Investigating the Effect of PTP1B Inhibitor on Leptin Signaling Pathway in High-Fat-Diet and Low Dose Streptozotocin-Induced Type II Diabetic Mice Model

by

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“Dedicated to My Loving Parents and Brothers”

DECLARATION

I hereby declare that the work presented in this thesis is my own effort and hard work; it is written and composed by me. No part of this thesis has been previously published or presented for any other degree or certificate.

Muhammad Tahir Ullah

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All praises and glories to Almighty "**ALLAH**" who says in the Holy Quran, "And your Lord is the most gracious who taught by the pen, taught man (those things) which He did not know". Countless Darood on Prophet **HAZRAT MUHAMMAD (Peace Be Upon Him),** who showed the path of knowledge to mankind and gave the lesson of seeking knowledge from cradle to grave.

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Muhammad Tahir Ullah

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List of Abbreviation

WAT	White adipose tissue
BAT	Brown adipose tissue
WHO	World Health Organization
BMI	Body mass index
TIDM	Type I Diabetes Mellitus
T2DM	Type II Diabetes Mellitus
CVD	Cardiovascular Diseases
NAFLD	Non-Alcoholic Fatty liver Diseases
JAK2	Janus Activated Kinase 2
STAT3	Signal Transducer and Activator of Transcription 3
NPY	Neuro Peptide Y
AgRP	Agouti-related Protein
POMC	Proopiomelanocortin
INSR	Insulin receptor
IR	Insulin resistance
PI3K	Phosphatidylinositol-3-Kinase
PIP2	Phosphatidylinositol 4,5-biphosphate
PIP3	phosphatidylinositol 3,4,5-triphosphate
AKT/PKB	Protein Kinase B
Mtorc	Mammalian Target of Rapamycin
FOXO1	Forkhead Box Protein O1
ATGL	Adipose Triglyceride Lipase
HSL	Hormone Sensitive Lipase
MGL	Monoacylglycerol Lipase

2-AG	2-Arachidonoyl glycerol
CBR	cannabinoid receptors
ARC	Arcuate Nuclei
TFs	Transcription Factors
CCD	Coiled-Coil Domain
ND	N-terminal Dimerization Domain
LD	Linker Domain
TAD	C-Terminal Transcriptional Activation Domain
DBD	DNA-Binding Domain
ER	Endoplasmic Reticulum
UPR	Unfolded Protein Response
PTP1B	Protein Tyrosine Phosphatase 1B
SOCS3	Suppressor of Cytokine Signaling 3
IRS1	insulin receptor substrate 1
IRS2	insulin receptor substrate 2
HFD	High Fat Diet
NF-KB	Nuclear Factor Kappa B
LIF	Leukemia inhibiting Factor
CNTF	Ciliary Neurotrophic Factor
GH	Growth Hormone
IL-6	Interleukin-6
TLR4	Toll like Receptor 4
VMH	Ventromedial Hypothalamus
DMH	Dorsomedial Hypothalamus
DM	Diabetes Mellitus

IDF	International Diabetes Federation
DKD	Diabetic Kidney Diseases
ROS	Reactive Oxygen Species
NO	Nitric oxide
FFAs	Free Fatty Acids
STZ	Streptozotocin
GLUT2	Glucose Transporter 2
IL-18	Interleukin 18
IL-2	Interleukin 2
TCA Cycle	Tricarboxylic acid cycle
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
BGL	Blood Glucose Level
PBS	Phosphate Buffer Saline
SH2	Src homology 2
BBB	Blood-Brain Barrier

Abstract

Obesity and diabetes are complex metabolic disorders characterized by elevated levels of fats and glucose, respectively. Currently, Protein Tyrosine Phosphatase 1B (PTP1B) is known for its negative regulation of insulin and leptin signaling. A low dose of streptozotocin (STZ) combined with a High Fat Diet (HFD) results in endoplasmic reticulum stress that results in the cleavage of PTP1B which increases the level of active PTP1B in the cytosol, causing the dephosphorylation of tyrosine-mediated receptors. Shred of evidence showed that PTP1B is overexpressed in both hypothalamus and adipocytes, disrupting the leptin signaling pathways in both organs. Hence, we predict that the inhibition of PTP1B might be a potential therapeutic target in the relieving of leptin resistance. Numerous studies have reported that Dodonaea Viscosa extract has hypoglycemic, anti-inflammatory, and anti-PTP1B properties. Compound 5, 7-dihydroxy-3, 6- dimethoxy-2- (4-methoxy-3- (3-methyl-2-enyl) phenyl)-4H -chromen-4- one extracted from the Dodonaea Viscosa was analyzed and showed anti-diabetic and anti-leptin resistant properties. We have divided our experimental animals into three groups named Group I, (Normal), Group II, (STZ-HFD induced diabetic group), and Group III, (STZ-HFD-compound treated group). Real-time PCR data showed that leptin lipolytic (PPARα, HSL, and MGL), and energy homeostasis markers (STAT3 and POMC) are found downregulated in both adipose and hypothalamus, respectively followed by their upregulation in the compound-treated group. We explored the downstream cascade of leptin signaling and its role in anorexic signaling in the hypothalamus and lipolysis in adipose tissue. Interestingly, we observed that both activities were dysregulated in the STZ-HFD model and were potentially reverted in the compound-treated group. Likewise, PTP1B and inflammatory markers (*IL*-6 and *IL*-1β) expression was significantly reduced in Group III. Collectively, the inhibitory effect of our compound helps to determine that, our compound not only inhibits the activity of PTP1B but also affects its regulated pathways same as our target leptin signaling pathway. Taken together, our compound ameliorates the diabetic condition by targeting insulin signaling mediators and our study demonstrates that by targeting PTP1B we might also encounter leptin signaling.

Key words: Leptin resistance, PTP1B, streptozotocin, HFD, Dodonaea Viscosa, Viscosol.

1. INTRODUCTION

1.1. Adipose

Adipose tissue, conversationally known as ‘‘fat’’ is an extensive heterogeneous and adaptable organ. While generally and historically viewed as a passive site for storage of energy, currently it is understood that adipose tissue controls and regulates various aspects of entire body physiology, which includes intake of food, insulin sensitivity, immune response, body temperature, maintenance of energy level. A pivotal property of adipose tissue is its high level of plasticity (A Sakers *et al*., 2022). To date numerous types of adipose tissues have been reported, specifically white adipose tissue (WAT), Brown adipose tissue (BAT) and Beige, that are present in different anatomical positions all through the body (A Chait and LJ Den Hartigh 2020).

Adipose tissue is composed of many diverse sorts of cells which functions coordinately and also secretes various types of hormones, cytokines and chemokines. Roughly 30% of the cells inside adipose tissue are adipocytes and the rest of the cells are stromal cells, immune cells, fibroblasts, macrophages, pre-adipocytes, and endothelial cells. (DE Chusyd *et al*., 2006). Warm blooded animals have two main sorts of adipose tissue; WAT and BAT. WAT have the largest proportion of total body adipose tissue and is present around major and important organs and blood vessels subcutaneously and abdominal cavity (ME Piche *et al*., 2020).

Across ageing adipose tissue quantity and dissemination also changes. Adipose tissue become dysfunctional with the increased number of inflammatory cytokines and also its concentration decreases when the anti-inflammatory cytokines amount decrease (E Zoico *et al*., 2019). The World Health Organization measures that about 1 billion individuals are overweight around the world, and 300 million individuals are obese (WHO). This information is alarming due to the fact that obesity can disrupt the function of different organ systems (YC Chooi *et al*., 2019), can also short life span of an individual and furthermore, epidemiology investigations have recorded high body mass index (BMI) as a risky aspect for different set of diseases which includes Corona, T2DM, cardiovascular diseases (CVD), Non-alcoholic fatty liver disease

(NAFLD) different type of cancers and chronic kidney disease (BM Popkin *et al*., 2020; Lauby-Secretan B *et al*., 2016). These all problem arise when there is disruption in adipose tissue function (Lauby-Secretan B *et al*., 2016). The escalating number of over wight and obesity is now a matter of concern, nearly one-third of the whole world population is now either have prey to overweightness and obesity (YC Chooi *et al*., 2019). Obesity severely affects the whole-body physiology and also contributes to develop different serious conditions in the body including diabetes (GM Singh *et al*., 2013). WHO has announced the obesity as a risk for human health. Obesity is not restricted to age it can occur to any person at any stage of life and at any age (YC Chooi *et al*., 2019).

Table 1.1 Characteristics of different adipose tissues.

	WAT	**BAT**	**Beige/brite**	**References**
Location	Visceral and subcutaneous	Interscapular, Suprarenal and neck region	Supraclavicular, cervical, within inguinal WAT	A Thirupathi *et al*. (2019); M Soundarrajan *et al* (2020)
Morphology	Large adipocyte	Small adipocyte	Small adipocyte	M Cedikova *et al*. (2016); A Thirupathi *et al*. (2019)
Lipid droplet	Large, single	Small, multiple	Multiple small lipid droplet	Q Zhu *et al.* (2019); MD Lynes *et al*. (2018)
Origin/development	Pdgfr-α progenitors	Myf5+ progenitors	----	Brown *et al.* (2014); Harms and Seale (2013)
Functions	Store energy	Heat production	Adaptive thermogenesis	M Cedikova *et al*. (2016); Harms and Seale (2013)
UCP-1	Almost undetectable	++	Upon stimulus++	Cedikova *et al.* (2016);

				Harms and Seale (2013)
Mitochondria	++	++	Upon stimulus++	Cedikova *et al*. (2016)
Adipocyte-Specific gene	MEST, GRAP2, TCF21, NANT, APOL7C, DAPL1, STAP1, RIP140, TLE3, Rb	DIO2, KNG2m, COX7A1, BMP7, CIDEA, CPT1B, ZIC1, SCL27A2, ELOVL3	Tbx1, Tmem26, CD137	M Hassan *et al*., (2012); C ME Rafols (2014); Harms and Seale (2013); CH Saely *et al*. (2012)
Vascularization	Low	Abundant	Cold stimulus tends to increase angiogenesis	M Hassan *et al*., (2012)
Obesity	Positive effect	Negative effect	Negative effect	Cedikova *et al*. (2016)
Activators	HFD	Thyroid hormone, cold, Bmp8b, Mmp7, natriuretic peptide	FGF21, irisin, catecholamines, thiazolidinediones	Harms and Seale (2013); Cedikova *et al*. (2016)
Iron content	Low	Abundant	Upon stimulation (Abundant)	Harms and Seale (2013)
Enriched markers	Ang, Resistin, LPL, G3PDH	UCP-1, Eva1, Pdk4, Ebf3, Hspb7	Tmem26, Tbx1, Shox2	Q Zhu *et al.* (2019); Cedikova *et al*. (2016); Harms and Seale (2013)

1.1.1. Leptin

Leptin is a 16 KDa hormone and was discovered in 1994 by Freidman *et al*. The word "Leptin" was originally coined from the Greek word 'leptos' which literally means 'thin' (AG Izquierdo *et al*., 2019). It is mainly produced and secreted by WAT while a minor concentration of leptin is also secreted by fibroblast (K Watanabe *et al*., 2019), mammary gland (K Rehman *et al*., 2018), placenta (M Schanton *et al*., 2018), skeletal muscle (M Schonke *et al*., 2018), ovary (SF de Medeiros and RJ Rodgers 2021), stomach (K Inagaki-Ohara *et al*., 2019), pituitary gland (GV Childs *et al*., 2021). It is encoded by Lep (Ob) gene which resides on chromosome 7q32.1 (Zhang *et al*., 1994; Li *et al*., 1999).

Leptin belongs to type I helical family of proteins which is linked to interleukins, growth and prolactin hormone. It consists of 4 antiparallel helices, forming a transmembrane structure consisting of 5-6 turns (F Zhang *et al*., 2005). Inactive and non-functional leptin protein has 167 amino acids sequence whereas only 146 amino acid sequence is present in functional and mature leptin protein (JB Funcke *et al*., 2014). Leptin executes its biological activities by binding to its receptor (Ob-Rb), encoded by LepR, which is located on Chr:1p31(M Wasim 2016). Leptin regulates and control body mass, food intake, reproductive functioning and plays an imperative role in angiogenesis, pro-inflammatory responses, fetal growth and lipolysis (M Obradovic *et al*., 2021). Leptin also controls metabolic activities by acting on peripheral tissues like adipose tissues (S Carter *et al*., 2013).

