 0.85% saline	13.0 ± 4.47	18.0 ± 5.07	5.0± 7.07▲‡	7.80 ± 4.76	8.80 ± 1.64^{+}	1.0± 3.93▪
Fresh plasma + 1.5mg hydergine	11.84 ± 2.04	36.2 ± 13.77^{+}	24.40± 13.40▲!	11.80 ± 2.04	36.20 ± 13.77^{+}	24.40± 13.48
Fresh plasma + 3.0mg hydergine	24.0 4.18	43 ± 13.03^{+}	19.0± 15.57▲	27.20 ± 7.22	15.20 ± 6.64^{+}	9.60± 3.28
Fresh plasma + 4.5mg hydergine	30.60 ±14.63	59.0 ± 20.43^{+}	28.40± 18.70▲	8.60 ± 4.15	41.0 ± 11.4^{+}	32.40± 7.33▪!~
Fresh plasma + 71.4IU/kg heparin	24.0 ± 4.18	41.0 ± 4.18^{+}	17.0± 7.58▲	6.60 ± 4.77	38.2 0± 19.33^{+}	31.60± 15.46▪

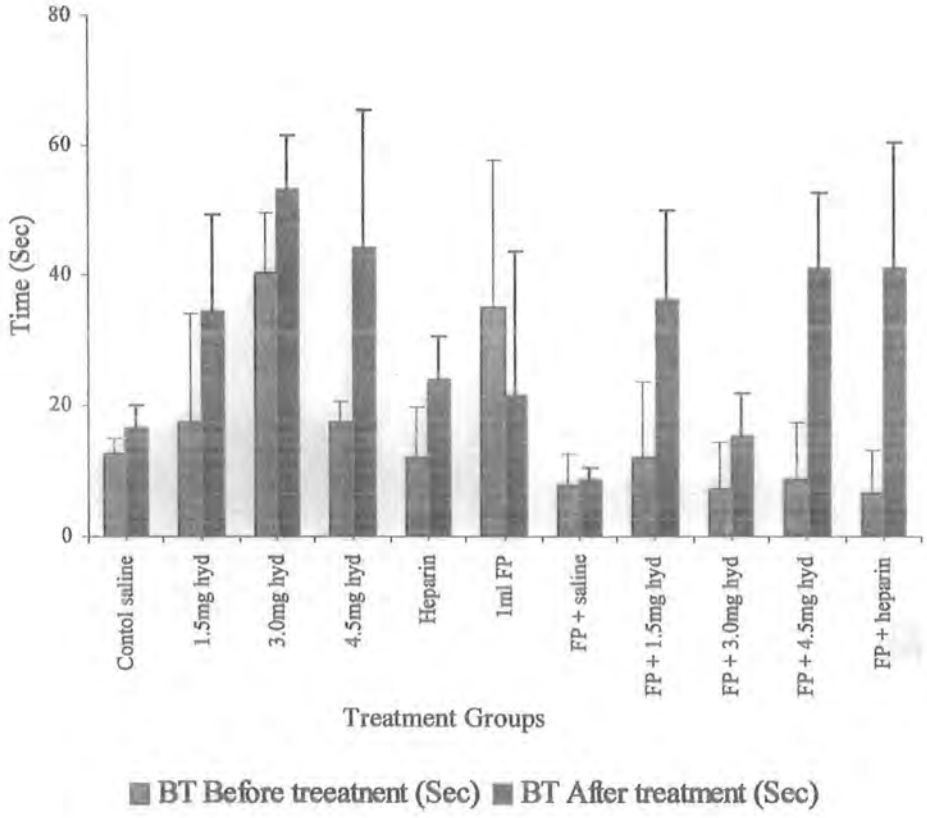
* Significantly different from control
▪ Significantly different from 1.5mg hydergine
▲ Significantly different from 3.0mg hydergine
‡ Significantly different from 4.5mg hydergine
! Significantly different from fresh plasma
~ Significantly different from fresh plasma+3.0mg hydergine
$^{+}$ Significantly different before and after treatment at P< 0.05

Figure 3 **Clotting time of animals before and after the administration of different doses of hydergine, heparin and fresh plasma.**



hyd: Hydergine, FP: Fresh plasma, Sec: Seconds
*Significantly different from control

Figure 4 **Alteration in bleeding time of animals before and after treatment to different doses of hydergine, fresh plasma and heparin.**



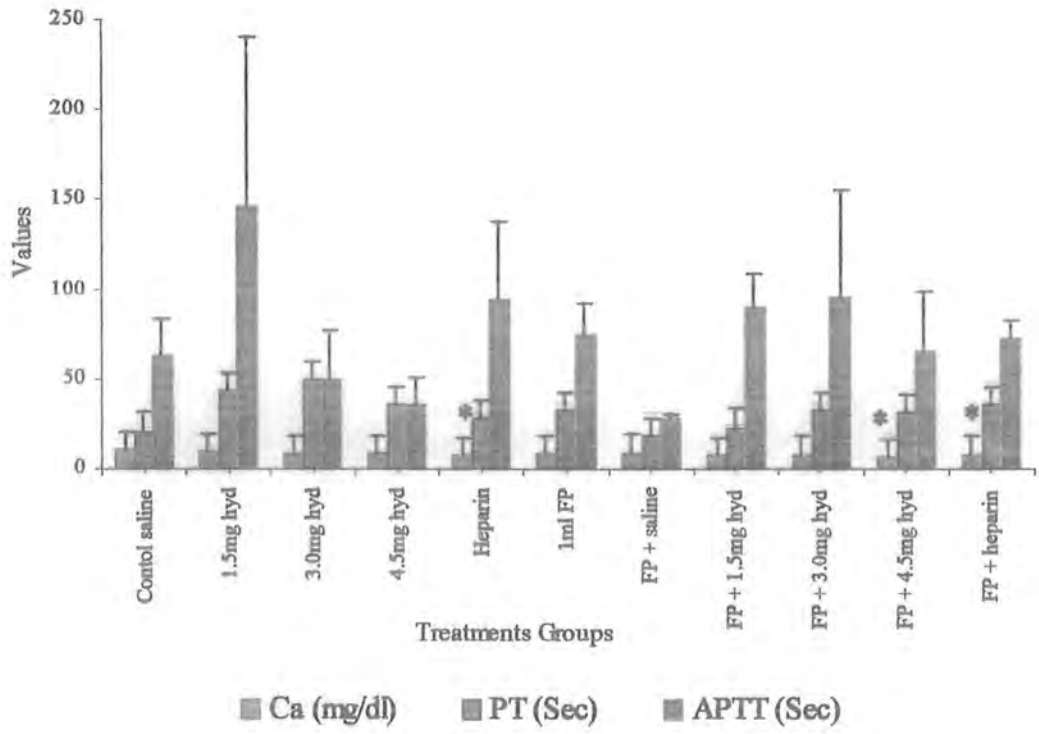
hyd: Hydergine, FP: Fresh plasma, Sec: Second

Table 3 **Prothrombin time, international normalized ratio, activated partial thromboplastin time and plasma calcium concentration of animals exposed to different doses of hydergine, fresh plasma and fresh plasma with hydergine.** (Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	Prothrombin Time (Seconds)	International normalized ratio	Activated partial thromboplastin time (Seconds)	Calcium concentration (mg/dl)
Control (1ml 0.85% saline)	21.0 ± 10.67	0.92 ± 0.56	62.40 ± 21.05	10.76 ± 1.31
1.5mg hydergine	43.20 ± 43.39	2.18 ± 2.46	146.0 ± 93.81	9.24 ± 0.31
3.0mg hydergine	49.40 ± 27.35	2.55 ± 1.59	19.4 ± 27. 35▪	8.76 ±0.74
4.5mg hydergine	35.60 ± 15.75	1.73 ± 0.87	35.6 ± 15.75	8.8 ± 0.51
71.4 IU/kg heparin	28.40 ± 4.21	1.37 ± 0.21	94.6 ± 42.70▪	7.2 ± 0.50*
1ml Fresh plasma	32.60 ± 6.42	1.59 ± 0.34	74.6 ± 17.55	8.74 ± 0.98
Fresh plasma + 1ml 0.85% saline	18.40 ± 5.02	0.81 ± 0.25	28.0 ± 2.73▪	8.96 ± 2.53*
Fresh plasma + 1.5mg hydergine	23.20 ± 2.86	1.08 ± 0.40	89.40 ± 18.55	7.06 ± 0.93*
Fresh plasma + 3.0mg hydergine	32.20 ± 9.57	1.58 ± 0.51	95.0 ± 59.86	8.10 ± 1.44*
Fresh plasma + 4.5mg hydergine	31.20 ± 16.57	1.53 ± 0.89	65.0 ± 32.97	6.56 ± 0.51
Fresh plasma + 71.4IU/kg heparin	35.20 ± 9.75	1.73 ± 0.52	72.80 ±9.06	7.90 ± 1.40*▪

* Significantly different from control
▪ Significantly different from 1.5mg hydergine

Figure 5 **PT, APTT and plasma calcium concentration of animals injected intraperitoneally with different doses of hydergine, heparin and fresh plasma.**



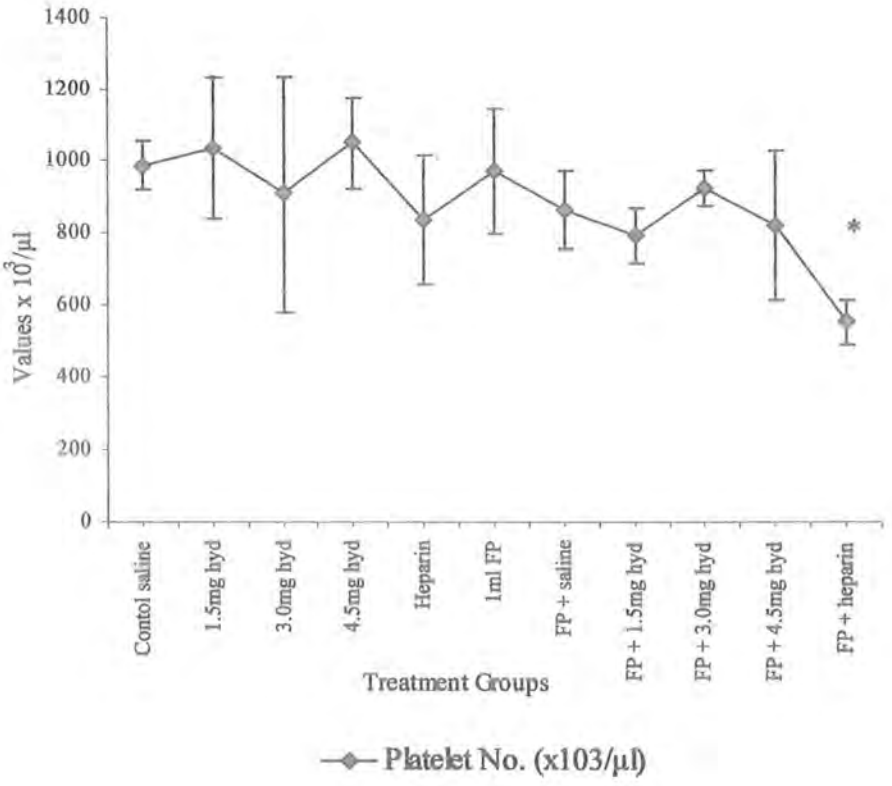
hyd: Hydergine, FP: Fresh plasma, Sec: Seconds
* Significantly different from control

