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ANTIDIABETIC AND HEPATOPROTECTIVE ACTIVITIES OF *BOMBAX CEIBA* EXTRACT IN OBESE RATS WITH METABOLIC SYNDROME

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ABSTRACT

Increased global obesity prevalence is a serious issue as it causes several other chronic metabolic disorders, including diabetes mellitus, one of the major global pandemics a few days ago. Obesity increases the propensity by several folds to develop insulin resistance and type 2 diabetes mellitus (T2DM). Our aim was to investigate the hypoglycemic, hypolipidemic and hepatoprotective activities of *Bombax Ceiba* extract on the biochemical and histological evaluation in obese rats fed High-carbohydrate high-fat diets (HCHFD) to prevent of metabolic diseases that cause obesity.

Sixty male rats were divided randomly (12 rats/ group) into 5 experimental groups. First (control) group: 12 rats were fed with standard diet. 2nd group (Bombax) rats were fed on standard diet and *Bombax ceiba* extract (400 mg/kg body weight) was administrated daily orally. The third group (HCHFD), rats were fed on HCHF diet whereas rats in the fourth group Bombax + HCHFD (Prophylactic) rats fed on standard diet and take *Bombax ceiba* extract (400 mg/kg body weight orally daily for 8 weeks then fed on HCHF diet and take *Bombax ceiba* extract to the end of experiment. Fifth group HCHF + Bombax (Treatment) rats were also fed on HCHF diet then take *Bombax ceiba* extract (400 mg/kg body weight daily). The experiment period was 20 weeks. Blood glucose, insulin, HOMA-IR, lipid profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), adropin,interleukin-6 (IL-6), retinol binding protein-4 (RBP-4), salusin-α and salusin-β were measured**.** Also histopathology for aorta tissue was done.

Our findings have shown significant increases in glucose, HOMA-IR, cholesterol, triglycerides, AST, ALT, IL-6, RBP-4 and salusin-β levels with significant decrease in insulin, high density lipoprotein (HDL), adropin and salusin-α, in HCHFD group in comparison with the control group,these findings improved in prophylactic and treated groups after treatment with *Bombax ceiba* extractin compare to HCHFD group.

Our study concluded that *Bombax ceiba* extract has potential hypoglycemic, hypolipidemic and hepatoprotective activities on the prevention of metabolic diseases resulting in rats fed HCHF diet and the histological evaluation confirm our biochemical results.

Keywords: Bombax, Salusins, Adropin, RBP-4, Metabolic syndrome, Rats.

Introduction

Metabolic syndrome (MetS) was conceptualized in people susceptible to cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) on the basis of a constellation of risk factors, such as elevated fasting plasma glucose (FPG), atherogenic dyslipidemia, elevated blood pressure, and abdominal obesity. A number of studies have shown a strong association between MetS and its components and the risk of a T2DM incident. Clinical consequences of MetS should therefore be concentrated on multifactorial interventions to reduce the risk of T2DM (Lee *et al*., 2020).

Symptoms of metabolic syndrome such as hypertension, dyslipidemia, impaired glucose tolerance, excess fat deposition, increased proinflammatory markers, and reduced antioxidant defenses were caused by high-carbohydrate, high-fat diet (HCHFD) in rats (Badawy *et al*., 2020).

Salusins are two neuropeptide hormones synthesized from preprosalusin, 28-amino acids Salusin-alpha (Sal- α) and 20 amino-acids Salusin-beta (Sal- β) (Watanabe *et al*., 2008). Atherosclerosis is likely to be prevented by Salusin- α, while salusin- β may act as a potential proatherogenic factor. These two peptides induce opposite effects on macrophage foam cell formation (Zhou *et al*., 2012 and Zhou *et al*., 2014). Interestingly, salusins are also expressed in human atherosclerotic plaques (Watanabe *et al*., 2008). Salusins were found in biological fluids studied in children and adolescents with essential hypertension, such as blood and urine and they found that serum salusin- β positively correlated with triglycerides (TG) level and TG/HDL cholesterol ratio levels. Increased salusin- α expression is correlated with better lipid profiles, while increased expression of salusin- β is correlated with poorer lipid profiles. It remains unclear the exact relationship and mechanisms by which salusins influence lipid metabolism and atherosclerosis (Niepolski *et al*., 2015).