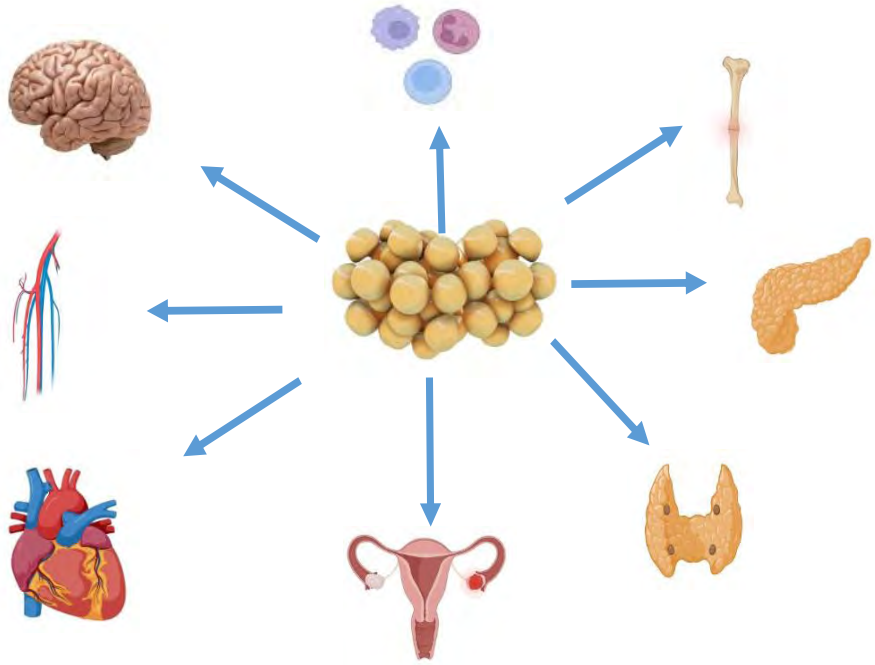

Figure 1.1 Localization of Leptin receptors and their functions.

1.1.2. Leptin Receptors

Leptin receptors have six isoform such as, Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re and Ob-Rf, these all are due to the result of alternate splicing (A Nunziata *et al*., 2019). These all are different from one another by only a single bond COOH terminus, except Ob-Rb which is long isoform of leptin receptor all the remaining isoforms are truncated (A Nunziata *et al*., 2019). Isoform Ob-Rb is majorly expressed in feeding center of hypothalamus, where it performs the function of controlling and regulating feeding behavior and metabolic rate (M Szyszka *et al*., 2007). This isoform of leptin receptors is also present in the peripheral tissues (T Trinh *et al*., 2021). Isoform Ob-Ra and Ob-Rb are present in all the tissues of mammals, but isoform Ob-Rb is very specific and only expresses in Hypothalamus and adipocytes (Pennington *et al*., 2022). Long isoform of leptin receptor Ob-Rb is the only receptor that can initiate the leptin signaling cascade, Ob-Rb activation leads to activation of Janus activated

kinase 2 (JAK2) which turns phosphorylate two tyrosine residues and signal transducer and activator of transcription 3 (STAT3) (H Liu *et al*., 2021).

1.1.3. Leptin and Energy Homeostasis

Leptin is reported to regulate food intake via its action on hypothalamus. Various studies of animal models have reported that hypothalamus is the key center for controlling and regulation of body weight and food intake (S Yang *et al.*, 2017). Leptin is secreted in the bloodstream by adipocytes, and to perform its action, leptin has to cross blood-brain barrier (BBB), acts on the hypothalamus and informs about body energy status (T Trinh *et al*., 2021). Soon after binding to the Ob-Rb, it then regulates the activity of a number of different hypothalamic neurons and expresses different orexigenic and anorexigenic neuropeptides. Leptin regulates different neuro peptides in the brain such as Neuro peptide Y (NPY), Agouti-related protein (AgRP), Proopiomelanocortin (POMC), orexin, galania, melanin-concentrating hormone (S Kumano *et al.,* 2003). It is reported that concentration of leptin hormone secreted by adipocytes have direct relation with mass of adipose tissue under physiological condition (S Carter *et al*., 2013).

Leptin is also involved in different processes such as increase glucose metabolism, suppressing insulin hyper exocytosis and also having role in fat metabolism (AA Ghadge *et al*., 2017). Different reports stated that leptin acts on *β*-cells and suppresses insulin secretion causing damaging and even destruction of the insulin receptor, consequently promoting the synthesis of glucose in the liver (D Kraus *et al.*, 2010; M Y Wang *et al*., 2010; WS Chen *et al*., 2009). Deficiency of leptin or leptin receptor prompts, insulin insensitivity, increases glucose concentration and leads to the development of obesity in ob/ob humans and mice (KD Niswender *et al*., 2007 and AG Diwan *et al*., 2018).

1.1.4. Leptin Signaling in Adipose

Conditions like overweightness and obesity are increasing day by day globally and their main source is fat depots in our body. Among the various health effects, this condition can increase the risk of other Diseases such as, CVD, hypertension, T2DM, fatty liver, dyslipidemia, obstructive sleep apnea, certain types of cancers, musculoskeletal disorders, many of these shorten lifespan (AG Izquierdo I 2019).

Leptin signaling starts when Ob-Rb gets activated by interacting with leptin molecule and down signaling cascade begins after that. The very first protein which is activated by Ob-Rb is JAK2 (BD Manning and A Toker 2017). JAK2 is family member of protein Tyrosine Kinases. The JAK family of protein tyrosine kinases is composed of four members (JAK1, JAK2, JAK3, and TYK2) that are specifically activated in response to different cytokines (JN Ihle 1995). JAK2 phosphorylates Insulin receptor substrate 1 and 2 (IRS1/2) and Phosphatidylinositol-3-Kinase (PI3K) is phosphorylated by IRS1/2 (H Liu *et al.,* 2021). PI3K family are divided into 3 classes (class I, II and III). Amongst them, PI3K class I have numerous vital roles and perform important activities (H Guo *et al*., 2015). PI3K Class I is a heterodimer and is classified into classes IA and IB, based on molecular structure differences (M Graupera *et al.,* 2013). PI3K once activated, it phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) to convert it into phosphatidylinositol 3,4,5-triphosphate (PIP3) on the inner side of the cell membrane and thus recruits signaling protein such as AKT/Protein Kinase B (PKB) (TF Franke *et al*., 1997).

AKT consists of 3 domains: middle kinases, and regulatory carboxy-terminal domain and pleckstrin homology (PH), translocation of AKT is regulated by PH domain (P Abeyrathnaet *et al*., 2015). AKT can be divided into 3 isoforms (AKT1, AKT2, AKT3). AKT1 is ubiquitously expressed, AKT2 is mainly expressed in adipose tissues, skeletal muscles and liver, and AKT3 is expressed in brain and testes (P Abeyrathna *et al*., 2015; JR Krycer *et al.,* 2010). Leptin activated AKT signaling pathway activates Mammalian target of Rapamycin (mTORC) which in turn activates Hormone-sensitive lipase (HSL), Adipose triglyceride lipase (ATGL) and Peroxisome proliferator-activated receptor alpha (PPARα) (RS Ahima and JS Flier 2000).

Forkhead box protein O1 (FOXO1) showed very significant role in the regulation of lipolysis in adipose tissues by regulating the expression of Adipose triglyceride lipase (ATGL). The role of FOXO1 in lipolysis is confirmed by knocking out FOXO1 gene in 3T3-L1 adipocytes, after that process of lipolysis and expression of ATGL is halted (P Chakrabarti *et al.*, 2009). Role of leptin mediated Hormone sensitive lipase (HSL) is studied by Takahashi *et al.*, stated that HSL is a under control regulation of leptin and mediates lipolysis and is more prominent at supra-physiological hyperleptinemia. By using HSL knockout mice the role of leptin in HSL expression had been cleared, HSL is partly mediated by leptin (M Shimabukuro 2017).

Monoacylglycerol lipase (MGL) impacts lipid metabolism by somewhere around 2 mechanisms. To start with, it catalyzes the MGL into glycerol and unsaturated fatty acids. These two products of MGL are used for energy purposes or synthesis purposes. Second function of MGL is to degrade 2-arachidonoyl glycerol (2-AG), the most plentiful endogenous ligand of cannabinoid receptors (CBR). MGL knockout mice shows increase 2-AG amount in adipocytes (U Taschler *et al*., 2011).

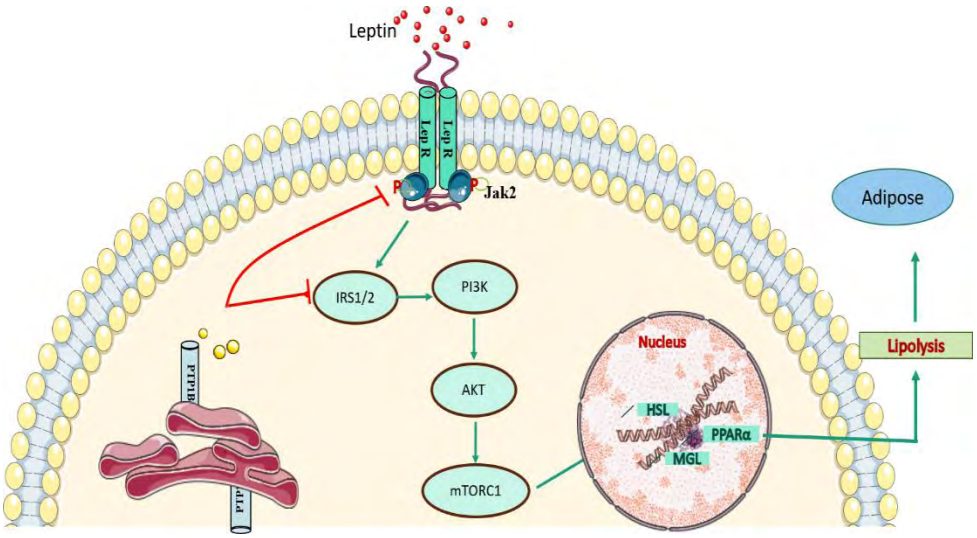

Figure 1.2 Activation of leptin receptor and downstream mediators.

1.2. Brain

Brain is the most superior and mysterious organ of the human body, controls almost all the basic and important functions of the body (RR Bimonte 1998), such as memory, breathing, senses, hunger, vision and controls the body temperature (JM Henshaw 2012). At broader level brain is comprised of mainly three parts such as cerebrum, cerebellum and the brainstem (LW Swanson 2000). Brain has two significantly different regions, outside portion and darker portion is known as gray matter while the inner and light portion is called white matter (V Pando-Naude *et al*., 2021). Brain has also outer protective covering known as meninges which are composed of dura matter, pia matter and arachnoid matter (K Dasgupta *et al*., 2019).

1.2.1. Hypothalamus

Hypothalamus have 14 gm of weight, constitute a very minute portion of the brain but performs very diverse and basic activities of life such as energy expenditure, feeding and digestion, electrolytes balance, regulation of body temperature, reproduction, sleep wake cycle (CB Saper and BB Lowell 2014). It is now understood that feeding center is present in the hypothalamus, in 1930s and 1940s a lesion was developed in the hypothalamus which disrupts feeding pattern and causes obesity and hyperphagia (CB Saper and BB Lowell 2014; JH Jeong *et al.,* 2017). Specific receptors for leptin action are present in arcuate nuclei (ARC), ventromedial, dorsomedial and premammilary nuclei in the hypothalamus. Leptin concentration decreased due to low fats in the body and insulin resistance which results in increased appetite and decreased energy expenditure (P Seoane-Collazo *et al*., 2020).