Table 4 **platelet count, platelet distribution width, mean platelet volume and platelet large corpuscular ratio of animals exposed to different doses of hydergine, fresh plasma and fresh plasma with hydergine.**
(Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	Platelet count ($10^3/\mu l$)	Platelet distribution width (fL)	Mean platelet volume (fL)	Platelet large corpuscular ratio
Control (1ml 0.85% saline)	985.4 ± 67.45Ψ	8.46 ± 0.52Ψ	7.22 ± 0.37Ψ	5.92 ± 1.43Ψ
1.5mg hydergine	1034.6 ± 198.53Ψ	8.24 ± 0.35Ψ	7.08 ± 0.14Ψ	6.02 ± 0.79Ψ
3.0mg hydergine	907.4 ±327.13Ψ	8.84 ± 1.87Ψ	7.10 ± 0.58Ψ	6.28 ± 2.06Ψ
4.5mg hydergine	1046.8 ±128.26Ψ	8.22 ± 0.48Ψ	6.94 ± 0.18Ψ	5.16 ± 0.96Ψ
71.4 IU/kg heparin	833.3 ± 176.76	8.14 ± 0.47Ψ	6.94 ± 0.30Ψ	5.66 ± 0.85Ψ
1ml Fresh plasma	967.8 ± 174.57Ψ	8.12 ± 0.40Ψ	6.90 ± 0.23Ψ	5.16 ± 0.90Ψ
Fresh plasma + 1ml 0.85% saline	857 ±108.34Ψ	8.14 ± 0.32Ψ	6.88 ± 0.24Ψ	5.18 ± 0.84Ψ
Fresh plasma + 1.5mg hydergine	789.6 ± 74.48	8.36 ± 0.41Ψ	7.06 ± 0.27Ψ	5.98 ± 1.18Ψ
Fresh plasma + 3.0mg hydergine	920.2 ± 47.70Ψ	8.12 ± 0.10Ψ	6.96 ± 0.21Ψ	5.50 ± 0.28Ψ
Fresh plasma + 4.5mg hydergine	818 ± 206.30	8.58 ± 1.40	6.94 ± 0.97Ψ	7.02 ± 4.97
Fresh plasma + 71.4IU/kg heparin	549.80 ± 63.10*	9.24 ± 0.91	8.22 ± 0.94	10.78 ± 2.67*

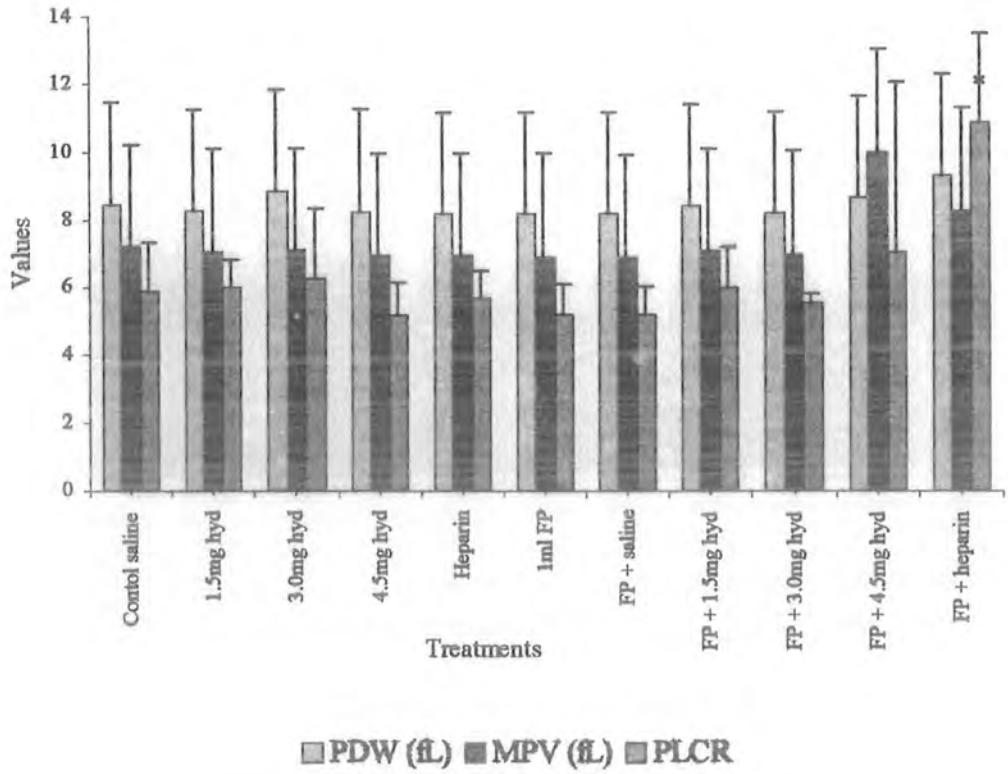
* Significantly different from control
Ψ Significantly different from fresh plasma+ heparin

Figure 6 **platelet counts of the rats exposed to different doses of hydergine, heparin and fresh plasma.**



hyd: Hydergine, FP: Fresh plasma
* Significantly different from control

Figure 7 **Assessment of variation of PDW, MPV and PLCR of the animals exposed to different doses of hydergine, fresh plasma and heparin.**



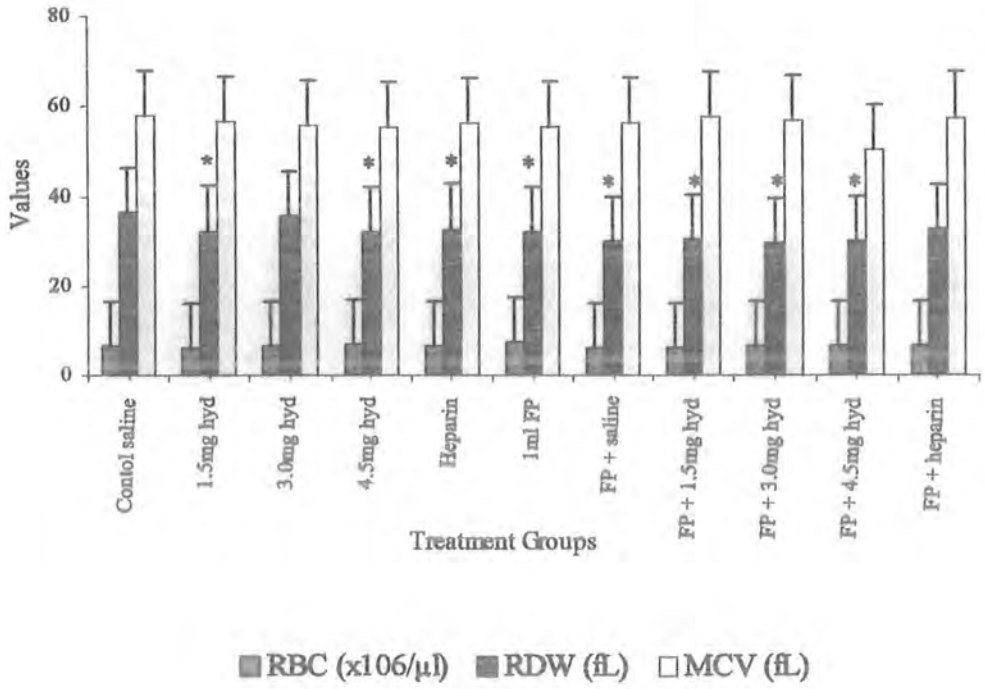
hyd: Hydergine, FP: Fresh plasma, fL: Famtolitres
* Significantly different from control

Table 5 **RBC, RBC distribution width and mean corpuscular volume of animals exposed to different doses of hydergine, fresh plasma and fresh plasma with hydergine.**
(Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	RBC ($10^6/\mu l$)	RBC distribution width (fL)	Mean corpuscular volume (fL)
Control (1ml 0.85% saline)	6.55 ± 0.91	36.20 ± 1.66	57.82 ± 1.50
1.5mg hydergine	6.00 ± 1.07	32.18 ± 1.58*▲	56.76 ± 1.76
3.0mg hydergine	6.40 ± 1.47	35.62 ± 1.48▪	55.66 ± 2.11
4.5mg hydergine	7.00 ± 1. 06	31.9 ± 1.13*▲	55.38 ± 0.76
71.4 IU/kg heparin	6.32 ± 0.73	32.62 ± 0.87*▲	56.30 ± 0.86
1ml Fresh plasma	7.16 ± 0.83	31.94 ± 0.81*▲	55.40 ± 1.11
Fresh plasma + 1ml 0.85% saline	5.85 ± 0.48	29.64 ± 0.61*▲	56.32 ± 1.03
Fresh plasma + 1.5mg hydergine	5.88 ± 0.60	30.18 ± 1.68*▲	57.32 ± 1.68!
Fresh plasma + 3.0mg hydergine	6.61 ± 0.37	29.26 ± 0.46*▪▲	56.74 ± 1.20
Fresh plasma + 4.5mg hydergine	6.45 ± 0.65	29.90 ± 1.96*▲	50.18 ± 1.26!
Fresh plasma + 71.4IU/kg heparin	6.31 ± 0.31	32.40 ± 0.57▲	57.28 ± 0.64

* Significantly different from control
▪ Significantly different from 1.5mg hydergine
▲ Significantly different from 3.0mg hydergine
! Significantly different from fresh plasma

Figure 8 **Graph showing variability in RBC, RDW and MCV of animals administered with different doses of hydergine, fresh plasma and heparin.**