During the study of obese insulin resistant mice, adropin was identified as a new factor linking nutrient intake

signals with metabolic homeostasis. It is a small peptide encoded by the gene related with energy homeostasis that is illustrated primarily in the liver and brain. Adropin is involved in the regulation of glucose homeostasis and lipid metabolism (Kumar *et al*., 2008). Human, mouse, and rat adropin amino acids sequencing are 100 percent similar. The half-life has of adropin has still not been defined. The half-life of this peptide is believed to be as short as several minutes (Aydin, 2014). The role of adropin in metabolism is supported by the finding that synthetic peptide therapy improves glucose homeostasis, fatty liver, and dyslipidemia seen in animal models of obesity (Kumar *et al*., 2008). In addition, lower levels of adropin were associated with insulin resistance in both humans and mice (Butler *et al*., 2012). Elevated levels of adropin were noticed in mice fed a high fat and low carbohydrate diet, whereas decreased levels were reported in mice fed a low fat and high carbohydrate diet. In addition, adropin was protected by the regulation of lipid and glucose metabolism against obesity-associated hyperinsulinemia and hepatosteatosis (Kumar *et al*., 2012).

There is no clearly defined relationship between adropin, insulin resistance and glucose tolerance (Wu *et al*., 2014). Changes in adropin expression were examined in the different tissues of rats after streptozotocin induced diabetes. Adropin is involved in the endothelial function and retardation of atherosclerosis by up-regulating endothelial nitric oxide synthase (eNOS). Peptide may therefore be a new regulator of eNOS via these dual pathways (Lovren *et al*., 2010). Butler *et al*. (2012) In MetS patients after gastric bypass surgery, adropin levels were shown to be negatively correlated with triglycerides, LDL cholesterol, and apolipoprotein B (ApoB) levels. Moreover, in his research, positive correlations were found between adropin with HDL cholesterol and free fatty acids. This author assumed that adropin might influence lipids metabolism (synthesis or clearance).

Initially, retinol-binding protein 4 (RBP4) was identified as a liver-secreted hormone and acted as a transport protein for vitamin A to simplify the transport of retinol to peripheral tissues from the liver. Latest studies, however, have shown that RBP4 could be a novel adipokine with possible interference in pathogenesis of insulin resistance and type 2 diabetes mellitus (Jin *et al*., 2020).

During exercise, interleukin (IL)-6 is secreted from skeletal muscle (SkM) during exercise and has been shown to influence liver metabolism. However, it is not known whether SkM IL-6 is implicated in regulating exercise training-induced counteraction of changes in metabolism of carbohydrates and lipids in the liver in when feeding with high-fat diet (HFD) (Kristófa *et al*., 2019).

The discovery of a powerful, unharmed and cost-effective hypoglycemic agent is essential. Since ancient time, diabetes therapy has been carried out orally with many other medicinal plants or their extracts based on their folkloric reputation. The use of traditional plants as a treatment for diabetes has also recently been recommended by WHO (Kumar and Kumar, 2011). In developing and developed countries, herbal medicine and herbal drugs are used gradually increasing over the last few years (Hemant *et al*., 2009). Therefore, in the treatment of human diseases searching for powerful pharmacologically active compounds from natural sources such as medicinal plants or their extracts, leading to the discovery of different useful drugs with valuable therapeutic activities (Alim *et al*., 2012). We have selected *Bombax ceiba* (B. *ceiba*) plant, which has multiple biological effects, including hypoglycemic activity.

Bombax ceiba is a lofty, deciduous tree, a member of the family Malvaceae. It is found worldwide in temperate and tropical Asia, Africa and Australia and commonly known as the silk cotton tree. Therapeutic potentials against various diseases such as diabetes, infections, asthenia, polyurea, glycosuria and hepatic toxicity are reported in different parts of this plant. Although various portions of *B. ceiba* has different therapeutic activities, including hypoglycemic, anti-inflammatory, analgesic, antioxidant, antimicrobial and hypotensive activity, but only little research has been reported to evaluate the effect of ethanolic extract of *B. ceiba* young roots on diabetes and liver toxicity (Rehman et *al*., 2017).

Our objective was to investigate the hypoglycemic, hypolipidemic and hepatoprotective activities of *Bombax Ceiba* extract on the biochemical and histological evaluation in obese rats fed HCHF diet for the avoidance of obese-causing metabolic diseases.