1.2.1.1. Leptin signaling in hypothalamus

Inside the brain, leptin follows up on numerous populaces of Ob-Rb neurons fundamentally in the hypothalamus and brain stem (Patterson *et al*., 2011; Scott *et al*., 2009). In the hypothalamus, leptin acts on several neurons which have Ob-Rb including those neurons which are lateral to hypothalamic area and the dorsomedial, ventromedial, ARC and ventral premammilary area. (Patterson *et al*., 2011; Scott *et al.,* 2009). All these sites contain several differential forms of leptin receptors, every

one of which contributes interestingly to leptin activity. The most reported site for leptin signaling is ARC (Hayes *et al*., 2010; Schwartz *et al*., 2000).

Binding of leptin ligands to Ob-Rb brings conformational changes in Ob-Rb that trigger autophosphorylation of JAK2 (Banks *et al.,* 2000; Kloek *et al.,* 2002). Phosphorylated JAK2 turn phosphorylates multiple tyrosine motifs Tyr985, Tyr1077 and Tyr1138 (Banks *et al*., 2000; Gong *et al*., 2007). Phosphorylated tyrosine motifs have Src homology 2 (SH2) that recruits explicit SH2-containing effector protein to receptor intercede consequent signaling (Banks *et al*., 2000; Gong *et al.,* 2007).

After phosphorylation of Tyr1138 brings about recruitment of STAT3 to leptin receptor and gets phosphorylated (pSTAT3) and stimulation by JAK2 (Banks *et al*., 2000; White *et al*., 1997). STATs protein is diverse a group of transcription factors (TFs) and consists of 7 members (STAT1, 2, 3 ,4, 5a, 5b and 6), have a vital role in transcription regulation and signal transduction (F Dandoy-Dron *et al*., 1995; S Sano *et al*., 1999; Y Cui *et al*., 2004). STAT3 was first reported in 1994, highly conserved, like other STAT proteins STAT3 also consist of six residues: coiled-coil domain (CCD) for protein-to-protein cross talk, N-terminal dimerization domain (ND), SH2 domain essential for STAT3 activation, linker domain (LD) that regulates DNA-binding stability, C-terminal transcriptional activation domain (TAD) and central DNA-binding domain (DBD) (H Liu *et al.*, 2021). Once pSTAT3 activated, it translocases into the nucleus, where it targets several genes (Bjorbaek, *et al*., 1999).

STAT3 after translocation into nucleus promotes gene expression of POMC neurons (MA Cowley *et al.*, 2001; MW Schwartz *et al*., 2000; JK Elmquist *et al*., 2005), whereas downregulates the expression of AgRP gene and can also inhibits AgRP neurons (Y Aponte *et al.*, 2011; MJ Krashes *et al.*, 2013). Dependably, ob/ob and db/db mice showed decreased expression level of *mPOMC* and increase level of *mAgRP* (MW Schwartz *et al*., 1997 and TM Mizuno *et al*., 1999). Knockout of *mAgRP* neurons and reduction in *mAgRP* expression leads to lean mice (GA Bewicket *et al*., 2005; E Gropp *et al.*, 2005). Contrarywise, knockdown of *mPOMC* neurons and their reduce expression bring obesity in mice (N Balthasar *et al*., 2004). This

underlines the significance of leptin-stimulated *mAgRP* and *mPOMC* in regulation of energy expenditure and food intake (AG Uner *et al*., 2019).

AgRP and NPY are present in close vicinity (Betley *et al*., 2013; Bouret 2017), the main orexigenic neurons that produce hunger signaling during energy deficiency and food intake (MS Vohra *et al*., 2021). AgRP and NPY are potent appetite inducer neurons located ARC nucleus (Aponte *et al*., 2011; Krashes *et al*., 2011). Various high-level procedure has been utilized to dig out the orexigenic role of NPY, knockout of NPY in mice model showed decrease food intake (Wu *et al*., 2012), while presence of NPY neuron revealed increase food consumption (Aponte *et al*., 2011; Krashes *et al*., 2011).

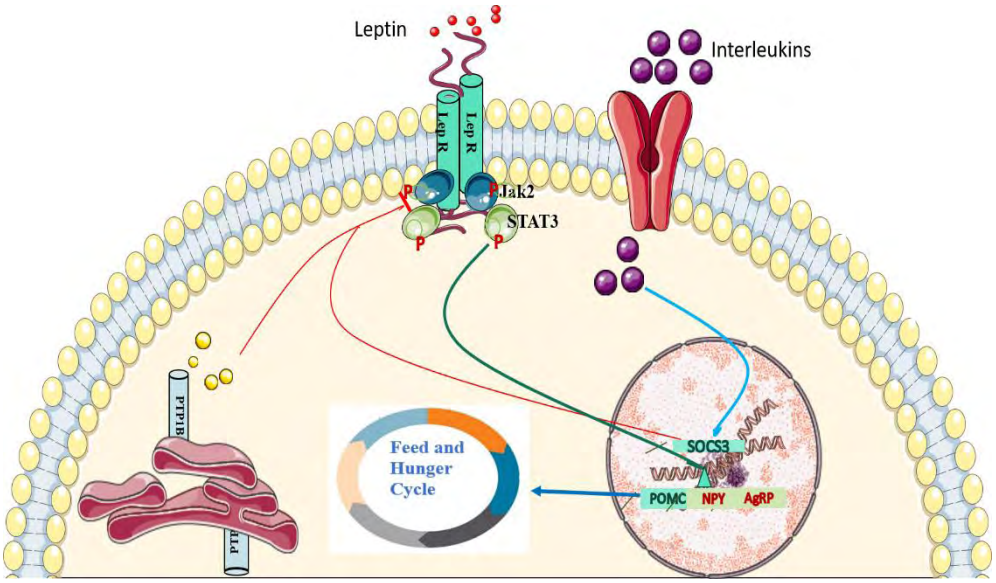

Figure 1.3 Diagrammatical sketch of activation of leptin receptor via leptin ligand, and regualtion of SOCS3 by *IL*-6.

Decrease leptin availability to brain is also considered as a significant factor for leptin resistance (WA Banks *et al*., 2004). For normal biological functioning of leptin signaling, it is prerequisite for leptin to cross BBB by a particular and saturable protein transporter protein (AG Izquierdo *et al*., 2019). Experimental data showed that leptin receptor functionality is mandatory and their absence results in loss of function of leptin in the brain (SM Hilemane *et al*., 2002). Henceforth, if the leptin level is

unnecessarily high, it might prompt leptin saturation and further diminish the proportion of leptin transport via BBB and results in leptin resistance (WA Banks *et al*., 2000; WA Banks *et al*., 2002).

1.3. Endoplasmic Reticulum and Leptin Resistance

Endoplasmic reticulum (ER), responsible for various functions in the cell such as lipid biosynthesis, protein biosynthesis, xenobiotic detoxification and also responsible for cellular Ca+2 storage (X Chen *et al*., 2021). ER homeostasis permits secretion of plasma membrane and also organelle proteins with proper tertiary structure with the help of molecular chaperons and different enzymes. Under pathological conditions, ER cannot perform its function of protein folding and the misfolded, unfolded proteins cannot be sent to Golgi Bodies and thus that proteins gather in the ER lumen. This phenomenon is known as ‘‘ER stress’’ (DL Eizirik *et al*., 2008).

ER stress is reported in causing impairment of leptin signaling pathway, which is brought about by accumulation of mis or unfolded proteins that activates unfolded protein response (UPR) (Cakir *et al.,* 2013). This process improves the ER capacity of protein folding, the degradation of unfolded proteins and also causes to minimize the entry of new proteins to solve its folding defects. ER stress diminishes the post-transcriptional changes of POMC and also disrupts its synthesis (Cakir *et al*., 2013). It is reported that, pharmacologically induced hypothalamic ER stress in mice cause to surges PTP1B concentration and Suppressor of cytokine signaling 3 (SOCS3) and thus causes leptin resistance (Cakir *et al.,* 2013). Obesity-related hypothalamic ER stress have a potent role in central leptin resistance (Cakir & Nillni 2018a).

During ER stress condition, PTP1B expression is increased in the cell, dephosphorylates the Ob-Rb and also IRS1/2 which results in both leptin and insulin resistance, respectively. PTP1B gene knockout mice are observed and shows higher sensitivity of leptin receptor as there is none dephosphorylation of leptin receptor take place then (CL White *et al.*, 2009). Expression of PTP1B is reported higher in leptin resistance indicating that PTP1B contribute to leptin resistance (DL Morris *et al.*, 2009). ER stress and inflammation disrupts leptin receptiveness to neurons by impeding leptin signaling pathway. As of late UPR and ER stress have arisen a

significant link in causing leptin resistance (L Ozcan *et al.*, 2009 and X Zhang *et al.*, 2008).

1.3.1. Role of HFD in Leptin resistance

Maximum number of humans and rodents who are obese generally have extremely high concentration of leptin in plasma. Nevertheless, this endogenous higher leptin concentration may not inrease utilization of energy or decrease apetite. There are uncommon cases of single-gene mutations which are accountable for obesity in humans, but the most common cause of obesity is thought to have from polygenic interaction with the environment (M Tshop *et al*., 2001). This condition is known as "leptin resistance". There are several hypothesis regarding leptin resistance but two of them are widely accepted which are that leptin fails to reach its target (hypothalamus in brain) (WA Banks *et al*., 2003) and there is disruption of 0b-Rb based signaling cascade (H Munzberg *et al*., 2005).

Activated pro-inflammatory cytokines are evidence that leptin resistance in hypothalamus is caused by high-fat-diet (HFD) (Thaler JP *et al*., 2012). In particular, only in one day rodents on HFD induced inflammation in hypothalamus. The major cytokines of innate-immunity/inflammation NF-KB and IKB kinase-B (IKKB) are strongly expressed in hypothalamus (Zhang X *et al.,* 2008). It has been observed that after only one day rodent exposure to HFD or a single ICV injection of fatty acid or glucose, IKK*β*–NF-KB signaling cascade become active. Therefore, HFD induced hypothalamus inflammation may be an initial in the on setting of leptin resistance by over nutrition (HFD) (Thaler JP *et al*., 2012 and Posey KA *et al*., 2009). ER stress in POMC and peripheral tissues in obese mice, signifies that ER stress and HFD are associated with metabolic disorders (M Schneeberger *et al*., 2013; JP Thaler *et al*., 2012). Prominently, IKK*β*/NF-KB expression is initiated by HFD via ER stress in hypothalamus, which prompts leptin resistance (X Zhang *et al*., 2008). In addition, IKK*β* expression enhances SOCS3 promoter activity, impedes the leptin signaling by blocking phosphorylation of JAK2 (MC Maeso Fortuny *et al*., 2006; X Zhang *et al*., 2008). The expression of SOCS3 isn't just initiated by leptin, yet additionally by LIF, ciliary neurotrophic factor (CNTF), growth hormone (GH), interleukin-6 (*IL*-6) and

different cytokines may induce SOCS3 (AR Lubis *et al*., 2008). Bjorbaek *et al.* reported that serum containing different cytokines might activates SOCS3, which thus can cause leptin resistance.

Increased expression and activity of hypothalamic Toll like receptor 4 (TLR4) is observed during chronic HFD feeding (Ropelle *et al*., 2010). Expression of TLR4 on astrocyte and microglia is observed by keeping rodents on HFD, this may trigger hypothalamic inflammation via inflammatory cytokines. These inflammatory cytokines specifically induce inflammation in AgRP and POMC neurons, mostly by upregulation of IKKβ–NF-KB signaling which congregates with PTP1B expression and stimulated leptin signaling to induce SOCS3, which results in leptin resistance (KCG De Git *et al*., 2015).