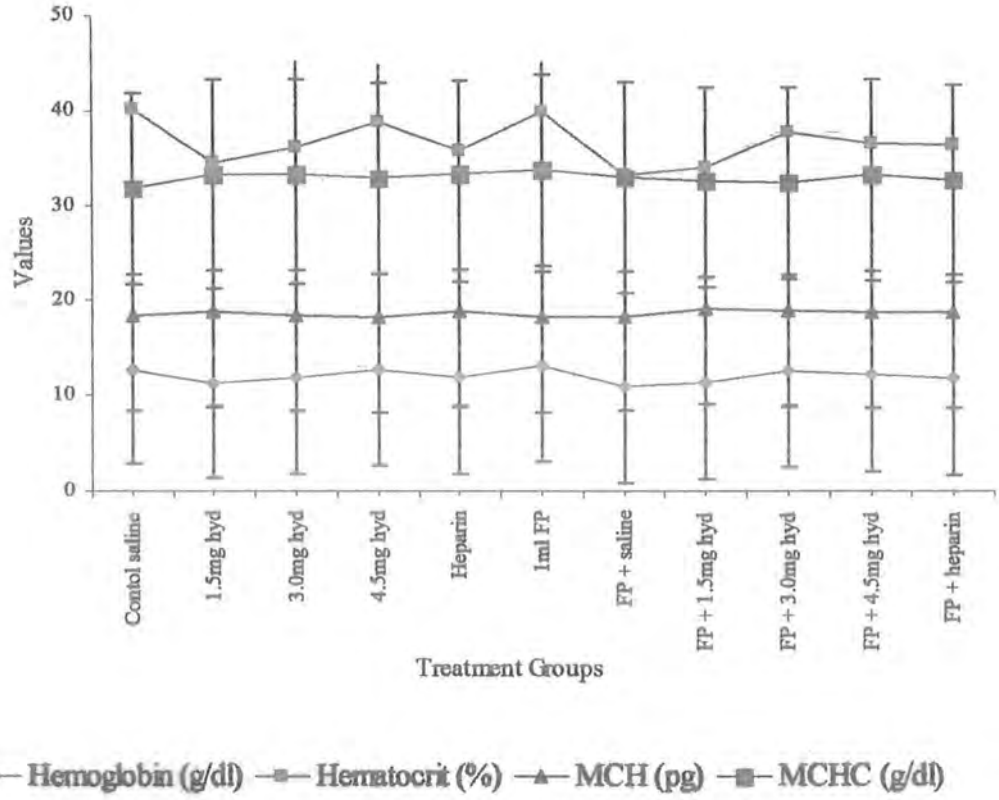


hyd: Hydergine, FP: Fresh plasma, fL: Famtolitres
*** Significantly different from control**

Table 6 **Hemoglobin, Hematocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of animals exposed to different doses of hydergine, fresh plasma and fresh plasma with hydergine.**
(Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	Hemoglobin (g/dl)	Hematocrit (%)	Mean corpuscular hemoglobin (pg)	Mean corpuscular hemoglobin concentration (g/dl)
Control (1ml 0.85% saline)	12.78 ± 0.56	40.20 ± 1.68	18.38 ± 0.52	31.80 ± 0.47
1.5mg hydergine	11.36 ±1.96	34.34 ± 6.76	18.89 ± 0.17	33.22 ± 0.98
3.0mg hydergine	11.8 ± 2.35	36.02 ± 9.11	18.46 ± 0.77	33.18 ± 2.21
4.5mg hydergine	12.7 ± 1.76	38.78 ± 5.97	18.14 ± 0.67	32.74 ± 0.95
71.4 IU/kg heparin	11.94 ± 1.33	35.64 ± 4.66	18.90 ± 0.24	33.1 ± 0.66
1ml Fresh plasma	13.09 ± 1.52	39.72 ± 5.06	18.22 ± 0.14	33.56± 0.66
Fresh plasma + 1ml 0.85% saline	10.78 ± 1.15	33. 0± 3.09	18.34 ±0.58	32.86 ± 0.47
Fresh plasma + 1.5mg hydergine	11.24 ± 1.33	33.80 ± 4.27	19.08 ± 0.69	32.28 ± 0.75
Fresh plasma + 3.0mg hydergine	12.46 ± 0.58	37.54 ± 2.21	18.84 ± 0.44	32.22 ± 0.43
Fresh plasma + 4.5mg hydergine	12.0 ± 1.43	36.34 ± 4.46	18.56 ± 0.43	33.02 ± 0.73
Fresh plasma + 71.4IU/kg heparin	11.72 ± 0.46	36.14 ± 1.66	18.60 ± 0.42	32.44 ± 0.48

Figure 9 **Hematocrit, hemoglobin levels, MCH and MCHC of animals exposed to different doses of hydergine, heparin and fresh plasma.**



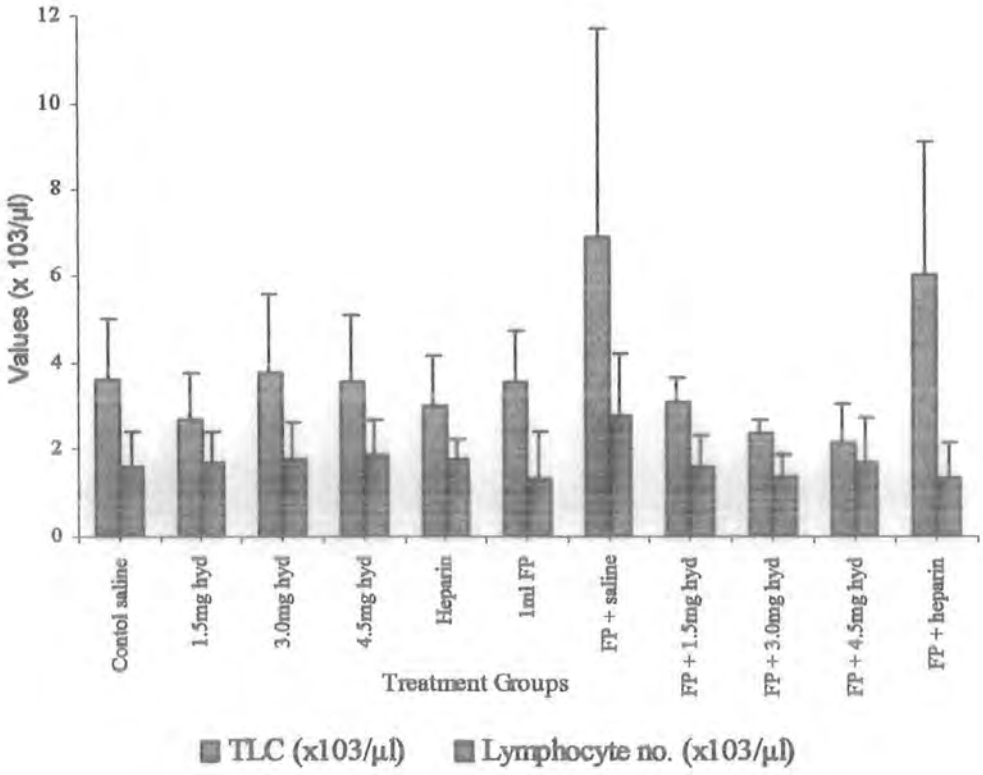
hyd: Hydergine, FP: Fresh plasma

Table 7 **Mean TLC, lymphocyte percentage and lymphocyte number of animals exposed to different doses of hydergine, fresh plasma and fresh plasma with hydergine.** (Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	Mean TLC ($10^3/\mu l$)	Lymphocyte percentage (%)	Lymphocyte number ($10^3/\mu l$)
Control (1ml 0.85% saline)	3.62 ± 1.38	43.76 ± 12.14	1.60 ± 0.83
1.5mg hydergine	2.70 ± 1.07	60.92 ± 10.21	1.68 ± 0.75
3.0mg hydergine	3.76 ± 1.83	62.74 ± 13.02	1.80 ± 0.86
4.5mg hydergine	3.56 ± 1.54	54.54 ± 7.10	1.88 ± 0.79
71.4 IU/kg heparin	3.02 ± 1.15	62.48± 13.90	1.80 ± 0.45
1ml Fresh plasma	3.58 ± 1.18	44.52 ± 13.40	1.36 ± 1.05
Fresh plasma + 1ml 0.85% saline	6.90 ± 4.79	44.54 ± 9.73	2.76± 1.45
Fresh plasma + 1.5mg hydergine	3.08 ± 0.57	66.90 ± 22.60	1.6 ± 0.75
Fresh plasma + 3.0mg hydergine	2.38 ± 0.30!	63.48 ± 23.53	1.38 ± 0.50
Fresh plasma + 4.5mg hydergine	2.16 ± 0.88!	88.0 ± 3.59*‡!◊	1.72 ± 1.01
Fresh plasma + 71.4IU/kg heparin	6.0 ± 3.08	58.38 ± 21.30	1.36 ± 0.79

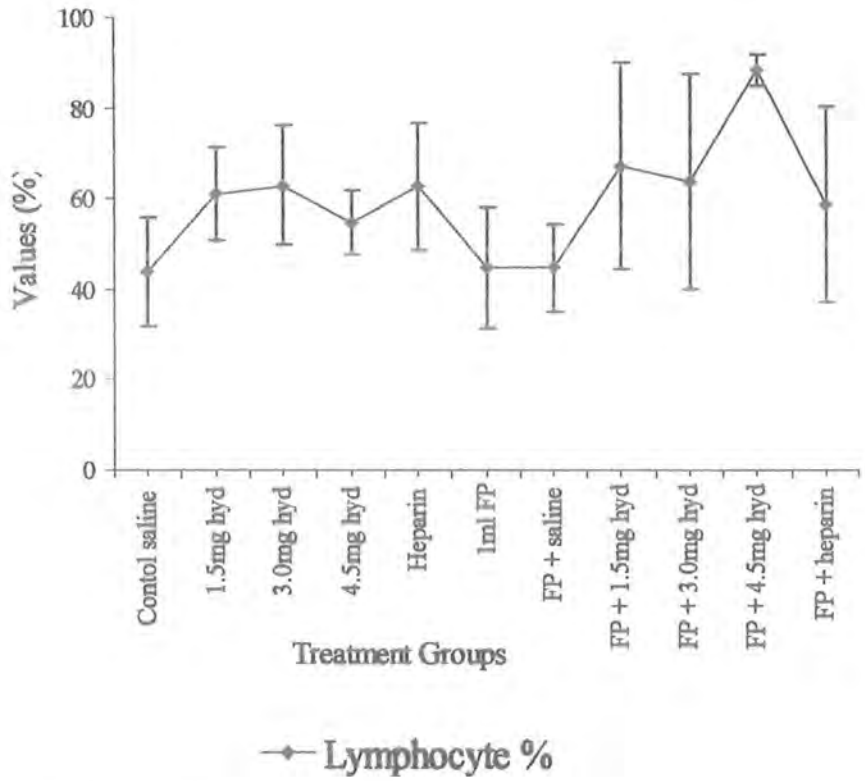
*Significantly different from control
‡ Significantly different from 4.5mg hydergine
! Significantly different from fresh plasma
◊ Significantly different from fresh plasma+1.5mg hydergine