Materials and Methods

Plant Materials

Worldwide chemical studies have shown that *Bombax ceiba* roots are used for therapeutic reasons due to its high content of lupeol, β-sitosterol and sesquiterpenes, which are beneficial for the treatment of certain diseases. In addition to the above active ingredients, the different parts of *Bombax ceiba* have been known to contain many important phytoconstituents substances, including alkaloids, glycosides, flavonoids, steroids, saponins, phytosterols and triterpenoids (lupeol and beta-sitosterol), phenolic compounds and tannins **(Jalapure and Gadge, 2011).**

Preparation of Ethanolic Extract

The dried coarse powders was soaked in an in amber-colored extraction bottle with 1.5 L of 95 percent ethanol. With occasional shaking and stirring, the bottles were sealed and kept at room temperature for 7 days. The extracts were purified via cotton followed by Whatman No.1 filter paper and concentrated under reduced pressure at 50°C with a rotary evaporator (Bibby Sterlin Ltd, UK) to provide brown-colored, extract of powdery crude.

Qualitative Phytochemical Profiles

Test for Carbohydrate (Benedict's test)

1 ml of *Bombax ceiba* extract solution was added to 5 ml of Benedict's reagent and boiled for 2 minutes and then cooled. The presence of carbohydrates is shown by the formation of a red precipitate.

Test for Saponins (Foam test)

In a test tube, 10 mg of *Bombax ceiba* extract was taken and vigorously shaken with 5 ml of H_2O. The producing of persistent foam confirms that saponins are present.

Test for Flavonoid, Phenol and Tannin (Ferric chloride test)

In 2 ml of water, 5 mg of the *Bombax ceiba* extract was dissolved. 1 ml of neutral 5 percent solution of ferric chloride

was added. The presence of flavonoid, phenols and tannin is indicated by a dark green color.

Test for Proteins (Biuret Test)

3-5 mg of *Bombax ceiba* extract was added in 4 percent NaOH and a little drop of 1 percent $CuSO_4$ solution then added. A violet or pink color confirms that protein is present.

Test for Steroids (Liebermann-Burchard test)

An extract of *Bombax ceiba* was dissolved in 1 ml of chloroform. We added 2 ml of acetic anhydride and 1 ml of concentrated sulfuric acid. The development of a greenish color solution shows that steroids are presence.

Test for Phytosterol (Salkowski's reaction)

2 ml of extract of *Bombax ceiba*, 2 ml of chloroform and 2 ml of concentrated H2SO4 were added and vigorously shaken. The chloroform layer is greenish yellow fluorescence, confirming that phytosterol is present.

Test for Amino Acids (Ninhydrin test)

2 ml of ninhydrin solution was added to an aliquot of the diluted extract. The formation of a violet color indicates that amino acids are presence.

Test for Glycosides (Keller- Killani Test)

Approximately 2 ml of extracts were taken in a test tube and 1 ml of glacial acetic acid was carefully added, consisting of a trace amount of FeCl3 to the extract and 1 ml of concentrated H2SO4. At the junction of two layers, a reddish-brown color is formed, and in the presence of glycosides, the upper layer turns to bluish green.

Test for Alkaloids (Wagner's Test)

Bombax ceiba was injected into a test tube. 5 ml of 1 percent HCl was added to the test tube in a stream bath and stirred. Some drops of Wagner's reagent were added after the solution was filtered. The formation of a reddish brown precipitate indicates that alkaloids are present.

Preparation of Bombax Dose

Using sterilized water, a dose (120 mg/kg body weight) of standard metformin is prepared by. *Bombax ceiba* extract is dissolved in 10 percent Dimethyl sulfoxide (DMSO) in order to prepare a 400 mg/kg body weight concentration dose (Khurshid *et al*., 2018).

Experimental study design: (Duration of experiment = 20 weeks)

The Ethics Committee of the National Research Centre approved all the experimental protocols. Sixty male rats (4-6) weeks old, weighing 95 - 106 g, were obtained from Animal House of the National Research Centre. The rats were split randomly into 5 experimental groups (12 rats/ group). 1st group (control): 12 rats were fed with standard diet. 2nd group (Bombax) rats were fed on standard diet and take *Bombax ceiba* extract (400 mg/kg body weight daily) orally. 3rd group (HCHF), rats were fed on HCHF diet whereas rats in the 4th group Bombax + HCHFD (Prophylactic) rats fed on standard diet and take *Bombax ceiba* extract 400 mg/kg body weight orally daily for 8 weeks then fed on HCHF diet and take *Bombax ceiba* extract to the end of experiment . 5th group HCHF + Bombax (Treatment) rats were also fed on HCHF diet and take *Bombax ceiba* extract (400 mg/kg body weight daily). The experiment period was 20 weeks.