1.3.2. Role of Protein Tyrosine Phosphatase 1 B in Leptin Resistance

PTP1B is a member of PTPs family, plays different vital biological roles and perform various functions of cell (B Sharma *et al.,* 2020). PTPs are very diverse group of enzymes, which involves in the catalysis, the removal of phosphate moiety from phosphorylated protein from tyrosine residue (M Kim *et al*., 2018). PTPs family has been well studies and until now it is estimated that PTPs family includes 107 human gene which are well characterized (B Sharma *et al.*, 2020). The main subfamily consists of 21 non receptor types and 17 receptor type. PTP1B is group into the receptor type of 1st sub-family which catalyzes the dephosphorylation process the PTPs substrates (RH Reddy *et al.,* 2017). Up until this point, PTP1B has been reported to involve in causing different disorders including insulin resistance, leptin resistance, CVD and cancers (Sharma *et al*., 2020).

PTP1B is anchored in the ER membrane at C-terminus by the hydrophobic tail, whose main function is to dephosphorylate leptin and insulin signaling pathway (FG Haj *et al*., 2002). Leptin receptor is activated when a specific ligand is bind to it, which is leptin hormone itself, when the receptor activated, the down cascade of signal transduction is started such as the JAK2 activates, phosphorylates the tyrosine residues Y985, Y1077 and Y1138 at first place and then STAT3 (MF Andreoli *et al.,* 2019). PTP1B causes leptin resistance by dephosphorylating JAK2, the very first step

to start the signaling cascade of leptin (JM Zabolotny *et al*., 2002). Unphosphorylated JAK2 thus cannot activates the STAT3 which a key step of leptin signaling, such as STAT3 translocases into the nucleus and then different protein expression and inhibition take place (H Liu *et al*., 2021). Over expression of PTP1B, lowers the activity and even inhibit Ob-Rb to perform it signaling cascade (CL White *et al*., 2009). PTP1B is expressed in ventromedial hypothalamus (VMH), ARC and dorsomedial hypothalamus (DMH), and neuron-specific and systemic deletion of PTP1B increased leptin sensitivity and decreases adiposity in mice (KK Bence *et al*., 2006; A Cheng *et al*., 2002; JM Zabolotny *et al*., 2002). PTP1B expression in leptin resistant animals is upregulated, proposing that PTP1B likewise adds to leptin resistance (CD Morrison *et al*., 2007 and CL White *et al*., 2009).

1.4. Diabetes

Diabetes mellitus (DM) is a type of metabolic disorder in which individuals experience high blood glucose levels because their bodies do not respond to, or produce insufficient insulin that assists with balancing out the glucose (Khan *et al*., 2019). Over the preceding few decades, the number of diabetic patients increased in both developed and developing countries. The International Diabetes Federation (IDF) recently estimates that 537 million people worldwide have diabetes. It is expected that the number of diabetic individuals will increase to 634 million by 2030 and 7.83 million by 2045 (IDF Diabetes Atlas 9th edition 2021).

The etiological study of diabetes primarily divided DM into two principal types: T1DM and T2DM which represent 85% of the all-out DM (Forouhi *et al*., 2019). Vascular complexities like CVD and diabetic kidney diseases (DKD), diabetic neuropathy, and retinopathy are the main sources of dismalness and mortality in people with diabetes (Morrish *et al*., 2001). T1DM, which is brought about by a complete or close to total lack of insulin, T2DM is depicted by the presence of insulin antagonism with a deficient compensatory surge in insulin secretion (Solis-Herrera *et al*., 2018).

The existence of autoantibodies against the *β*-cells and the absence of insulin production are biological markers of T1DM (LS Geiss 2014). T2DM is linked with

physical inactivity, aging, and obesity (Berbudi A *et al*., 2020). Due to both deficient insulin activity (insulin resistance) and impeded insulin production by islet *β*-cells in the pancreas. This condition brings about high glucose levels in the bloodstream (Brestoff J. R. and Artis D. 2015).

1.4.1. Causes of Diabetes

T2DM is a complex metabolic problem with diverse contributing features including different genes, epigenetics, environment, lifestyle, and diet (GG Kang *et al*., 2019). T2DM is also linked with the most serious diseases like hypertension, and hyperlipidemia which may cause insulin resistance in individuals (N Holman *et al*., 2015). These factors are involved in the production of Reactive Oxygen species (ROS) in the mitochondrial matrix which disturbs the cell redox balance and produce oxidative stress which is not present in normal condition (M Valko *et al*., 2007). The pancreatic *β*-cells have low potency to overcome endogenous oxidative stress, making them highly vulnerable to oxidative stress (J Wang and H Wang 2017). Consequently, an abundance of ROS production in *β*-cells prompts a low concentration of insulin secretion. Excess Nitric oxide (NO) production can initiate apoptosis of *β*-cells of the pancreas and halt insulin production and secretion (S Tangvarasittichai 2015).

The pathophysiological connection between T2DM and obesity is mainly accredited to two aspects IR and insulin deficiency (JP Felber and A Golay 2002). HFD contributes to the increased concentration of free fatty acids (FFAs) and followed by excess of plasma glucose level, a major marker for IR (AG Ampofo *et al.,* 2020). When the level of FFAs exceeded from normal, then the extra FFAs are stored around different organs such skeletal muscles, liver and pancreas. This results in the mitochondrial dysfunction and turn release of toxins and ROS and causes damage to the cells and leads to impaired metabolism of glucose, affects secretion of insulin and damage *β*-cells (N Ouchi *et al.,* 2011).

1.5. Streptozotocin and its Effect

Streptozotocin (STZ) was first identify by group of researchers at Upjohn laboratory in Michigan 1959 in the fermented broth of streptomyces achromogenes (J Capdevila *et al.,* 2022). STZ (2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose) is a very potent alkylating antineoplastic drug that is very destructive to *β*-cell, it has a molecular mass of 265 g/mol and a blend of *α*- and *β*-stereoisomers (Eleazu *et al*., 2013). It is a potent alkylating agent of DNA in both mammalian and bacterial cells (Eleazu *et al*., 2013). STZ destroy pancreatic *β*-cells leading to hyperglycemia, hyperinsulinemia and following T2DM. After 72 h of administration its effect can be seen relying upon dose administered (Eleazu *et al*. 2013).

Glucose transporter 2 (GLUT2) can block by STZ because of its chemical structural analogue to N-acetyl glucosamine, *β*-D-(acetylamino)-2-deoxy-glucopyranose and glucose, so as result it accumulated specifically in *β*-cells (Eleazu *et al*., 2013). GLUT2 are moreover present in the kidney and liver however less significantly so STZ explicitly targets the pancreas (Bouwens and Rooman, 2005). STZ can target *β*-cells of pancreas and causes immunological response by means of releasing of decarboxylase autoantigens and glutamic acid, when it injected in high doses (Eleazu *et al.,* 2013). And in such cases, pancreatic *β*-cells are destroyed, and hyperglycemia is instigated prompting to pancreatic islets inflammation (Boni-Schnetzler and Meier 2019).

The disrupted *β*-cells evidently releases impaired DNA that acts on different types of cells, such as endothelial, epithelial, macrophages, neutrophil-type containing Toll-like receptor 9 (TL9) receptors on their surfaces. When TL9 receptor enacted the nuclear TF, NF kappa B (NF-KB) that forms the heterotrimer P50-P65-kappa B to upregulate the inhibitory components (ikb) (Vitseva *et al.,* 2008; Naamane *et al.,* 2007). After that NF-KB (P50-P65) sans iKb cross the nuclear membrane and enter into nucleus and upregulate gene expression of pro-form of several inflammatory genes which are NF-B dependent, such as *IL*-18, *IL*-2 tumor necrosis factor alpha (TNF-α) (A Stutz *et al.,* 2009).

This mechanism produce a large amount of Nitric oxide (NO) and ROS. These reactive species damages the DNA or mutate the DNA which have to be reversed. The poly (ADP-ribose) polymerase I, DNA repair enzyme activated but it consumes all the energy stored in the *β*-cells (K Van Dyke *et al*., 2010). STZ inhibits ATP production by blocking the TCA cycle directly and through NO in beta cells (Nukatsuka *et al*., 1990; Sofue *et al.,* 1991). Superoxide produced due to STZ affects mitochondrial activity and increase xanthine oxidase activity (Turk *et al.,* 1993). Xanthine oxidases use ADP as a substate and produce uric acid along with superoxide (hydrogen peroxide and hydroxyl radicals) in feedback manners (Nukatsuka *et al.,* 1990 and Takasu *et al.,* 1991) and as a result necrosis occur and cell dies. After cell death several pro inflammatory cytokines deployed to the site of necrosis and that's the reason that diabetes and leptin resistance is linked with inflammation (K Van Dyke *et al.,* 2010 and Thaler JP *et al.*, 2012).

1.6. PTP1B Inhibitors

Tyrosine residue phosphorylation of proteins is the key mechanism to regulate cell growth, regulation, and differentiation of cells. It is a reversible mechanism which is opposes by PTPs (AK Tamrakar *et al*., 2014; AJ Barr *et al*., 2010). Besides, defective and distorted activity of PTPs and PTKs causes abnormal tyrosine residue phosphorylation processes prompting to various and serious disorders such as inflammatory disorders, diabetes, cancer and leptin resistance (AK Tamrakar *et al.,* 2014; Y He *et al*., 2005; P Heneberg 2009). PTP1B is placed in intracellular PTP, negatively impact insulin and leptin signaling (Y He *et al*., 2005). It is reported that PTP1B is responsible for dephosphorylation of tyrosine residue (pY1162/pY1163) of IRS-1 and IRS2 (A Haque *et al.,* 2011).

Recently PTP1B is used as potent drug target for treatment of leptin resistance and T2DM (Tamrakar *et al*., 2014). In this specific circumstance, albeit several PTP1B inhibitors have been studied, but the polar and highly conserved PTP1B catalytic domain makes it a quite sluggish to achieve cell permeability and selectivity for PTP1B inhibitors (Kennedy and Ramachandran 2000; Feldhammer *et al.,* 2013; Panzhinskiy and Nair 2013; Sun *et al*., 2016). For signal transduction, Ob-Rb employs

JAK2 to downstream it downstream signaling cascade. Hypothalamus expresses PTP1B, which dephosphorylates Ob-Rb and associated kinases JAK2. Leptin signaling is inhibited by PTP1B by dephosphorylating JAK2 and thus PTP1B inhibition can reverse the leptin resistance (A Cheng *et al*., 2002 and JM Zabolotny *et al.,* 2002).

Recently, compounds isolated from plants have shown different biological activities, such as anticancer, anti-obesity, anti-diabetic, anti-inflammatory, antihypertension, anti-Alzheimer, antioxidant and antimicrobial (Kamohara 2016; Tsoukalas and Engin 2018; Salehi *et al.,* 2019; Wu C *et al.,* 2006). It is reported that 16αH-17-isovaleryloxy-ent-kauran-19-oic acid extracted from plant species Acanthopanax koreanum is a strong inhibitor of PTP1B with IC50 value 7.1 to 0,9 μM in a noncompetitive fashion, while ent-kaur-16-en-19-oic acid and acanthoic acid can inhibit the PTP1B in dose-dependent manners (Tsoukalas and Engin 2018). Along with these compound, 17-isobutyryloxy-kauran-19-oic, Ent-16βH and 17-acetoxy-18-isobutyryloxy-kauran-19-oic acid secluded from Siegesbeckia glabrescens can inhibit PTP1B expression at 30 μg/ml (Kim *et al*., 2006). Compound, hueafuranoid A, isoated from Antarctic lichen Huea sp, has been reported to inhibit PTP1B activity with IC50 value of 13.9 μM (Y Cui *et al*., 2012).

1.7. Dodonaea Viscosa

Dodonaea Viscosa (D. viscosa) is a medicinal plant first reported from Australia and is local to western America. The plant is broadly dispersed in the specific areas of Mexico, Northern Mariana Island, New Zealand, South America, Florida, Virgin Island, India, Pakistan, Africa, and somewhere else. Presently, it is present in most of the nations. The particular plant is now present throughout the tropical and sub-tropical countries (Al Oraimi *et al.,* 2013).