Figure 10 **TLC and lymphocyte number of the animals treated differently with hydergine, heparin and fresh plasma.**



hyd: Hydergine, FP: Fresh plasma

Figure 11 **Effect of different doses of hydergine, heparin and fresh plasma on the lymphocyte percentage of the exposed animals.**



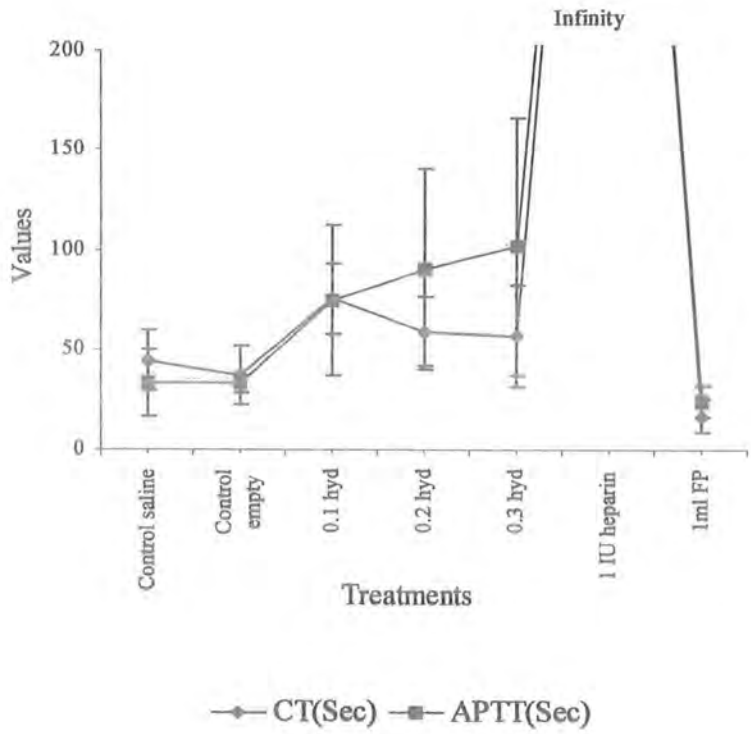
hyd: Hydergine, FP: Fresh plasma

Table 8 ***Ex vivo* coagulation study of blood exposed to different solutions, aspired from single animal.**
(Values are expressed as mean ± standard deviation, P=0.05)

Treatments (In all groups N=5)	Clotting Time (Seconds)	Activated Partial Thromboplastin Time (Seconds)	Calcium concentration (mg/dl)	Prothrombin Time (Seconds)	International Normalized Ratio
1ml 0.85% saline	44.0 ± 15.16	33.0 ± 16.80	11.64 ± 0.77	32.2 ± 13.57	1.56 ± 0.68
Control with no addition	37.0 ± 14.83♦	32.8 ± 4.40	12.14 ± 0.84	24.4 ± 9.44	0.91 ± 0.29
0.1 mg/ml hydergine	75.0 ± 17.32 Φ	74.5 ± 37.42	10.86 ± 0.45 Φ	61.0 ± 67.21	3.18 ± 3.90
0.2 mg/ml hydergine	59.0 ± 17.46 Φ	90.0 ± 50.46	10.64 ± 0.47♪ Φ	34.4 ± 12.52	1.70 ± 0.66
0.3 mg/ml hydergine	57.0 ± 25.39 Φ	101.6 ± 64.55	10.69 ± 0.55♪ Φ	30.0 ± 12.24	1.36 ± 0.71
1IU/ml heparin	Did not clot	Did not clot	10.48 ± 0.51♪ Φ	85.0 ± 49.49	4.50 ± 2.82
1ml fresh plasma	17.0 ± 8.36 ♦	24.4 ± 8.01♠	12.75 ± 0.73	13.2 ± 7.98	0.49 ± 0.29

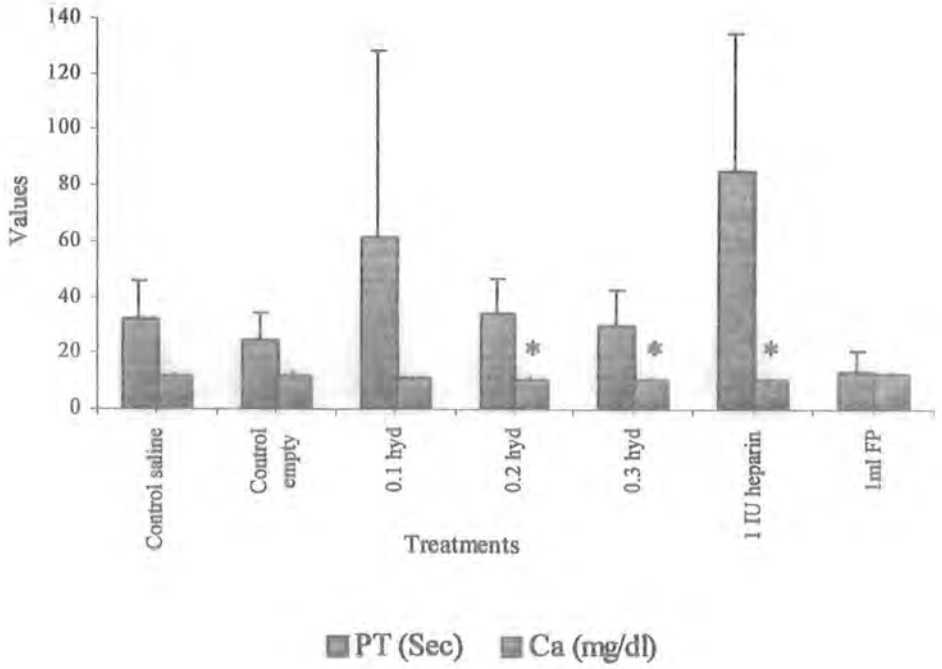
⌂ Significantly different from 0.85% saline
♪ Significantly different from empty tube
♠ Significantly different from 0.3hydergine
♦ Significantly different from heparin
Φ Significantly different from fresh plasma

Figure 12 **Comparison of CT and APTT of the solutions plasma exposed to different doses of hydergine, fresh plasma and heparin in *ex vivo* experimentation system.**



hyd: Hydergine, FP: Fresh plasma

Figure 13 **PT and plasma calcium levels in the solutions exposed to different concentrations of hydergine, heparin and fresh plasma in *ex vivo* part of study.**



hyd: Hydergine, FP: Fresh plasma
* Significantly different from empty control

DISCUSSION

DISCUSSION

The present study was carried out to investigate the anticoagulatory effects of hydergine, an extract of ergot of rye and a proved cognition enhancer. Ergot mesylates (hydergine) are among the most frequently prescribed medications through out the world and have been in use as putative cognition enhancers (Schneider and Tariot, 1997).

Currently, the bleeding time increased significantly in rats exposed to high doses of hydergine. The observation is similar to another study where heparin, a known anticoagulant significantly prolonged the bleeding time in a dose dependent manner whereas the blockade of tissue factor/factor VIIa function does not result in a prolonged bleeding time. Thus dissociation of antithrombin and antihemostatic effect indicates that inhibition of the coagulation system at its initial stage represents a promising approach for the development of new anticoagulants (Himber *et al.*, 1997).

It has been observed that long-term anticoagulant therapy substantially reduces the risk of stroke in myocardial infarction patients. The increased risk of bleeding complications associated with anticoagulant therapy is offset by a marked reduction in ischemic events. The risk of intracranial bleeding is directly related to the intensity of anticoagulant treatment (Aida *et al.*, 2004). Another study indicated that a thrombolytic agent activated protein C (APC) did not adversely affect haemostatic function or produce intracerebral hemorrhage. In contrast, bleeding and intracerebral hemorrhage are potential life-threatening complications with thrombolytic treatment with tissue plasminogen activator or antiplatelet treatment with heparin (Masayoshi *et al.*, 2001). Moreover, ximelagatran, which is the first oral anticoagulant of a new class of anticoagulants called direct thrombotic inhibitors, requires routine blood monitoring as it increases the risk of bleeding when being used for venous thromboembolism management and stroke prevention in patients with atrial fibrillation (Kwok *et al.*, 2005).

The most significant effect of antiplatelet therapy is that it may increase bleeding, thus it can reduce the risk of recurrent stroke or transient ischemic attack (David and Pancal, 2003). Compared to aspirin, oral anticoagulant significantly decreases the risk of stroke, ischemic shock and cardiovascular events for patients with non-vascular chronic or peroxysomal atrial fibrillation but modestly increases the absolute risk of major bleeding. The balance of benefits and risks varies by patient sub group (Van walraven *et al.*, 2002).

In the current study hemorrhages were observed in the eyes of the hydergine treated animals and the tails of these animals started bleeding from the cut edges. A history of mucocutaneous bleeding often indicates abnormal platelet function that can be associated with a normal, increased or decreased platelet count. Simple procedures such as platelet count, peripheral blood smears and a platelet function-screening tests are often required for more specific analyses. Platelet dysfunction does not directly affect the anticoagulation proteins however; the laboratory evaluation of platelet dysfunction should also include some basic coagulation assays such as PT and APTT to exclude a coagulopathy as the reason for bleeding (Kandice and George, 2002).

In the study under consideration, hydergine decreased the clotting efficiency of the animals. The clotting time of the animals treated with hydergine with and without a prior exposure of fresh plasma was prolonged. Similarly, in a previous study the addition of frozen fresh plasma to whole blood *in vitro* after systemic heparinization significantly prolonged the activated clotting time (Wilhelmi *et al.*, 2001). Moreover, the present finding appears parallel to a previous observation where another cognition enhancer; *Ginko biloba* has shown to inhibit platelet function (Chung *et al.*, 1987). Studies have been performed to determine whether *Ginko biloba* significantly affect bleeding time or other measures of blood coagulation (Kudolo, *et al.*, 2002; Baldit, *et al.*, 2003). While the results have generally failed to find any anti-clotting effect, prudence still suggests that *Ginko biloba* should have not been used by anyone during the periods before and after surgery or labor and delivery or by those with bleeding problems such as hemophilia. It is also at least remotely possible that ginko could cause bleeding problems if combined with any natural blood thinners such as garlic, policosanol and high dose vitamin E (Kohler,

et al., 2004). Moreover, ingestion of fresh garlic and garlic powder may have additive effects with anticoagulants or platelet aggregation inhibitors leading in one case to a life threatening hemorrhage (Sanjay and Subir, 2002).