Diet-induced Metabolic Syndrome in Rats

In this study, feeding rats with a high-carbohydrate high-fat (HCHF) diet was used to induce a model to more closely mimic the changes associated with human metabolic syndrome (endothelial dysfunction, diabetes, obesity along with nonalcoholic fatty liver disease) according to Christopher *et al*. (2007). Also, standard diet was prepared according to Reeves (1997) (Table 1).

Table 1 : Components of high-carbohydrate high-fat diet (HCHFD) and standard diet (low fat diet):

Product	HCHFD		Standard Diet	
	gm%	kcal%	gm%	kcal%
Protein	20	17	14.2	17.7
Carbohydrate	50	43	73.1	75.9
Fat	21	40	4.0	9.4
Total (kcal/gm)	**4.68**	**100**	**3.85**	**100**

Sampling: Animals were fasted overnight; three ml of blood was aspirated under formalin anesthesia from the peripheral vein of the tail, and then centrifuged for 15 min at 3000 rpm to obtain clear serum which stored at -80°C till the day of the evaluation. Serum lipid profile and glucose level were measured immediately once every week to follow up the induction of obesity and diabetes**.**

Anthropometric measurements: The weight of the rat in grams (gm) was measured by digital balance once every week.

A-Biochemical parameters measured are:
Determination of Fasting Glucose level:

The serum glucose level was measured by standard commercial colorimetric enzymatic assays (BioMerieux, Marcy l'Etoile, France; Roche Diagnostics, Basel, Switzerland).
Based on the Passing and Bablok, (1983) methods.

Determination of Insulin level:

According to Yallow and Bawman (1983), serum insulin levels were estimated by an enzyme-linked immunosorbent assay (ELISA) using BioSoure INSEASIA Co. (Nivelles, Belgium) Kit.

Insulin resistance was calculated from the following equation:

HOMA-IR formula (homeostatic model assessment for insulin resistance) = fasting insulin (μIU/m) X fasting glucose (mg/dl) / 405 Mathews *et al*. (1985).

Determination of Lipid profile level:

The serum levels of total cholesterol (TC), high density lipoprotein (HDL-cholesterol) and triglycerides (TG) were measured colorimetric enzymatic assays (BioMerieux, Marcy l'Etoile, France; Roche Diagnostics, Basel, Switzerland) according to the method of Kwang *et al*. (2007), Lopez-Virella (1977) and Cole *et al*. (1984) respectively.

Determination of liver enzymes:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by standard commercial colorimetric enzymatic assays (BioMerieux, Marcy l'Etoile, France; Roche Diagnostics, Basel, Switzerland) according to the method of Reitman and Frankel. (1957).

Determination of serum level of adropin:

According to Topuz *et al.* (2013) using the manufacturers protocols (R&D systems), the levels of adropin (myokine) in the samples were determined using ELISA for rats.

Determination of serum level of interleukin-6 (IL-6):

The levels of IL-6 (Pro-inflammatory adipokine) in the samples were determined using ELISA for rat according to Kimura and Kishimoto, (2010) using the manufacturers protocols (R&D systems).

Determination of serum level rat of retinol binding protein-4 (RBP-4):

The levels of RBP-4 (Pro-inflammatory adipokines) in the samples were determined using ELISA for rat according to Rosales *et al.* (1996) using the manufacturers protocols (R&D systems).

Determination of serum level rat of salusin-alpha and salusin-beta

The levels of salusin-alpha (Sal-α) and salusin-beta (Sal-β) in the samples were determined using ELISA for rat according to Sato *et al.* (2006) & Sahin and Aydin. (2013) respectively using the manufacturers protocols (R&D systems).

Histopathological:

Aorta specimens from all animals were immediately dissected after death and fixed for at least 72 hours in 10 percent neutral-buffered formal saline. All the specimens were washed for half an hour in tap water and then dehydrated, cleared in xylene and embedded in paraffin, in ascending alcohol grades. Serial sections of 6 µm thick were cut and stained with haematoxylin and eosin (Drury and Walligton, 1980) for histopathological investigation after one month of feeding. Images were captured and processed using version8.0 of Adobe Photoshop.

Statistical Analysis

All analysis was performed on a personal computer using the social science statistical package (SPSS) version 9 software. A mean ± standard error (SE) was expressed for all numeric variables. Comparisons of means were made using the independent-sample T test. Pearson's correlation coefficient was obtained and a 'p' value <0.05was regarded as statistically significant.