The plant is widely distributed as a single-stemmed or multi-stemmed, small tree and height about 7m tall. Plant leaves are different in shapes and size and for the most part obviate, yet few of them are lanceolate. Leaf size of plant is around 4-7.5 cm long and 1-1.5 cm wide with dark green tone. The leaves are organized alternately and secretes a white gummy substance. Typically, the flowers are around 2.5 cm long, yellow

color but every so often yellow change to red. The flower. The flower is of specific gender, both stamin and carpel are not identified in a single plant (MA Hossain 2019), in a rare condition both can be found in plant at same time (Al Oraimi *et al.,* 2013).

M. Khan *et al.* (1992) and R.A and Mothana *et al*. (2010) reported that Leucocyanidins a Methanolic extract of plant contains different bioactive compounds like tannins, terpenoids, volatile oil and flavonoids. Whereas ethanolic extract of plant showed the presence of flavonoids, alkaloids, saponins, phytosterols, triterpenoids and steroids (R.A. Mothana *et al*., 2010). Flavanoids, saponins, tannins, steroids and terpenoids were identified from aqueous extract of plant (N.U. Prakash *et al*., 2012; D.Lawal *et al*., 2012).

Table 1.2 Classification of Dodonaea viscosa.

Classification	
Kingdom	Plantae
Sub-kingdom	Tracheobionta
Division	Magnoliophyta
Class	Angiosperms
Sub-Class	Rosids
Order	Sapindales
Family	Sapindaceae
genus	*Dodonaea*
Specie	*Dodonaea viscos*

1.7.1. Evaluation of Dodonaea Viscosa on PTP1B inhibition

XH Wang *et al*. (2018), reported that extract from Dodonaea Viscosa is a potent inhibitor of enzyme PTP1B, Triterpenoids 1 to 6 extracted from the particular plant and were tested in vitro their role in inhibition of PTP1B and Triterpenoids 1, 2, 5 and 6 show positive effect on PTP1B inhibition with IC50 values ranges from 23.7±2.2 to 35.7±9.3 μM. P Muthukumran *et al*., (2011) have confirmed the role of Dodonaea Viscosa on PTP1B inhibition, by treating the male Wister rats which were diabetic.

Butanol and methanol extracts from the particular plant were given to rats. The glucose tolerance of rats was significantly increases in rats in both short and prolong treatment.

Dodonaea viscosa leaves and stem contain nine polyphenolic compound 1 to 9, every of them were studied through spectroscopic analysis also included HREIMS and 2D NMR. Consequently, it is shown that plant have PTP1B inhibitory properties, all the compounds which were isolated exhibit significant dose-dependent inhibition with IC50 ranges from 13.5 TO 57.9 μM. Viscosol (4) from among them shown promising result on inhibition of PTP1B with IC50 value 13.5 μM. To uncover the mechanism behind the inhibitory effect a very detailed kinetic studied was conducted and HPLC-DAD-ESI/MS analysis shown that compound 1 to 9 had the potential to inhibit PTP1B (Z Uddin *et al.*, 2018). All the peaks obtained by LC-DAD-ESI/MS analysis were characterized and it was found that compound 4 is the most potent which is 5,7-dihydroxy-3,6-dimethoxy-2-(4-methoxy-3-(3-methyl-but-2-enyl)-phenyl)-4 chromen-4-one. The whole methanolic extract of D. Viscosa showed potent PTP1B inhibition (Z Uddin *et al.,* 2018).

The potent bioactive compound (molecular formula C23H24O7), with a molecular weight of 412.1522. PTP1B inhibitory activity of all the 9 compounds was analyzed by hydrolysis of p-nitrophenyl phosphate, monitored by spectrophotometer. All the 9 compounds have an IC50 value of 13.5-57.9 μM. Compound 4, potent inhibitor of PTP1B have an IC50 value of 13.5 μM and exhibits more fold inhibitory activity than other isolated compounds. Also, from the kinetic analysis, it was seen that compound 4 effectively blocked free enzymes as compared to the enzyme-substrate complex which is evident from KI = 4.1 ± 0.2μM and KIS = 26.4 ± 0.4μM respectively (Z Uddin *et al*., 2018).

1.8. Aims and Objectives of the study

In this study, we determine the role of leptin signaling in food intake and lipolysis, and the potential role of flavonoid compound 5,7- dihydroxy-3,6-dimethoxy-2-(4-methoxy-3-(3-methyl but-2-enyl)-phenyl)-4H-chromen-4-one, to revert Leptin signaling in a HFD and low dose streptozotocin-induced type 2 diabetic model.

1.8.1. Specifics aims of the study

1) To study the role of flavonolic compound in reverting the Leptin signaling in STZ-HFD leptin resistance mice.
2) To evaluate the role of leptin signaling in food intake in hypothalamus.
3) To determine effect of leptin signaling on lipolysis in adipose tissue.

2. Materials and Methods

2.1. Animals

Our experiment was conducted on mice (Mus musculus) as an animal model. Experimental studies were performed on male mice C57BL/6, whose weight ranges from 25 to 40 mg (8 to 12 weeks). All these mice were kept for 1 week and acclimatized in the animal facility center of Quaid-i-Azam University. Mice were provided with standard pellet food and water and conditions of the room include 12 hours light/dark cycle, 27°C.

2.2. Ethical approval

The study was permitted by the institutional Bioethics committee of Quaid-i-Azam University, Islamabad. All the experiments were performed according to the standard protocols.

2.3. Groups

The experiment was performed on total of 9 mice, which were separated into three groups, each containing 3 mice. Group I, (Normal), The normal group was provided with a normal pellet diet and water. Group II, (STZ-HFD induced diabetic group) was placed on HFD alongside STZ injection intraperitoneally for 5 successive days (40mg/body weight), for the induction of diabetes. Group III, (STZ-HFD-compound treated group) was administered the same as group II with the administration of our compound (5,7-dihydroxy-3,6-dimethoxy-2-(4-methoxy-3-(3-methyl-but-2-enyl)-phenyl)-4H-chromen-4-one) (33.3 mg/Kg) intraperitoneally for the treatment after the induction of diabetes (Table 2.1). The total percentage of normal pellet diet were as 22.2% protein, 4.1% fat, and 12.1% carbohydrates whereas, HFD consists of 25% protein, 17% carbohydrate, and 17% cholesterol.

Table 2.1 Grouping of mice.

Sr.No.	Groups	Names
1	Group I	Normal
2	Group II	STZ-HFD induced
3	Group III	STZ-HFD-compound treated

2.4. Induction of Diabetes and Compound treatment

As stated above, mice were separated into 3 groups and each group consists of 3 mice. The normal or positive control group was kept on normal standard diet and water. Normal group was administered with a single dose of saline (500μl) intraperitoneally. The fasting blood glucose concentration of control mice was measured by a glucometer (ACCU-CHEK Instant S, Roche Diagnostic, Mannheim, Germany), for 7 days at fasted condition for 4 to 6 hours. Regular and repeated low dose of streptozotocin (Bioworld, CAT # 41910012-3) were used to generate the T2DM mice model.

In STZ-HFD induced T2DM model, all mice of group II were kept on overnight fasting before the injection of STZ and normal water was provided. The streptozotocin (Bio plus Fine Research Chemical, CAT # 41910012-3, Bioworld), was dissolved in saline (500 μl), vortex and was administered intraperitoneally according to the body weight of mice (40mg/Kg), for 5 repeated days. After every STZ injection, the mice group was provided with 10% glucose, along with HFD. After the 5th day, normal water was provided to the mice and 10% glucose water was removed. To develop T2DM model leading to leptin resistance, mice take 9 to 10 days. Only those mice were validated as diabetic, who's fasting BGL was higher than 250mg/dl. The BGL concentration was measured and monitored on regular bases using a glucometer until euthanization.

Group III was also administered intraperitoneally with STZ for five consecutive days and was kept on HFD and 10% glucose water. The induction of diabetes in group III is like group II (STZ-HFD induced). After confirming that mice are diabetic, HFD was removed. On the very next day (11th day), compound (5,7-dihydroxy-3,6-dimethoxy-2-(4-methoxy-3-(3-methyl-but-2-enyl)-phenyl)-4H-chromen-4-one) isolated from Dodonaea Viscosa first dissolved in 1% dimethyl sulfoxide (DMSO) was given to each mouse (33mg/kg, 1mg/mice) by a single intraperitoneal injection (500μl). After administering our compound, mice were closely observed for 7 days, and fasting BGL were measured all over that week. After checking the fasting BGL on the 17th day, group III was euthanized for further analysis. During the whole experimental days, all the group's weights were strictly measured. Their mRNA expression was studied using RT-qPCR.

2.5. Serum Blood Glucose Analysis

Our experiment lasted for 21 days and during this period, the BGL of mice was measured regularly by keeping them fasting for 4-6 hours. We measured the BGL using a commercial glucometer (ACCU-CHEK Instant S, Roche Diagnostic, Mannheim, Germany) from the tail vein.

2.6. Blood collection and serum separation

After the induction of T2DM leading to leptin resistance and treatment with our compound, all the mice were anesthetized with chloroform. Blood was collected by cardiac puncture from the mice with the help of a 23G needle/1ml syringe. Blood was transferred to a 4ml gel and clot vacutainer (BD # 366643, Lot No # 7327961, Becton, Dickinson and company, USA). Tube was then centrifuged (Hermile Labortechnit GmbH Siemensstr-25 D-78564, Wehingen) at 6000 rpm for 10 minutes. The serum was present in supernatant, so we collected it, and stored those sera at -20°C for further usage.

2.7. Mice Dissection

All the mice were kept on overnight fasting, the BGL was checked in all the mice. Throughout the dissection, a cooling chain was maintained to ensure a better quality of RNA. A wooden dissection board was used, and mice were dissected using sterilized surgical tools. Required organs, brain and adipose tissues were isolated from the mice. The organs were washed with chilled PBS buffer (pH 7.2-7.4) and chilled distilled water, respectively and stored at -80°C for further investigation.

2.8. RNA extraction and purification

RNA from adipose tissue and hypothalamus was extracted by using RNA Kit (PureLink TM, RNA Minikit, Invitrogen by Thermifiesher Scientific, Cat No # 1218301 8A). For RNA isolation, the tissue (100-150mg) was grind by using a mortar and pestle with liquid nitrogen. Lysis buffer (0.6-1ml) and β-mercaptoethanol (10μl) was added to the homogenate. The lysed tissues were transferred to the RNase-free Eppendorf tube. Additionally, the lysate was passed 5 to 10 times through a 21 G×1/4 (0.8 mm×32mm) gauge syringe needle for properly homogenization. The volume of 100-200μl of chloroform was added, tubes were shake vigorously for 15s and incubated at room temperature for 3 minutes. After incubation, centrifuged the tube at 12000g for 15 minutes at 4°C. The supernatant was transferred into a new RNase-free tube, and an equal volume of chilled 70% ethanol was added.

The homogenate was vortexed and 700μl volume was transferred in the spin cartridge, centrifuged at 12000g for 15s, the flow-through liquid was discarded and the spin cartridge was inserted in the tube. The above steps were repeated until all the sample homogenate passed through the spin cartridge. Furthermore, 700μl of wash buffer I was added and centrifuged at 12,000g for 15s. The flow-through was discarded. Wash buffer II with volume of 500μl was added and tube was again centrifuge under the same condition. The process was repeated, for a better quality of RNA and flow through was discarded. A dry spin was given at 12000g for 2 minutes to let the membrane dry properly before adding elution buffer (Nuclease-free water).

Finally, elution buffer with the volume of 35μl was added in the spin cartridge and incubate for 1 minute at room temperature. Then, centrifuged it at 12000g for 2 minutes, at room temperature, and step was repeated for proper elution. The purified RNA was checked by using the nanodrop machine (Colibri Spectrophotometer, Berthold Detection System GmbH 75173 Pforzheim, Germany). The purified RNA was then stored at -80 ºC until further use. All the procedure were performed at 4°C and the cooling chain was maintained to minimize the denaturation of RNA and contamination by RNases.