Certain studies have shown that nootropics are related to the increased bleeding tendency. Aspirin, Vitamin E and ginko biloba are proven antioxidants so is hydergine that protects brain against ischemia. There exists evidence that they even help to generate new synapses, however, all of these agents might be associated with the risk of bleeding. Ginko can cause vasodilatation and reduce the ability of blood to clot. It has been studied that daily low dose aspirin (81mg) given orally to the patient with newly diagnosed and relapsed multiple myeloma who were receiving thalidomide, reduced the incidence of venous thromboembolic events without an increase in bleeding complications (Rachid *et al.*, 2005). Ginger may counteract the effects of certain drugs that are used to treat high blood pressure or dysrhythmias. The herb might contribute to spontaneous bleeding if combined with anti-platelet anticoagulant drugs such as coumadin. Similarly, when combined with coumadin, ginseng can increase the risk of bleeding problems (Schmidt, 2004). However, no such studies in relevance to hydergine exist and its thrombolytic or antiplatelet activity has not been studied objectively as per see.

The wide spread adoption of international normalized ratio has been suggested as a method for standardization of PT which is a coagulatory assay used to measure the anticoagulant affect of warfarin (Mike, 2005). In the current study, there was no significant affect on international normalized ratio that was carried out to evaluate the prothrombin time. APTT and PT are two major methods for securing patient for bleeding tendency. Heparin is an anticoagulant commonly used for various clinical conditions and thus affects the coagulation profile. In patients with thrombosis, PT is not significantly influenced by heparin safe dose (Chen *et al.*, 2003).

Presently, animals exposed to hydergine displayed no significant increase in APTT. It is somewhat contradictory to another study in which bolus dose of argatroban and heparin has been shown to produce dose related increase in activated clotting time and activated

partial thromboplastin time within ten minutes of administration. Dissipation of anticoagulatory effect was approximately 4-times faster for argatroban than 100U/Kg heparin (Swan *et al.*, 2000). However in another group of experiments, the APTT of the heparin treated rats was prolonged significantly while in argatroban treated animals it was prolonged slightly (Onomura *et al.*, 2000).

It has been suggested earlier that the arterial thrombolytic effect of direct thrombin inhibitors, when compared with those of heparin, should be evaluated by the activated clotting time and not the APTT or thrombin generation assays. For a therapeutic APTT prolongation, thrombin inhibitors induce higher systemic anticoagulation than heparin and thus might unduly have higher bleeding liability (Jean *et al.*, 1995). An intravenous course of standard unfractionated heparin with the APTT to a desired length is the standard initial in-hospital treatment for patients with deep vein thrombosis but fixed dose subcutaneous low molecular weight heparin appears to be effective and safe (Koopman *et al.*, 1996).

During the current experiments, 3.2% sodium citrate was used as an anticoagulant for blood to carry out PT and APTT. This is critical concentration that affects the results of coagulation tests. If the concentration is raised to 3.8% especially in heparin treated subjects, the change in INR is 0.7. Therefore, the use of 3.2% citrate is recommended for all coagulation tests (Adcock *et al.*, 1997).

The prothrombin time and INR of the animals that were introduced to hydergine *ex vivo* were not significantly different from those of controls. Contrary to this the bleeding time was prolonged while platelet counts did not show any difference. This appears similar to the previous findings where thrombin time, partial thromboplastin time and prothrombin measured in plasma samples treated *ex vivo* with hirudin, were prolonged depending upon the plasma hirudin levels. Platelet count, fibrinogen levels and fibrinolytic system remained unchanged. Bleeding time prolonged twice at maximum hirudin levels (Markwardt *et al.*, 1984). INR is a worldwide routinely used factor in the monitoring of oral anticoagulation treatment. Among various vitamin K dependent plasma proteins, the

coagulation factors II, VII and X showed the most significant association with INR (Cezary *et al.*, 2003).

There is another evidence in which lower than expected thrombin inhibition by endogenous antithrombin action after full activation by heparin addition was found to be a common feature in patients who suffered from previous venous thrombotic events and may reflect a hitherto unrecognized thrombophilic alterations (Cristina *et al.*, 2002). However, in the present study, the clotting time and prothrombin time of the rats exposed to fresh plasma were reduced compared to the hydergine treated rats. This is somewhat parallel to a study where frozen plasma was given to babies having bleeding at the umbilical site; the prothrombin time was reduced significantly one hour after treatment and remained low even after 12 hours (Greisen and Andreasen, 2003).

In the present study *ex vivo* and *in vivo* plasma calcium levels of hydergine treated animals decreased significantly compared to controls. This is parallel to the findings of another vasodilator; the calcium antagonist Varapamil that has shown increased oxygen extraction of ischemic tissues in coronary and peripheral vascular disease (Bagger *et al.*, 1997). Bell *et al.* (1990) found a positive correlation between the extent of single platelet aggregation and calcium levels. Higher calcium levels cause clotting of blood samples. The removal of calcium by EDTA is required for the functional integrity of intracellular signaling pathways. Although EDTA is known to be a non-membrane permeating chelator, high concentrations of EDTA have been reported to alter membrane fluidity, an action that may in turn disturb intracellular calcium levels (Ptiri and Vijaya, 2000).

In the present study the clotting time of the samples exposed *ex vivo* to hydergine was increased while the plasma calcium concentrations were decreased. In another study, the blood samples obtained from different plastic vacuum tubes were recalcified and clotting time was determined by free oscillation rheometry. The clotting times for blood collected in vacutainer or vacuette tubes decreased with maximal effect after 30 minures (Ramstrom, 2005).

Computer simulation of chemical equilibria in multicomponent biological liquids have been a method applied to search for new low molecular weight endogenous blood anticoagulants with a mechanism of action based on reduction of the equilibrium concentration of biologically active calcium ions. The analysis of equilibria revealed four endogenous ligands capable of forming stable complexes with calcium including glutamate, lactate, citrate and ATP. Their anticoagulant properties have been confirmed by universal biological assay in rats in terms of plasma recalcification time and APTT (Nikolaeva *et al.*, 2005).

In the study under consideration, there was no significant change observed in the platelet number of the animal groups exposed to different doses of hydergine however, the control animals treated with heparin showed spontaneous thrombocytopenia in both cases i.e., to a single exposure as well as in case of prior fresh plasma administration. The results seen are parallel to a previous study where type II immune-mediated heparin induced thrombocytopenia in 2% of individuals exposed to heparin for more than four days (Gilaad *et al.*, 2005). Heparin induced thrombocytopenia is a serious and underestimated adverse drug effect (Franchini and Veneri, 2005). The behavior of hydergine treated rats was not similar to heparin-exposed rats. This conflict may be due to the difference of mechanism of action or the difference of half-life of the two drugs. Short-term exposure may have been the cause of no change in platelet number in response to hydergine exposure. Platelet dysfunction with a normal platelet count usually indicates a qualitative platelet disorder. These disorders should be evaluated in patients with a normal PT, APTT and platelet count (Kandice and George, 2002).

No significant effect was observed on mean platelet volume due to hydergine administration. However, mean platelet volume decreases with age but there is no difference between the genders. There is also a negative correlation between whole blood platelet number and mean platelet volume (Bancroft *et al.*, 2000).

RBC counts of hydergine treated animals were not significantly different however, RBC distribution width showed a significant decrease. Similar results were reported in an

earlier study conducted on horse where heparin was found to exert a profound influence on red blood cell mass. Three of heparin regimens (320, 240 and 160 U/Kg) resulted in a significant decrease in RBC number, platelet cell volume and total hemoglobin content. Platelet count was also reduced. The observed increase in the mean corpuscular volume was associated with decreased RBC number. Plasma proteins, serum billirubins, free hemoglobin, heptoglobin and urine hemoglobin values remained unchanged in all groups. Heparin anticoagulation therapy with the smallest dose (40 IU/Kg) had no detectable effect on the measured values nor did the saline solution (Duncan *et al.*, 1985).

An extract of ginko biloba is helpful in reducing viscosity and elasticity of the whole blood, show blood coagulation and inhibit platelet aggregation. Complete blood counts, biochemical profiles and urine analysis taken before and after treatment with piracetam did not reveal any change suggesting it to be a safe drug (Donma, 1998). In another study, fish oil supplemented with and without vitamin E did not affect RBC characteristics or exercise performance (Oostenburg *et al.*, 1997). Nitric acid, an oxygen scavenger like hydergine, has been suggested to have cardiovascular effects via regulation of red blood cell deformability (Melek *et al.*, 2003).

Thrombolytic therapy had no direct effect on intrinsic RBC aggregability in patients with acute myocardial infarction. Inhibition of RBC aggregation by thrombolytic therapy may result from the degradation of fibrinogen, key factor in the formation of RBC aggregates, and from the generation of fibrinogen degradation products capable of deaggregating RBC (Ben *et al.*, 2002). In a study in which the effects of unfractionated and fractionated heparin on red blood cell aggregability were examined *in vitro*, the results showed that heparin did not increase this rheological parameter but showed a slight tendency to lower it (Martinez *et al.*, 2000). Moreover, when repeated deoxy-oxy cycling forms extremely stretched sickle shaped cells, the erythrocyte membrane becomes susceptible to oxidative injury by reactive oxygen species. The protection of the erythrocyte membrane from such an oxidative injury would prevent the membranes from becoming leaky to calcium ions thus inhibiting the activation of the calcium activated potassium efflux channels and the formation of dense cells (Tsuyoshi and Tomoko, 2001).

Presently, there was no significant variation in the hematocrit in any of the groups treated with hydergine. This corresponds to the finding that EDTA had no effect on the packed cell volume and the white blood cell count and differential counts produced with EDTA were similar to those of non- anticoagulated blood smear (Hanley *et al.*, 2004). In a study where bleeding was induced in a group of rabbits, heparin and a human antibody for factor IX caused a reduction in hematocrit, but it was explained due to the substantial bleeding and not the anticoagulant exposure because less bleeding did not induce change in hematocrit (Canio *et al.*, 2002).

It has been suggested that prothrombin time of patients taking warfarin should be monitored more frequently. Patients with other risk factors complicated by hemorrhage should undergo periodic examinations including determination of hematocrit and hemoglobin levels for bleeding (Emile, 1999). Acute and chronic doses of aspirin, 150mg/kg for 25 weeks and 600mg/kg for 7 days reduced RBC count, hemoglobin and other cell indices while leukocyte counts were increased in treated mice. Both doses suppressed the rate of erythropoiesis in bone marrow whereas the rate of leucopoiesis appeared to increase in the treated animals. Aspirin in both acute and chronic doses induce anemia associated with leucocytosis in mice; the anemia does not seem to be induced due to alterations in iron metabolism (Merchant and Modi, 2004). This observation does not show an agreement with the hydergine treated rats since no such changes were observed in these blood parameters which may either be due to very low concentrations of the drug or very short term exposure. High doses of hydergine and longer exposure will clear the conflict.

However, the results of a meta-analysis indicated that a three month course of low molecular weight heparin is as effective and safe as a corresponding period of oral anticoagulant treatments and may thus be considered as valuable alternative option for patients in whom oral anticoagulant treatment appears contradicted or problematic (Lorio *et al.*, 2003).

The removal of hydergine from blood begins after 1.5 hours that lasts for about 15 hours (Eckert *et al.*, 1978). In a study it was proved that in the hospitalized patients with acute coronary artery disease, the ideal anticoagulant should be delivered by intravenous or subcutaneous injections and dosed easily so that it does not require frequent monitoring and can be reversed immediately and predictably. Rapid reversal of drug activity can be achieved by formulation of a drug as an infusible agent with a short half-life, or by administration of a second agent, an antidote that can neutralize the activity of a drug (Christopher *et al.*, 2004). One of the thrombolytic agents, used in routine is recombinant hirudin that has a terminal half-life of 2 to 3 hours. The APTT correlates well with plasma levels of hirudin and allows close titration over a wide range of anticoagulation; while thrombin time is relatively insensitive for monitoring hirudin administration. At anticoagulant levels effective in experimental thrombosis, a 6-hour infusion of hirudin is well tolerated and safe in a predominantly male group of patients with stable coronary atherosclerosis (Zoldhelyi *et al.*, 1993).

Hydergine has been prescribed for the treatment of dementia. Primary mechanism of action of the medicines that treat certain forms of dementia include modifications in neurotransmitter synthesis, inhibition of neurotransmitter uptake and enzyme induced neurotransmitter break down, anticoagulant and anti-platelet activity, enhanced blood flow and glucose metabolism (Diamond *et al.*, 2003; Olin *et al.*, 1999).

In traumatic brain injury, patients show hypercoagulation even when they were treated with anti-thrombin and clotting onset time of the patients were monitored for three months. The results indicated that clotting onset time may be clinically relevant variable with prognostic value able to monitor the degree of hypercoagulation over time (Ungersteadt *et al.*, 2003). The success of thrombolytic therapy for acute stroke has demonstrated that neurological outcome can be improved with timely treatment. Antithrombin and antiplatelet agents represent promising therapeutic approaches for stroke management. Antiplatelet therapy has modestly improved out come in both acute stroke and in secondary stoke prevention, although bleeding or other adverse events associated with antithrombin therapy have largely negated their potential benefit The

drugs that are to be used should also allow the maintenance of cerebral blood flow, blood brain barrier integration and neuronal function (Martin, 2000). The optimal drug for the prevention of venous thrombosis is one that is efficacious with minimal bleeding and easy to administer (Ray, 2003). Despite the advances with low molecular weight heparin, however, more potent anticoagulants are still required. Likewise there is also a need for safer oral anticoagulants that do not require routine coagulation monitoring (Shannon and Jeffery, 2005b).

CONCLUSION

The present study was carried out to investigate the anticoagulatory effects of hydergine. The results of *in vivo* experiments showed that short-term exposure of acute doses of hydergine caused a significant increase in the clotting time and bleeding time while plasma calcium levels were reduced. However, there occurred no significant change in PT, INR and APTT. Blood cell counts including platelet count, PDW, MPV, PLCR, RBC, RDW, hemoglobin, hematocrit, MCH, MCHC, TLC, and lymphocyte number did not demonstrate any significant change in response to hydergine treatment. But lymphocyte percentage elevated significantly in the animal groups exposed to high doses of hydergine.

Ex vivo experiments showed that the blood samples treated with hydergine had prolonged clotting time and APTT while plasma calcium levels, PT and INR were not changed. Thus the study concludes that short-term hydergine treatment caused an increase in bleeding efficiency. Further studies are however, required to evaluate the effect of long-term exposure of hydergine on the haemostatic system.

REFERENCES

REFERENCES

Adcock DM, Kressin DC, Marlar RA (1997). Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. American Journal of Clinical Pathology; 107(1): 105-10

Aida JA, Peter JK, Axel RW, Paul FV, Jan JJ, Jaap WD (2004). Risk of stroke during long-term anticoagulant therapy in patients after myocardial infarction. Annals of Neurology; 39(3): 301-7

Amenta D, Ferrante F, Franch F. (1988). Effects of long-term Hydergine administration on lipofuscin accumulation in senescent rat brain. Gerontology; 34(56): 250-6

Andreas FH, Mimoun A, Nicole D, Patrick A, Anne DZ (2000). Complete and long-term rescue of leisoned adult motoneurons by lentiviral-mediated expression of glial cell line-derived neurotrophic factor in the facial nucleus. The Journal of Neuroscience; 209(150): 5587-93

Andreas K (1993). Efficacy of cerebroprotective substances in the management of functional disorders induced by the cytotoxic brain edema-producing substance hexachlorophene. Nauyn-Schiedeberg's Archives of Pharmacology; 347: 79-83

Bagger JP, Helligose P, Randsback F, Henrik H, Skiv J (1997). Effect of Varapamil in intermittent claudication. Circulation; 95: 411-14

Baldit SC, Calpan H, Drouet L (2003). No alteration in platelet function or coagulation induced by EGb in a controlled study. Clinical Lab Hematology; 25: 251-3

Bancroft AJ, Abel EW, Mcleran M, Belch JJ (2000). Mean platelet volume is a useful parameter: a reproducible routine method using a modified Coulter thrombocytometer. Platelets; 11(7): 379-87

Barry HP, Alan JD, Yi Z, Stephen JP, Charles L, Nikolaus S, Alison B, Adriana D, Eric L, James M (2003). Cell specific mitotic defect and dyserythropoiesis associated with erythroid band 3 deficiency. Nature Genetics; 34: 59-64

Bechter, R and Schön, H (1988). Use of the whole-embryo culture system in drug safety assessment. Toxicololgy *in vitro*; 2(3): 195-203

Bell DN, Spain S, Goldsmith HL (1990). Extracellular free Ca^{++} accounts for the sex difference in the aggregation of human platelet in citrated platelet rich plasma. Thrombosis Research; 1(58): 47-60

Ben AR, Sheinman G, yedgar S, Elder A, Roth A, Berliver AS, Barshtein G (2002). Thrombolytic therapy reduces red blood cell aggregation in plasma without affecting intrinsic aggregability. Thrombosis Research; 105(6): 487-92

Berde, B, Stürmer, E (1978). Introduction to the pharmacology of ergot alkaloids and related compounds as a basis of their therapeutic action. Handbook of Experimental Pharmacology: Ergot Alkaloids and Related Compounds; 44: Berlin, Springer-Verlag: 1-29

Berga P (1986). Synergistic interactions between piracetam and hydergine in some animal models of cerebral hypoxia and ischaemia. Drug Research; 36: 1314-20

Bernard W S, Niou-Ching W, Clarence W (2001). White blood cell and platelet counting performance by hematology analyzers: A critical evaluation." Lab Hematology; 7: 255-66

Bertoni F C, (1994). Morphological alterations of synaptic mitochondria during aging. Annals of New York Academy of Sciences; 717: 137-49

Beutler E and Williams WJ (2001). Williams hematology. 6thed. New York: McGraw-Hill health professions division; 1941

Branconnier R (1983). The efficacy of the cerebral metabolic enhancers in the treatment of senile dementia. Psychopharmacology Bulletin; 19(2): 212-20

Buyukozturk A, Kanit L, Ersoz B, Mentes G, Hariri NI (1995). The effects of hydergine on the MAO activity of the aged and adult rat brain. European Neuropsychopharmacology; 5(4): 527-9

Cahn J, Borzeix M (1983). Cerbral blood flow and metabolism and neurological deficits in an experimental infarction. Application to the study of an ergot derivative. Press Medicine; 29:12(48): 3058-60

Canio JR, Surinder J, Leo D, Stuart B, Daniel K (2002). A human antibody that inhibits factor IX/IXa function potently inhibits arterial thrombosis without increasing bleeding. Arteriosclerosis, Thrombosis and Vascular Biology; 22: 517

Cedazo-Minguez A, Bonecchi L, Winblad B, Post C, Wong EH, Cowburn RF, Benatti L. (1999). Nicergoline stimulates protein kinase C mediated alpha-secretase processing of the amyloid precursor protein in cultured human neuroblastoma SH-SY5Y cells. Neurochemistry International; 35(4): 307-15

Cezary W, Jaeck G, Preemyslaw K (2003). Multivariant relationships between international normalized ratio and vitamin K dependent coagulation derived parameters in normal healthy donors and oral anticoagulant therapy patients. Thrombosis Journal; 1: 7

Chen CC, You JY, Ho LH (2003). The APTT assay as a monitor pf heparin anticoagulation efficacy in clinical settings. Advances in Therapy; 20(5): 231-6

Christopher P. Joseph D, george A, Shahid M, Rabekah R, george Q, Elizabeth S, William P, Bruce A (2004). Antidote-medicated control of an anticoagulant aptamer *in vivo*. Nature Biotechnology; 22: 1423-28

Chung KJ, Dent G, McCusker M, (1987). Effect of ginkolide mixture (BN 52063) in antagonizing skin and platelet responses to platelet activating factor in man. Lancet; 1: 248-51

Clark J, Bucher T, Waite R (1985). Analysis of the cardiovascular effects of co-derocrine (Hydergine). Journal of Pharmacology; 16 (3): 101-11

Colman, RW (2001). Hemostasis and thrombosis: Basic principles and clinical practice. 4th ed., Philadelphia: Lippincott Williams & Wilkins; (19): 1578

Copeland RL (1981). Behavioral and neurochemical effects of hydergine in rats. Archives of Iternational Pharmacodynamics; 252: 113-23

Cover C, Poulin J, Gustafson M, Wyant T, Gamble D, Kay M (1996). Posttranslational changes in band 3 in adult and aging brain following treatment with ergoloid mesylates, comparison to changes in Alzheimer's disease. Life Sciences; 58 (8): 655-64

Cristina L, Luigi P, Gualtiero P, Barbara L, Edoardo R, Sergio C (2002). Reduced inhibition of activated prothrombin by heparin and venousthromboembolism: heparin resistance revisited. Thrombosis; 87: 182-88

Cucinotta D, DeLeo D, frattola L, Trabucchi M, parnetti L (1996). "Dihydroergokryptine vs. placebo in dementia of Alzheimer's type: Interim study after a 1 year follow up. Archives of Gerontology and Geriatrics; 22: 169-180

Dahlback B (2000). Blood coagulation. Lancet; 355: 1627-32

David G, Pancal JG (2003). Platelets and cardiovascular diseases. Circulation; 108(13): 88

Dean W (1998). Hydergine, potential anti-aging drug, still highly recommended. Anti-aging Bulletin, International Anti-aging System; 3: 4

Diamond B, Johnson SK, Torsney K, Morodan J, Prokop BJ, Davidek D, Kraner P (2003). Complementary and alternative medicines in the treatment of dementia: An evidence based review. Drugs and Aging; 20: 981-98