2.9. cDNA Synthesis

The RNA sample from all the tissue were normalized. The normalized sample of 1 μg was used for the cDNA synthesis by using a cDNA synthesis kit (Maxima SYBR Green/ROX qPCR Master Mix (2X), Thermo scientific). A total volume of 20μl was prepared to synthesize 1000ng of cDNA. A master mix consisting of 2μl of dNTPs, 2μl of RT buffer, 1μl of RNase inhibitor, 1μl of reverse transcriptase enzyme, 0.8μl of oligo dT primer was prepared, and required RNA with the concentration of 1000ng was added. The remaining volume was maintained with nuclease-free water. The reaction mixture was then incubated for 1 hour at 37ºC in a PCR machine (T3 Thermoblock, Biometra, Germany). Afterward, were heated for 5 minutes at 95 ºC to inactivate the reverse transcriptase enzyme. cDNA was then stored at -20ºC.

2.10. RT-qPCR

Real-time qPCR was performed with SYBR Green PCR Master Mix (Thermo Fisher Scientific, CA, USA) by using MyGo Pro PCR system (MyGo PCR systems, IT-IS life sciences). The set of primers or desired target genes markers are given below in Table 2.4. The primers (100μM) were diluted by ratio of 1:10. Likewise, the cDNA was diluted with a ratio of 1:10. The total reaction of 10μl was prepared as given in the Table 2.2. PPiA was used as housekeeping gene. The program setting used in RT-PCR is mentioned in the Table 2.3. The relative mRNA expression of the target genes was analyzed by using the ΔΔCT method (X Rao *et al*., 2013).

Table 2.2 Recipe of the Master mix for RT-PCR reaction (10μl).

Sr.no	**Names**	**Concentration**
1	SYBR Green	2μl
2	Forward primer	1μl
3	Reverse primer	1μl
4	cDNA	6μl

Table 2.3 Profile of RT-PCR

Sr. no	**Stage**		**Incubation Temperature**	**Time**	**Cycles**
1	Hold		95 °C	60s	No
2	3 step Amplification		95 °C	15s	40
			60 °C	30s	
			72 °C	30s	
3	Pre-melt Hold		95 °C	10s	No
4	High Resolution Melting	Initial stage	60 °C	60s	No
		Final stage	97 °C	1s	

2.11. Statistical Analysis

To assess the significance in our data, we represent our data as mean ± SEM. The graphs were plotted by using Graph pad prism (Version 9). For statistical analysis, one-way ANOVA (comparison between the groups) and Tukey's Test (pairwise multiple comparisons) were performed. The p-value <0.05 were considered significant.

Table 2.4 List of Primers for RT-qPCR respective Tm (℃) and amplicon size (bp).

Sr.no.	**Primer name**	**Sequence (5' to 3')**	**Tm (℃)**	**Amplicon size (bp)**
1	mPi3k-F	GAGACAGGATGGGTCAAGGA	60	132
	mPi3k –R	CAAAGCAACACAGGAGAGCA	60.2	
2	mPtpn1-F	GCATAGGACAGTGGTAATGCG	60.5	123
	mPtpn1-R	AACTCACAGGGAAAGCAGAGG	60.8	
3	mTorc1-F	CCAGGAGGACATTTGTTCAGA	60.1	96
	mTorc1-R	CACTGAACACAGTAGAGCCAGTG	60.0	
4	mIrs1-F	AAGCACTGTGACACCGGAA	60.3	72
	mIrs1-R	CTTCGTGACCAGCTGTCCTT	60.4	
5	mIRS2-F	AAATGTGACTGGAGCAGCCT	59.	79 bp

			9	
	mIRS2-R	AAGAGAGATCCACCCATCCC	60.3	
6	mPPiA-F	TTGGTCCGAAGTAGCCACA	60.2	88
	mPPiA-R	GCCAAGCCTTTCTCGTTTC	59.9	
7	mAgRP-F	TGTGTAAGGCTGCACGAGTC	60.1	78
	mAgRP-R	GAAGCGGCAGTAGCACGTA	60,2	
8	mJAK2-F	AGACAGATGGGAAGGGAAGG	60.4	70
	mJAK2-R	CACACGCCTGCTGGTATTC	60.3	
9	mPOMC-F	GCAAGCGCTCCTACTCCAT	60.5	101
	mPOMC-R	CGACTCGTTCTCAGCAACG	60.8	
10	mSOCS3-F	CAAGGGGTGACCTGAAGAGA	60.2	89
	mSOCS3-R	TCTGGGGTGCAAGGGAT	60.0	
11	mSTAT3-F	AGCAGCCGAACCCCATA	60.2	96
	mSTAT3-	GCCCAGATTGCCCAAAG	60.	

	R		1	
12	mNPY-F	ATACTACTCCGCTCTGCGACA	60.1	77
	mNPY-R	TCTCAGGGCTGGATCTCTTG	60.5	
13	mPPARα-F	GCCGTTGCCACTGTTCA	60.4	110
	mPPARα-R	TACGCTCAGCCCTCTTCATC	60.5	
14	mLEPTIN-F	GGTCATACCCTGTGGAGGTG	60.2	96
	mLEPTIN-R	CACATCACATCACCCCTCAG	60.0	
15	mMGL-F	ATAACTGGGGCTCACTGCTCT	60.3	88
	mMGL-R	TCCTGAGGTAACAGCAAGGC	60.4	

3. Results

3.1. Mice mean body weight and fasting Blood glucose level

Insulin and leptin play a significant role in regulating the body glucose level (BGL) and body mass respectively. Therefore, we measure the mice's body weight and serum glucose level. The fluctuations in the body weight were plotted against the experimental days. A dramatic reduction in the body weight of STZ-HFD group was observed as compared to the control. While on treatment with our compound the trendline of body weight was noted like the control group (Figure 3.1). Normal pellet diet was given to the control group whereas HFD was given to both STZ and compound treated group. Ordinary One-Way ANOVA was performed and results were found significant, ****P-value <0.0001.

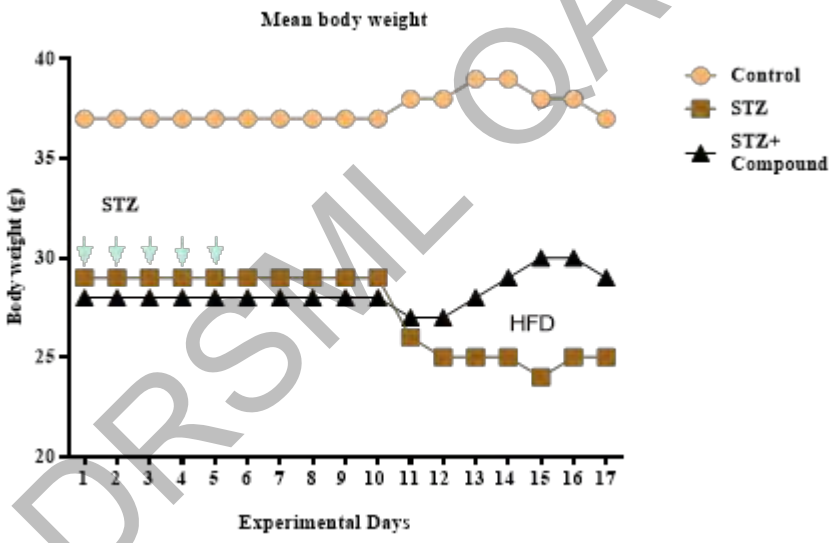

Figure 3.1. Mean body weight of all experimental groups, the 5-days dose induction of STZ drug is indicated with an arrow.

Further, fasting blood glucose level (BGL) was also estimated before the start of the experiment on day 1 and after that post-treatment. Mice were considered diabetic with a fasting BGL >250mg/dl. The blood glucose level of the STZ-HFD induced diabetic mice group was found to be significantly elevated as compared to the normal group, as shown in (Figure 3.2). After injecting with a compound, a gradual alleviation of BGL

has been seen in the STZ-HFD-compound treated group. The normal group was only given one saline injection (500μl). Five STZ injections were given intraperitoneally to both STZ-HFD induced, and STZ-HFD-compound treated groups. STZ-HFD-compound treated group was given an injection of the compound on day 11. We evaluated the gradual alleviation of BGL in the STZ-HFD-compound treated group. (P=0.7157), (p ***).

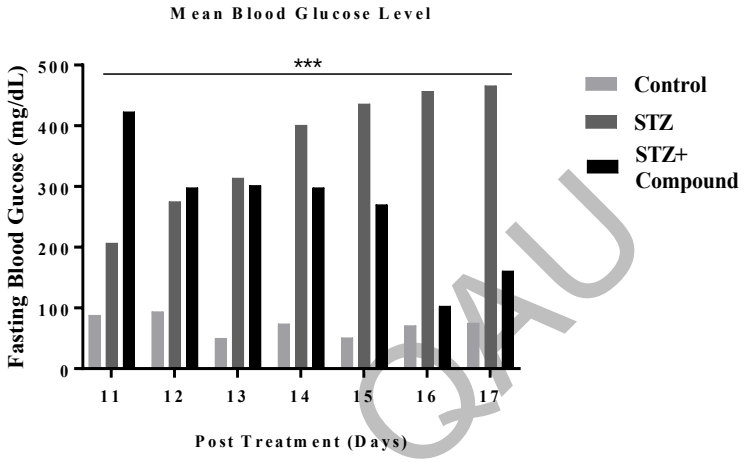

Figure 3.2 Mean fasting blood glucose level (BGL) of mice.

3.2. Targeted gene expression in Adipocytes

In mice adipocytes (control), STZ-HFD induced T2DM mice model (STZ-HFD) and STZ-HFD-compound treated mice model (STZ+C), we evaluate different genes at mRNA level, which were involved in the downregulating leptin mediated PI3K pathway such as inhibition of *De-novo* lipogenesis, halting of adipocytes differentiation, genes regulating fatty acid hydrolysis and genes important for changing structure of lipid droplets and those involve in adipocytes specified lipolysis. We have found that the expression of leptin hormone was significantly reduced in the STZ-HFD induced group as compared to the STZ-HFD-compound treated group. These results validate that our PTP1B inhibitor in the treated group was able to revert the leptin signaling pathway by inhibiting the PTP1B (Figure 3.3). The cDNA sample was run in triplicates and PPiA was used as a housekeeping gene. The data from three independent experiments were analyzed by a ΔΔCt method and the mRNA fold change was

obtained by using three respective groups. The data were represented as means ± SD. The differences between groups were analyzed by One-way ANOVA using Brown-Forsythe test and Bartlett's test. A ***p<0.0001 was considered statistically significant.

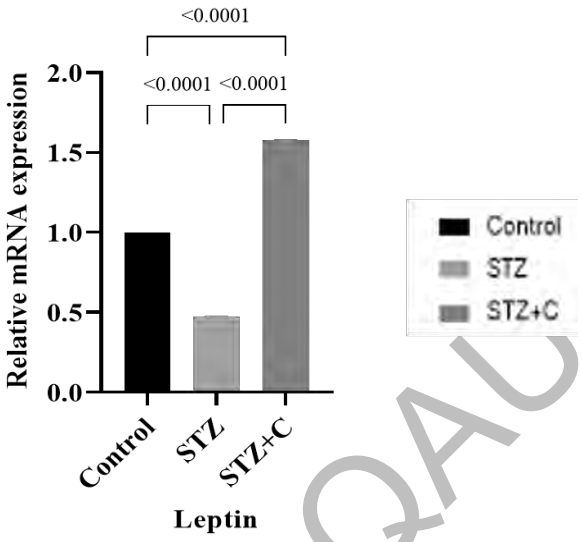

Figure 3.3 Leptin expression.

As we were interested in targeting PTP1B in the diabetic-induced model, so we measured the relative expression of *mPTP1B* and found a significant decrease in the expression in STZ-HFD-compound treated group as compared to the STZ-HFD group. Therefore, the bar graph (Figure 3.4) shows that our compound has an inhibitory effect on PTP1B. The data were represented as means ± SD. One-way ANOVA using Brown-Forsythe test and Bartlett's test was used as a statistical test and ***p<0.0001.