Dominiak P, Grevel J, Abisch E, Grobecker H, Dennler J, Welzel D (1988). Absolute systemic availability of a new oral formulation of co-dergocrine in healthy subjects. European Journal of Clinical Pharmacology; 35(1): 53-7

Dominiak P, Weidinger G (1991). Therapy comparison between the combination hydergine/nifodiplin and nifodiplin alone in patients with isolated systolic hypertension. Med klin (Munich); 86(1): 15-9

Donma MM (1998). Clinical efficacy of piracetam in treatment of breath-holding spells. Pediatric Neurology; 18(1): 41-5

Ducan SG, Meyers KM, Reed SM (1985). Reduction of red blood cell mass of horse: toxic effects of heparin anticoagulant therapy. American Journal of Veterinary Research; 44(2): 2271-6

Eckert, H, Kiechel, J, Rosenthaler, J, Schmidt R, Schreier, E (1978). Biopharmaceutical aspects. Handbook of experimental pharmacology: Ergot Alkaloids and Related Compounds. Berlin. Springer-Verlag; (44): 719-803

Elsener M, Ferrafiat M, de-Bruyn M, Jouhet P, Robert Y, Rouge P E (1988). Inhibition of post-partum physiological milk secretion by caffeine dihydroergocryptine and dihydroergotoxine. Review of French Gynecology and Obstetrics; 83(11): 697-701

Elwon O, Helmy A, tamawy M, Naseer M, Banhawy I, kader A, Elwan F (1995). "Hydergine in strokes. Journal International of Medicine Research; 23 (3): 154-66

Emile RM (1999). Current treatment options in cardiovascular medicine. Peripheral Arterial Diseases; 1: 27-34

Emmenegger, H, Meier-Ruge W (1968). The Actions of Hydergine on the Brain. Pharmacology; 1: 65-78

Erdincler D, karakic Y, Toplan S, Ocean S (1996). The effect of ginkgo biloba glycoside on the blood viscosity and erythrocyte deformability. Clinical Hemorheology; 16(3): 271-76

Eriksson P (1998). Neurogenesis in the adult hippocampus. Nature Medicine; 4(11): 1313-17

Franchini M, veneri D (2005). Heparin induced thrombocytopenia. Recent Program in Medicine; 96(9): 433-5

Fumio Gotoh, Hideo Tohgi, Shunsaku Hirai, Akiro Terashi, Yasuo Fukuuchi, Eiichi Otomo, Yukito Shinohara, Eiichi Itoh, Tamotsu Matsuda, Tohru Sawada\ (2000). Cilostazol stroke prevention study: A placebo-controlled double-blind trial for secondary prevention of cerebral infarction. Journal of Stroke and Cerebrovascular Diseases; 9(4): 147-57

Galeotti N, Ghelardini C, Bartolini A. (1996). Piracetam and aniracetam antagonism of centrally active drug-induced antinociception." Pharmacol biochemistry and behavior; 53(4): 943-50

Gennaro, A.R, (1995). Remington: The Science and Practice of Pharmacy. 19th ed., Easton, PA,Mack Publishing Co;

Gerd K, George K, Fred H (1997). More hippocampal neurons in adult mice living in an enriched environment. Gage in Nature; 386: 493-95

Gilaad GK, Braden M, Kevin M (2005). Heparin induced thrombocytopenia secondary to intraperitoneal heparin exposure. Nephrology Dialysis Transplantation; 20(11): 2561-62

Goldstein J (1996). CFIDS treatment protocol: Betrayal by the brain. 1st ed., Haworth Medical Press; 800-3

Greisen G, Andreasen RB (2003). Recombinant factor VIIa in preterm neonates with prolonged prothrombin time. Blood Coagulation and Fibrinolysis; 14(1): 117-20

Gruenward J, Brendler T, Jaenicke C (1998). Physicians Desk Reference for Herbal Medicine. 1st ed. Medical Economics Company, Inc.

Gunnar H, Ingela B (2004). Occurrence and quality of anticoagulant treatment of chronic atrial fibrillation in primary health care in Sweden; a retrospective study on electronic patient record. BMC Clinical Pharmacology; 4:1

Guyton, A. C and Hall, J. B (2006). Text book of medical physiology. 11thed. W. B. Saunders Co.

Hampton K, Preston F (1997). ABC of clinical hematology: Bleeding disorders, thrombosis, and anticoagulation. British Medical Journal; 314: 10-26

Hana P, Jan C, Michael H, Bohumil K, Ivana C, Alexandr J, Ladislav C (2002). Structure types of dihydroergotoxine mesylates. Collection of Czechoslovak Chemical Communication; 65 (8): 1329-38

Hanley CS, Hernandez DS, Bush S, Latimar KS (2004). Comparison of the effect of dipotassium ethylene diamine tetraacetic acid and lithium heparin on hematological values in the green iguana (*Iguana iguana*). Journal of Zoology and Wild Medicine; 35(3): 28-32

Heisler K, Cowley A, Kishi T, Tecott H, Fan W, Low J, Smart L, Rubinstein M;,Tatro B, Zigman M, ConeD, Elmquist K (2003). Central serotonin and melanocortin pathways regulating energy homeostasis. Annals of New York Academy of Sciences; 994: 169-74

Herrmann WM, Coper H (1987). Are nootropics a separate class of drugs? A differentiation in various models. Methods of Experimental Clinical Pharmacology; 9(3): 173-82

Himber J, Kirchhofer D, Riederer M, Tschopp T, Steiner B, Roux SP (1997). Dissociation of antithrombotic effect and bleeding time prolongation in rabbit by inhibiting tissue factor function. Thrombosis and Hemostasis; 78(3): 1142-49

Hirsh J, Dalen JE, Deykin D, Poller L (1992). Oral anticoagulants: mechanism of action, clinical effectiveness and optimal therapeutic range. Chest; 3102:28

Horowiz B, prince A M, Hammon J (1994). Viral safety of solvents/detergent-treated blood products. Blood Coagulation and Fibrinolysis; 5: 821-8

Huber, F., Köberle, S., Prestele, H., Spiegel, R (1986). Effects of long-term ergoloid mesylates (Hydergine) administration in healthy pensioners: 5-year results. Current Medical Research Opinion; 10(4): 256-79

Hughes JR (1976). An Ergot Alkaloid Preparation (Hydergine) in the Treatment of Dementia: A Critical Review of the Clinical Literature. Journal of the American Geriatrics Society; 24: 490-97

International Committee for the standardization of the hematology and International committee on thrombosis and hemostasis (1985). American Journal of Clinical Pathology; 88:779

Inzitari D, Erkinjuntti T, Wallin A, Ser T D, Romanelli M, Pantoni L (2000). Subcortical vascular dementia as a specific target for clinical trials. Annals of the New York Academy of Sciences; 903: 510-521

Iwatsuki K, Iijima F, Chiba S. (1983). Effects of hydergine on pancreatic exocrine secretion in the isolated, blood-perfused pancreas of the dog. Archives of International Pharmacodynamics Therapy; 261(2): 328-34

James F, Niegel L, Charles WB (2003). Clavicipitalean fungi. 1st Ed; Mercel dekker: 10

Jamieson DD, Taylor KM (1981). Effects of centrophenoxine, piracetam and hydergine on rat brain lipid peroxidation. Experientia; 37(8): 879-81

Jan C, Bohumil K, Alexander J, Ladislav C (2000). Crystal structures of dihydro-α-ergokryptine and dihydro-β-ergokryptine mesylates. Collection of Czechoslovak Chemical Communications; 65(8): 1329-38

Jean PC, Alain G, Thomas B, Tschopp, Sèbasstien R (1995). Activated clotting tie as an appropriate test to compare heparin and direct thrombin inhibitors such as hirudin or Ro 46-6240 in experimental arterial thrombosis. Circulation; 91: 1568-74

Kandice KM, George C (2002). The laboratory diagnosis of platelet disorders: An algorithmic approach. Archives of Pathology and Laboratory Medicine; 126(2): 133-46

Kenneth GM (2003). Thrombin formation. Chest; 124: 4S-10S

Kessler J., Thiel A., Karbe H., Heiss W. D (2000). Piracetam improves activated blood flow and facilitates rehabilitation of poststroke aphasic patients. Stroke; 31(9): 2112-6

Kirchner E (1989) "Stabilization of the cardiovascular system in organ donors." Anesthesiology ranimation; 4(2): 83-8

Kohlar S, Funk P, Kieser M (2004). Influence of a 7 day treatment with ginko biloba special extract EGs 761 on bleeding time and coagulation: a randomized placebo-controlled double blind study in healthy volunteers. Blood Coagulation and Fibrinolysis; 15: 33-9

Koopman M, Prandoni P, Piovella F, Ockelford P, Brandjes D, Vander M, gallus A, Simonneau G, Chesterman C, Prins M, Bossuyt (1996). Treatment of venous thrombosis with intravenous unfractionated heparin administered in the hospital as compared with subcutaneous low molecular weight heparin administration at home. New English Journal of Medicine; 334: 682-7

Kudolo GB, Blodgelt J (2000). Effect of ingestion of *Ginko biloba* extract on platelet aggregation and urinary prostanoid excretion I healthy and type-2 diabetic subjects. Thrombosis research; 108: 151-60

Kumar V, Singh PN, Muruganandam AV, Bhattacharya SK (2000). Effect of Indian hypericum perforatum linn on animal models of cognitive dysfunction. Journal of Ethno Pharmacology; 72(1-2): 119-28

Kwok L, Molckovsky D, Boucher M (2005). Ximelagatran; a new type of oral anticoagulant. International Journal of Technical Assessment and Health Care; 21(4): 480-6

Lorio A, Guercini F, Pini M (2003). Low molecular weight heparin for the long-term treatment of symptomatic venous thromboembolism: A meta-analysis of the randomized comparisons with oral anticoagulants. Journal of Thrombosis and Hemostasis; 1(9): 1906

Markwardt F, Nowak G, Steurzebecher J, Griessbach U, Walsmann P, Voger G (1984). Pharmacokinetics and anticoagulant effect of hirudin in man. Thrombosis and Hemostasis; 52(2): 160-3

Martin MB (2000). Stroke: Antithrombin versus antiplatelet therapy. Journal of Expert Opinion on Investigational Drugs; 9(2): 355-69

Martinez M, Vaya A, lapez C, CouSAngrona E, Mira Y, Azhar J (2000). High and low molecular weight haparins do not modify red blood cell aggregability *in vitro*. Clinical Hemorheology and Micro Circulation; 23(1): 67-70