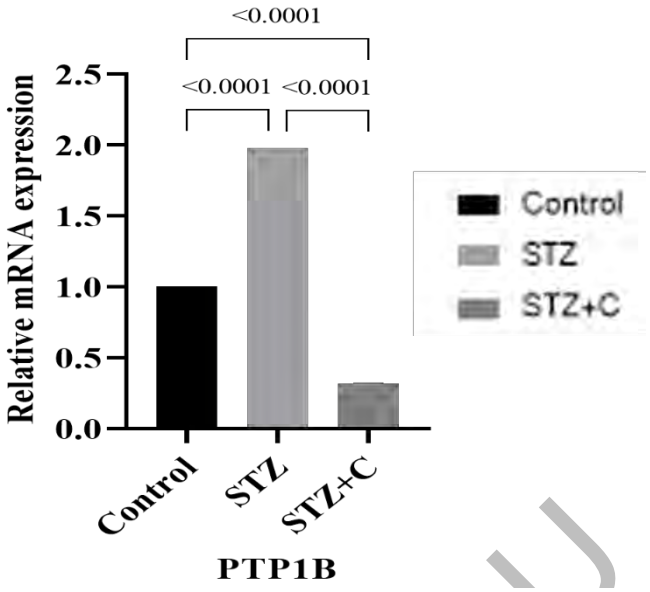

Figure 3.4 PTP1B expression.

The expression of insulin receptors IRS1, IRS2 and PI3K, AKT2, and mTORC1 were was significantly reduced in the STZ-HFD group but was found significantly high in the STZ-HFD-compound treated group (Figure 3.5). These results authenticate that PTP1B has a negative effect on the expression of insulin signaling mediators and found a significant increase after treating with our potential PTP1B inhibitor (compound). The data were represented as means ± SD. Statistical analysis was done by using One-way ANOVA using Brown-Forsythe test and Bartlett's test. The value of ***p<0.0001 was considered statistically significant.

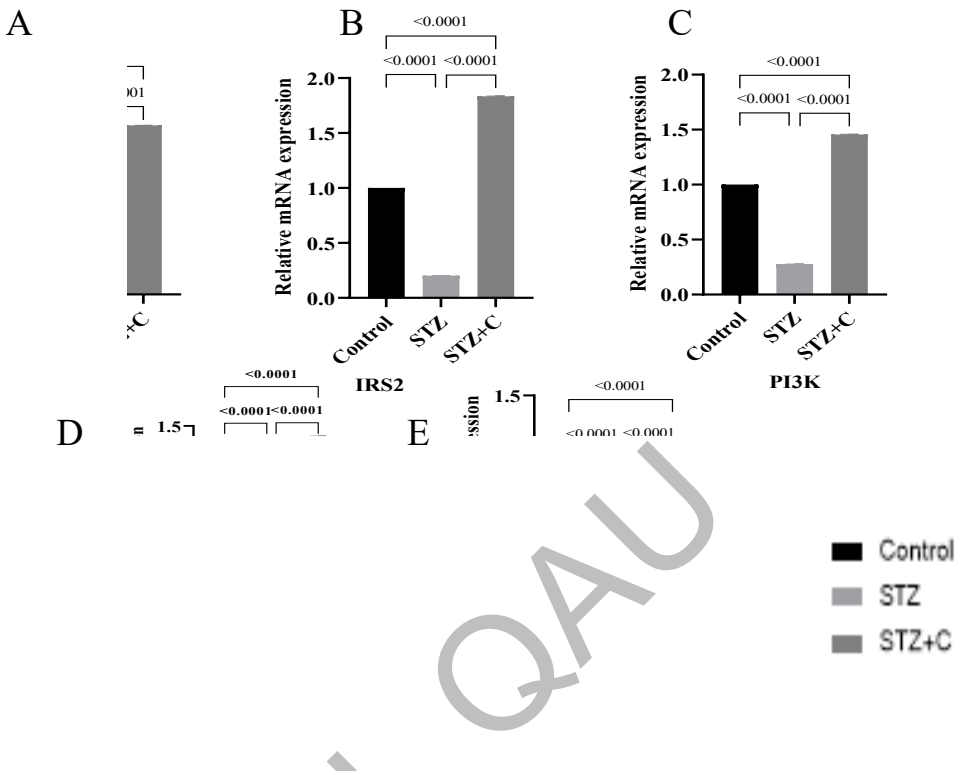

Figure 3.5 Leptin and Insulin-mediated signaling pathway expression. (A) Relative mRNA expression of IRS1. (B) Relative mRNA expression of IRS2. (C) Relative mRNA expression of PI3K. (D) Relative mRNA expression of AKT2. (E) Relative mRNA expression of m TORC1

Further, we have found the expression of leptin receptor (Lep R) and JAK2 (downstream mediators of leptin) was significantly increased in the STZ-HFD-compound treated group as compared to the STZ-HFD group (Figure 3.6). These results elaborate that the expression of Lep R and Jak2 was decreased during the diabetic state while, in our STZ-HFD-compound treated group the expression was increased showing the PTP1B inhibitor was successful able to revert the condition. The data were represented as means ± SD. Statistical analysis was done by using One-way ANOVA using Brown-Forsythe test and Bartlett's test. The value of ***p<0.0001 was considered statistically significant.

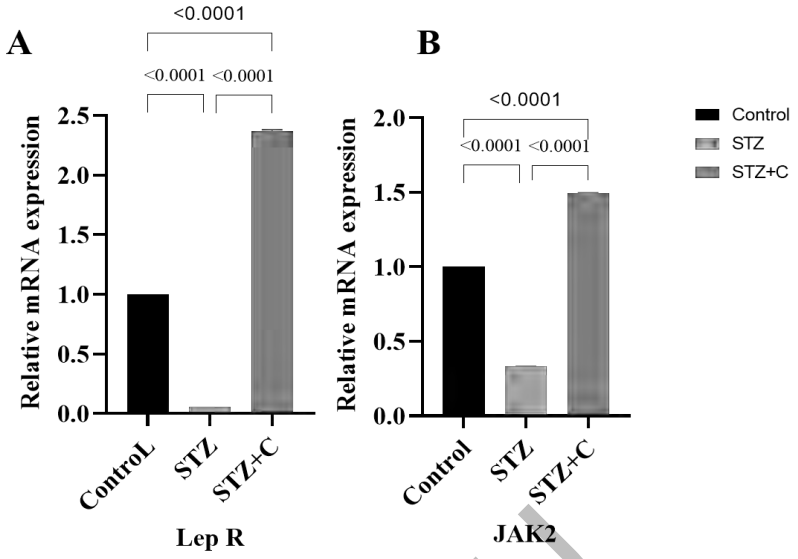

Figure 3.6 Expression of leptin signaling mediators. (A) Relative mRNA expression of Lep R. (B) Relative mRNA expression of JAK2.

To validate the role of leptin on lipolysis and adipose differentiation, we evaluate the gene expression which is involved in lipolysis and differentiation of adipocytes such as PPARα HSL, MGL, and the expression of such genes in the STZ-HFD group was significantly reduced and showed elevated expression in STZ-HFD-compound treated group (Figure 3.7). The results showed that our compound has the potential to negatively regulate the expression of PTP1B. The data were represented as means ± SD. For statistical analysis, we used One-way ANOVA using Brown-Forsythe test and Bartlett's test. The statistically significant value in our data was ***p<0.0001.

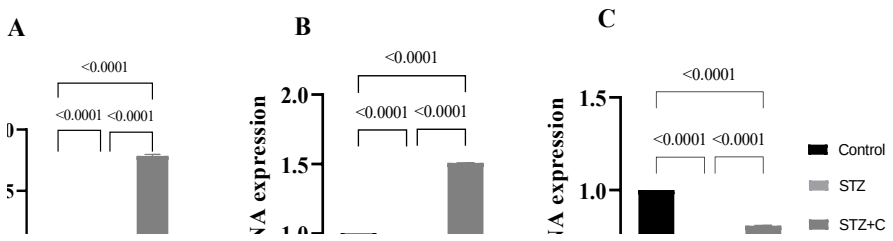

Figure 3.7 Effect of leptin on lipolysis. Relative mRNA expression of PPARα. (B) Relative mRNA expression of MGL. (C) Relative mRNA expression of HSL.

3.3. Targeted gene expression in hypothalamus

Various studies have supported the detrimental effect of PTP1B in the brain on leptin signaling that causes over-weightness and obesity in individuals. Our study was focused to find out the relation between PTP1B and leptin resistance. So, we checked the PTP1B expression at the mRNA level in the hypothalamus of the brain by performing qPCR. Its expression was significantly increased>2 folds in the STZ-HFD group and was found significantly alleviated in the STZ-HFD-compound treated group. Inflammatory markers were also checked like *IL*-6 and *IL*-1β at mRNA level which in turn causes the expression of SOCS3 negative regulator of leptin signaling. The relative mRNA expression of different genes which are under the regulation of leptin was studied in the hypothalamus by using PPiA as a housekeeping gene.

We have found the expression of Lep R, JAK2 and STAT3 were significantly elevated in the STZ-HFD-compound treated group as compared to the STZ-HFD group which showed reduced expression (Figure 3.8). These expressions validate that our compound has the potential to inhibit the expression of PTP1B. The data were represented as means ± SD. One-way ANOVA using Brown-Forsythe test and Bartlett's test was used for statistical analysis. The statistical significance in data was ***p<0.0001.

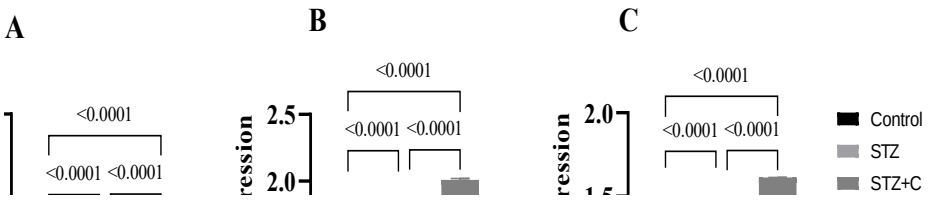

Figure 3.8 Leptin mediators expression. (A) Relative mRNA expression of Lep R. (B) Relative mRNA expression of JAK2. (C) Relative mRNA expression of STAT3.

Further, we have investigated the expression of PTP1B and POMC in the hypothalamus (Figure 3.9). The expression of PTP1B was significantly higher in the STZ-HFD group as compared to the STZ-HFD-compound treated group. The expression of POMC was significantly higher in the STZ-HFD-compound treated group whereas decreased expression in the STZ-HFD group. The significance in our data was ($P<0.0001$). The data were represented as means ± SD. We used One-way ANOVA using Brown-Forsythe test and Bartlett's test for statistical analysis. This result showed that our compound has significantly decreased the expression of PTTP1B and increased the expression of POMC.

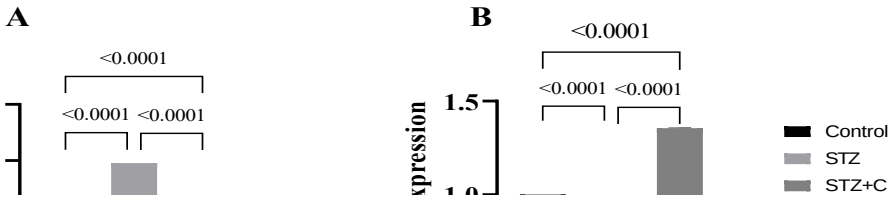

Figure 3.9 PTP1B and POMC expression. (A) Relative mRNA expression of PTP1B. (B) Relative mRNA expression of POMC.

Next, we have found the expression of NPY and AgRP (Figure 3.10). The expression of NPY and AgRP was significantly higher in the STZ-HFD group whereas decreased expression was displayed in the STZ-HFD-compound treated group. The result validates that during leptin resistance (STZ-HFD) where PTP1B expression is higher than normal, both annoreix peptide NPY and AgRP expression is elevated. The expression was then seen to be lowered in the STZ-HFD-compound treated group. The data were represented as means ± SD. We used One-way ANOVA using Brown-Forsythe test and Bartlett's test for statistical analysis. The statistical significance in data was ***p<0.0001.