Masayoshi S, Ram K, Arun A, Jose AF, Florence H, John H, Berislav VZ (2001). Annti-inflammatory, anti-thrombotic and neuroprotective effects of activated protein C in a murine model of focal ischemic stroke. Circulation; 103: 1799

Matter B (1976). Failure to detect chromosome damage in bone-marrow cells of mice and Chinese hamsters exposed in vivo to some ergot derivatives. Journal of International Medicine Research; 4: 382-392

Matyushichev V.B, Shamratova V.G.· Savrasova I.V (2000). Relationship between erythrocyte count and volume in humans and rats. Kluwer Academic Publishers; Bulletin of Experimental Biology and Medicine; 130 (9) : 840-842

McEvoy, G.K (1997). AHFS Drug Information. American Society of Hospital Pharmacists, Inc; 1002-3

Melek BK, Rosalinda BW, Herbert JM, Oguz KB (2003). The effects of nitric oxide on red blood cell deformability. American Journal of Hearing and Circulation Physiology; 10: 1152

Merchant MA, Modi DN (2004). Acute and chronic effect of aspirin on hematological parameters and hepatic ferritin expression in mice. Indian Journal of Pharmacology; 36(4): 226-30

Mike G (2005). Pharmacogenetics in the management of coumarin anticoagulant therapy: The way forward or an expensive diversion? PLOS Medicine; 2(10): 342

Miyanaga K (2005). Treatment of dementia. Nippon Ronen Igakkai Zasshi; 42(1): 49-51

Mizuno T, Tsukiya T, Taenaka Y, Tassumi E, Nishinaka T, Ohnishi H, Oshikawa M, Sato K, Shioya K, Takeiua Y, Takano H (2002). Ultra structural alterations in red blood cell membranes exposed to shear stress. Asaio Journal; 48(6): 668-70

Mondari C, Ducret T, Hausler A (1992). Elevated corticosteroid levels block the memory improving effects of nootropics and cholinomimetics. Psychopharmacology (Berl.); 108 (1-2): 11-5

Muller WE, Eckert GP, Eckert A (1999). Piracetam: Novelty in a unique mode of action. Pharmacopsychiatry; 32 (1): 02-9

Nandy, K., Schneider, F.H. (1978). Effects of dihydroergotoxine mesylate on aging neurons *in vitro*. Gerontology; 24: 66-70

Nikolaeva LS, Chirkov VV, Dobrynina NA, Lyapina LA, Pastorova VE (2005). "Investigations of the anticoagulant activity of several low molecular weight endogenous ligands of blood plasma by methods of computer simulation and universal biological assay. Pharmaceutical Chemistry; 39(2): 57-63

Olin J, Schneider L, Novit A, Luczak S (1999). Review: hydergine leads to greater global improvements on comprehensive rating scales in dementia. Evidence Based Mental Health; 2: 15

Onomura M, Tsukada H, Fukuda K, Kudama M, Nakamura H, Hosokawa M, Ohya M, Seino Y (2000). Effect of argatroban on trinitrobenzene sulfonic acid induced colitis. Journal of Gastroenterology and Hepatology; 15(8): 931-38

Oostenburg GS, Mensink RP, Hardeman T, Hornstra G (1997). Exercisese performance, red blood cell deformability and lipid peroxidation effects of fish oil and vitamin E. Journal of Applied Physiology; 83(3): 746-52

Pearson, D, Shaw S (1982). Life Extension: A Practical Scientific Approach. New York: Warner Books

Pham A, Faulhaber D, Misselwit F, Naumann E, Norden C, Martin G, Suessmilch R (1988). Alpha 2 adrenoceptors of blood platelets from hypertensive and normotensive rhesus monkeys. Folia Haematol Int Mag Klin Morphol Blutforsch.; 115(4): 465-8

Priti K and Vijaya S (2000). Ethyleneglycol-Bis-(β-amino ethyleneether) tetracetate as a blood anticoagulant: Preservation of antigen presenting cell function and antigen specific proliferative response of peripheral blood mononuclear cells from stored blood. Clinical and Diagnostic Lab Immunology; 7(4): 578-83

Rachid B, Liand G, Kandice KM, Gordan S, Bridget M, Erin Y, Mary A, Beth F, Rony A, Steven A, Jerome Z, Mohammad AH (2005). The role of aspirin in the prevention of thrombotic complications of thalidomide and anthracyclin based chemotherapy for multiple myeloma. Mayo Clinical Procedure; 80: 1568-74

Ramstrom S (2005). Clotting time analysis of citrated blood samples is strongly affected by the tube used for blood sampling. Blood Coagulation and Fibrinolysis; 16(6): 447-52

Rao, DB, Norris JR (1971). A double-blind investigation of hydergine in the treatment of cerebrovascular insufficiency in the elderly. Johns Hopkins Medical Journal; 130: 317-23

Ray TG (2003). Dyslipidemia, statin and thromboembolism a potential risk factor and a potential treatment. Current Opinion in Pulmonary Medicine; 9(5): 378-84

Roberts GT, Rand M J (1977a). Effects of some ergot derivatives in bone marrow of mice. Mutation Research; 56: 59-68

Roberts, GT, Rand, M.J (1977b). Chromosomal damage induced by some ergot derivatives. Mutation *in vitro* Research; 48: 205-214

Roberts, G.T, Rand, M.J. (1978). The dominant lethal effect of some ergot alkaloids. Mutation Research; 50: 317-325

Rodak B F (2002). Hematology: Clinical principles and applications. 2nd edition. W.B Saunders Company.

Ross I B, Alison S, Kerry T (2002). Hematology. Medical Journal of America; 176(1): 19

Saletu B, Grunberger J, Anderer R (1990). On brain protection of co-dergocrine mesylate (Hydergine) against hypoxic hypoxidosis of different severity: double-blind placebo controlled quantitative EEG and psychometric studies. International Journal of Clinical Pharmacology and Therapeutic Toxicology; 28(12): 510-24

Sanjay KB, Subir KM (2002). Effects of garlic on cardiovascular disorders: A review. Nutrition Journal; 1: 4

Sarah LA, Machin SJ (2004). Haemostasis. Surgery; 22(8): 200a-e

Schardein, J.L (1976). Drugs as Teratogens, Boca Raton, FL, CRC Press: 261

Schmidt LM (2004). Herbal remedies; the other drugs your patient take. Home Health Care Nurse; 22(3): 169-75

Schran, H.F, McDonald, S, Lehr R (1988). Pharmacokinetics and bioavailability of ergoloid mesylates. Biopharm. Drug Symposium; 9: 349-61

Shannon MB, Jeffery IW (2005a). Coagulation assays. Circulation; 112: 53-60

Shannon MB, Jeffery IW (2005b). New anticoagulants: beyond heparin, low molecular weight heparin and warfarin. British Journal of Pharmacology; 144: 1017-28

Sherkey J (1996). Treatment protocol. Betrayal by the brain. The Howarth medical press; 1: 800-3

Shneider L and Tariot P (1997). Cognition enhancer for Alzheimer's disease. Liberman Journal Eds Saunders: 1685-1702

Siegl H (1985). Renal and antihypertensive effects of co-dergocrine (hydergine) in rats. Journal of Pharmacology; 16(3): 113-27

Sinzinger H (1985). Double blind study of the influence of co-dergocrine on platelet parameters in healthy volunteers. European Journal of Clinical Pharmacology; 28(6): 713-6

Slagle, Mark A. (2001). Ergoloid mesylates (Hydergine). Southern Medical Journal; 94(7): 682-83

Spiegel R, Huber F,Koberle S (1983). A controlled long term study with ergot mesylates (hydergine) in healthy, elderly volunteers; result after three years. Journal of American Geriatric Society; 31(9): 544-55

Steurer G, Fitscha P, Ettl K, Sinzinger H (1989). Effects of hydergine on platelet deposition on active human carotid artery lesions and platelet function. Thrombsis Research; 155(5): 577-89

Swan SK, St Peter J, Lambrecht L, Hursting MJ (2000). Comparison of anticoagulant effects and safety of argatroban and heparin in healthy subjects. Pharmacotherapy; 20(7): 756-70

Tamura T, Satoh T, Minakami H, Tamada T (1989). Effect of hydergine in hyperprolactnimia. Journal of Endocrinology and Metabolism; 69: 470-4

Tareen, KI, Bashir, A, Saeed, K, Hussain T (1988). Clinical efficacy of codergocrine mesylate in children with learning difficulties. Journal of International Medicine Research; 16(3): 204-9

The American heritage dictionary of English language (2000). 4th edition, Houghton Mifflin Company

Tripodi A, Poller L, Van de besselaa A, Mannucci A (1995). "Proposed scheme for calibration of international reference preparations of thromboplastin for the prothrombin time. Thrombosis and Hemostasis; 75:1368

Tsolaki M, Pantazi T, kezis A (2001). Efficacy of acetylcholinestrase inhibitor, versus nootropics in Alzheimer's disease: A retrospective, longitudinal study. Journal of International Medicine Research; 29(1): 28-36

Tsuyoshi O, Tomoko O (2001). *In vitro* effects of aged garlic extract and other nutritional supplements on sickle erythrocytes. Journal of Nutrition; 131: 1085-92

Ungerstedt JS, Grenander A, Bredbacka S, Blomback M (2003). Clotting onset time may be a predictor of outcome in human brain injury: A pilot study. Journal of Neurosurgical Anesthesiology; 15(1): 13-18

Van walraven C, Hart RG, Singer DE, Laupacis A, Cannokys P, Koudstall P, Chang Y, hellemons B (2002). Oral anticoagulants vs aspirin in cardiovascular atrial fibrillation: An individual patient meta analysis. Journal of American Medical Association; 288(19): 2441-8

Wadworth, AN, Chrisp, P (1992). Co-dergocrine mesylate: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in age-related cognitive decline. Drug Aging; *2*(3): 153-73

Ward D (2002). Hydergine - Update - Still Highly Recommended. Imm Inst Org Nootropic Research; 19: 20

WHO expert committee on biological standardization (1983). 33rd report Technical report series 687, WHO Geneva

Wilhelmi M, Frankle U, Cohnert T, Weber P, Kaukemuller J, Fishcher S, Wahlers T, haverica A (2001). Coronary artery bypass grafting surgery without the routine

application of blood products: is it feasible? European Journal of Cardiothoracic Surgery; 19(5): 657-61

Yoshikawa M (1983). A dose response study with dihydroergotoxine mesylate in cerebrovascular disturbances. Journal of the American Geriatric Society; 31: 1 1-7

Zoldhelyi P, Webster MW, Fuster V, Grilt DE, Gasper D, Edward SJ, Cabot CF, Chesbro (1993). Recombinant hirudin in patients with chronic stable coronary artery disease. Safety, half life and effect on coagulation parameters." Circulation; 88: 2015-22