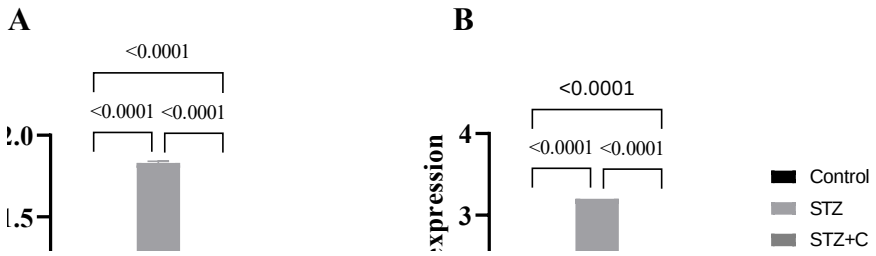

Figure 3.10 Orexigenic peptide expression. (A) Relative mRNA expression of NPY. (B) Relative mRNA expression of AgRP.

Further, we have found the expression of inflammatory markers *IL*-6, *IL*-1*β* and negative regulator of leptin SOCS3 (Figure 3.11). We have found increased expression of *IL*-6, *IL*-1*β* and SOCS3 in the STZ-HFD group whereas, the expression was significantly reduced in the STZ-HFD-compound treated group These results showed that the expression of inflammatory markers is reduced by inhibiting the PTP1B expression. The data were represented as means ± SD. For statistical analysis One-way ANOVA using Brown-Forsythe test and Bartlett's test was used. The statistical significance in data was ***p<0.0001.

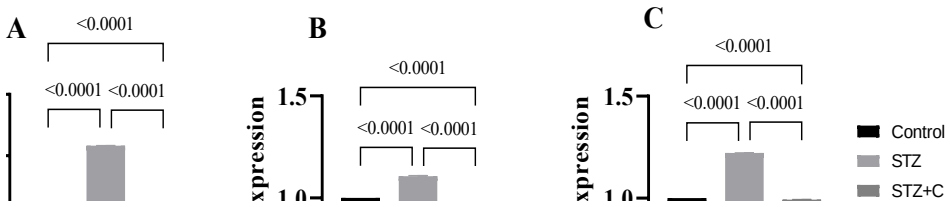

Figure 3.11 Expression of Inflammatory markers and negative regulators of Leptin. (A) Relative mRNA expression of *IL*-6. (B) Relative mRNA expression of *IL*-1*β*. (C) Relative mRNA expression of SOCS3.

4. DISCUSSION

T2DM is characterized by a persistent hyperglycemic and insulin resistance state (Tangvarasittichai, 2015). It is been characterized by disruptions in the metabolism of fats, carbohydrates, and protein that result in deficiencies of insulin secretion, insulin action, or both. In some conditions i.e., diabetes, the *β*-cells of the pancreas are destroyed which can affect the production of insulin and hence lead to diabetes (Alberti & Zimmet, 1998). Persistent hyperglycemia condition leads to a number of micro and macro-complications that collectively disrupt the body's homeostasis.

Adipose tissue is composed of a diverse variety of cells that are involved in the secretion of various types of hormones, cytokines, and chemokines. Adipose tissue is surrounded by a number of cells including stromal cells, immune cells, fibroblasts, and macrophages. With the discovery of the leptin hormone, adipose tissue is not only considered as a site for energy reservoir but its true essence are still unraveled (EE Kershaw and JS Flier 2004). Leptin has been studied enormously in order to find its relation between central and peripherals organs to identify its role in regulating the cycle of hunger and energy hemostasis (RS Ahima and JS Flier 2000). Literature shows that leptin has a direct effect in controlling body weight and improving the metabolic regulation of rodents (LA Campfield *et al*., 1995; JL Halaas *et al*., 1995; TW Stephens *et al*., 1995). The focus of our study was to explore the changes in the regulation of leptin hormone in high glycemic conditions. Recently, a protein named Protein Tyrosine Phosphatase 1B (PTP1B) is considered a new road in treating diabetes and its associated complications. PTP1B is involved in negatively regulating the signaling pathways that are mediated via tyrosine kinase. The main targets of PTP1B are insulin and leptin signaling pathways. A number of studies reported that ER stress and inflammatory cytokines are responsible for the enhanced expression of PTP1B in the liver, muscle, fat, and hypothalamus, which propagates leptin resistance and IR (Agouni *et al*., 2011). PTP1B enhanced expression in insulin-sensitive tissues has been reported in STZ-induced diabetic models of rats and mice (Mohammad Taghvaei *et al*., 2011). During the condition of ER stress, over expression of PTP1B take place which

inhibits the leptin signaling and subsequently results in leptin resistance (O Grunzdeva *et al*., 2019).

PTP1B inhibitors are found to be the potential and novel drugs for the medication of obesity and T2DM (Nurul Islam *et al*., 2013). PTP1B is commonly expressed in the human brain, liver, muscle, and adipose tissues (Zabolotny *et al*., 2008). PTP1B is involved in insulin and leptin resistance and its inhibition is the most potent therapeutic target in human diseases (Montalibet and Kennedy 2005).

The current study was designed to investigate the effect of inhibiting the PTP1B on leptin signaling specifically in the brain and adipose tissue. For this purpose, we have generated a mouse model having leptin resistance induced by feeding them with HFD and carbohydrate combination that causes a decrease in plasma leptin level (CL White *et al*., 2009). PTP1B is also found to be expressed in the hypothalamus of the brain (JM Zabolotny and KK Bence 2002). Multiple low doses of STZ in combination with HFD were used in generating a model having T2DM and leptin resistance. The toxic effects of STZ cause partial damage to the *β*-cells of the pancreas and triggered an inflammatory process, aggravating the damage. (Like & Rossini 1976). The increased level of PTP1B is reported in insulin-sensitive tissues in STZ-induced diabetes in mice and rats (Adeli *et al*., n.d.). Leptin signaling is inhibited by the expression of PTP1B, which directly dephosphorylates the tyrosine residues on leptin receptor (J Peng *et al*., 2021). PTP1B is overexpressed in obesity and in IR in adipose tissues and hypothalamus (Ahmad *et al*., n.d.). Overexpression of PTP1B can inhibit the leptin signaling pathway and thus causes leptin resistance in the hypothalamus (O Gruzdeva *et al*., 2019).

In our present study, we evaluate the role of PTP1B on signaling pathways and also examine the protective role of the PTP1B inhibitor in dysfunctional leptin and insulin signaling (N Krishnan *et al*., 2018). As predicted, our PTP1B inhibitor in the compound treated group was able to revert the leptin hormone by inhibiting the PTP1B and we found an increased mRNA expression of leptin, and a decrease expression of PTP1B which goes in line with our hypothesis. Furthermore, we evaluated the insulin sensitivity in adipocytes and found a significant retrieval from insulin resistance to normal insulin signaling pathway. Insulin signaling initiators such as *mIRS1, mIRS2,*

mPI3K, and *mAKT2* expression were found significantly enhanced in our compound-treated group. Our study is also supported by other studies that PTP1B deletion in the adipose leads to enhanced glucose uptake and elevates adipose insulin sensitivity (M Teimouri *et al*., 2022; LA Méndez-Garcia *et al*., 2018). A study reported that during leptin resistance *mTORC1* expression was also reduced (G Marwarha *et al*., 2010). In our study, we found a similar case, the expression of *mTORC1* was reduced in the STZ-HFD case but was significantly increased in our compound treated group.

Additionally, to explore the role of leptin on lipolysis and adipose differentiation and evaluate how PTP1B is able to regulate the expression of the genes involved in lipolysis and differentiation of adipocytes. We measured the relative expression of *mPPARα, mHSL, mMGL*, and found a significant reduction in the STZ-HFD group and elevated expression in STZ-HFD-compound treated group. Our study is also supported by a study that during leptin resistance the expression of *PPARα* is reduced and once the leptin signaling pathway is reverted the expression increases (A Yadav *et al*., 2013; MY Wang *et al*., 1999). Shred of evidence shows that leptin has a potent role in lipolysis by expressing *mATGL, mMGL*, and *mHSL* (Y Li *et al*., 2008). Our study also supports the literature as we also found a decreased level of *mHSL* and *mMGL* in the leptin resistance model, but the concentration was significantly increased after treating with our compound.

Various reports have supported the negative effect of PTP1B in the brain on leptin signaling. Our study also focused to find out the relation between PTP1B and leptin resistance in the brain. Therefore, we checked the PTP1B expression at the mRNA level in the hypothalamus of the brain and found an increase in expression in our disease model and significant decrease in the compound treated group. Moreover, we also investigate the anorexic effect of leptin by measuring the expression of *mNPY* and *mAgRP* peptides, which is a potent stimulator of food intake. We found an elevated level of expression of the said peptides, but their expression was reduced in our compound treated group.

Our study was also supported by the study that concludes that leptin suppresses *mNPY* and *mAgRP* during normal signaling pathways (A Guzman *et al*., 2019; H Munzberg *et al*., 2020). Some pieces of evidence reported the decreased expression of *mPOMC*

neurons during leptin resistance (IV Romanova *et al*., 2018). A reduction in the expression of the *mJAK2* and *mSTAT3* has been observed in leptin resistance (H Liu *et al*., 2021). The same was observed in our study, where we found a decreased level of concentration in leptin-resistant model but an expression of the said gene mimics the normal level after treatment with our compound. The expression of leptin receptors is also reduced during our study, and this has been supported by other studies that stated that during leptin resistance the expression of leptin receptors in the hypothalamus is also reduced (J Wauman *et al*., 2011). We also found a decreased concentration of *mPOMC* in the STZ-HFD case while increased expression was observed in the compound-treated group. Further, we have analyzed the expression of inflammatory markers *mIL-6, mIL-1β*, and negative regulators of leptin *mSOCS3*. We found an increased expression of *mIL-6, mIL-1β*, and *mSOCS3* in the STZ-HFD group whereas, it was significantly reduced in the STZ-HFD-compound treated group. These results showed the expression of inflammatory markers is reduced by inhibiting the PTP1B expression. Our results were consistent with the previous report which stated that *IL*-6 overexpression can also cause the expression of *mSOCS3* (AR Lubis *et al*., 2008).

Summarizing the whole study postulate that the T2DM mice model with a low dose of STZ and HFD, causes inflammation and over-expression of PTP1B. Overexpression of PTP1B also causes leptin resistance; leptin is an anorexic hormone that tends to balance food intake and energy consumption by regulating the hypothalamus and adipocytes. Our phytochemical flavanolic compound viscosol having anti-inflammatory activities, also have PTP1B inhibitory activity. To conclude, our compound can be a potential drug to relieve inflammation, insulin- and leptin resistance and this will help to develop a new pharmacological drug for the treatment of T2DM and leptin resistance by specifically inhibiting PTP1B.

5. Conclusion

Our study reveals the effect of PTP1B on the leptin signaling pathway in the combined diabetic and leptin resistance model via measuring the leptin signaling mediators involved through a multifaceted mechanism involved in the disruption of the hunger cycle and energy homeostasis. The flavanolic compound viscosol (5, 7-dihydroxy-3, 6-dimethoxy-2-(4-methoxy-3-(3-methyl but-2-enyl) phenyl)-4H-chromen-4-one) has anti-diabetic and anti-leptin resistant properties. The present study confirms that the therapeutic administration of our compound is successfully involved in the inhibition of *De-novo* lipogenesis, halting of adipocytes differentiation, genes regulating fatty acid hydrolysis, formation of lipid droplet structure, feeding sensation, and anorexic effect in adipose and the brain. The compound also exhibited a protective effect against inflammation via downregulating the expression of inflammatory markers *(IL*-6, *IL*-1β). Conclusively, our results reinforced our hypothesis that PTP1B is involved in the leptin signaling pathway and by targeting PTP1B, not only the hyperglycemic condition was reverted, but it might also help in regulating the leptin and its downstream signaling in adipose and the brain.

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