Results

Table 2 : Antioxidant activity of total extract of *Bombaxcebia*

Antioxidant activity of total extract of *Bombaxcebia*	
Concentration (PPm)	**% Inhibition**
0	0
1	46.89
2	58.82
4	61.58
8	61.58
16	66.1
32	70.62
64	71.75
IC_{50}	**72.77**

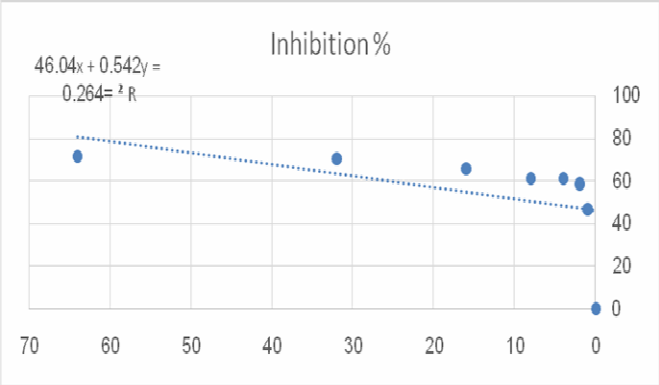

Fig. 1 : Antioxidant activity of total extract of *Bombax cebia*

Table 3 : Phytochemical constituents of ethanolic extract of *Bombax cebia*

Phytochemicals Constituent	Detection test	Result
Saponins	Foam	-
Sterols	Libermann-Burchard	+++
Phytosterol	Salkowski	-
Flavonoids, phenolic compound and Tannins	Ferric chloride	+++
Proteins	Biuret	-
Alkaloids	Mayer Wagner's	++
Carbohydrates	Benedicts	-
Amino acids	Ninhydrin	-
Fixed oils and Fatty acid	Spot	-
Cardiac glycosides	Keller-Killani	+

Table 4 : Fasting glucose, insulin and HOMA-IR in different studied groups:

	STD Control n=12	STD+ Bombax n=12	HCHFD n=12	Bombax+ HCHFD (Prophylactic) n=12	HCHFD+ Bombax (Treatment) n=12
Glucose(mg/dl)	89.5 ± 1.5 [a]	95.5 ± 2.8[b]	222 ±3.4 [c]	97.5± 2.3 [b]	133.5 ± 2.9[d]
Insulin (µIU/ml)	14.6 ± 0.8[a]	13.6 ± 1.9[a]	7.4 ± 0.5[b]	12.9 ± 0.9 [a]	10.4 ± 0.8 [c]
HOMA-IR	3.2 ± 0.5 [a]	3.2± 0.3 [a]	4.1 ± 0.7 [b]	3.1 ± 0.4 [a]	3.4 ± 0.6 [a]

The values are expressed as mean ± standard error (SE). In each group, n denotes the number of rats. Different letters (superscripts a, b, c, d) indicate statistical differences (One-way ANOVA with post hoc test. a value of $p<0.05$ was considered to be statistically significant).

Significant increases in glucose and HOMA-IR levels were shown in Table (4) with a marked decline in insulin level in the HCHFD group when compared with control group; these results improved after treatment with *Bombax ceiba* extract compared to HCHFD group and the prophylactic group showed remarkable results by returning insulin and HOMA-IR to normal levels.

Table 5 : Lipids profile and liver enzymes in different studied groups:

	STD Control n=12	**STD+ Bombaxn n=12**	**HCHFD n=12**	**Bombax+ HCHFD (Prophylactic) n=12**	**HCHFD+ Bombax (Treatment) n=12**
Cholesterol (mg/dl)	65.2 ± 5.04 [a]	60.48 ± 8.1 [a]	133.4 ± 11.2[b]	72.5 ± 2.65 [c]	99.2 ± 7.4 [d]
Triglyceride (mg/dl)	70.58 ± 2.5[a]	71.6 ± 3.8[a]	176.5 ± 3.6[b]	90.6 ± 2.6[c]	120.7 ±3.5[d]
HDL-cholesterol (mg/dl)	58.3 ± 2.7 [a]	59.3 ± 3.6 [a]	40.5 ±3.9 [b]	50.8 ± 2.6 [a]	48.3 ± 2.9 [a]
AST(U/L)	130.1±5.8[a]	125.9±6.7[a]	388.5±12.9[b]	135.75±7.6[a]	144.81±6.1[c]
ALT(U/L)	55.3±5.9[a]	50.9±4.6[a]	181.9±6.8[b]	59.6±4.1[a]	64.4±3.8[c]

The values are expressed as mean ± standard error (SE). In each group, n denotes the number of rats. Different letters (superscripts a, b, c, d) indicate statistical differences (One-way ANOVA with post hoc test. a value of $p<0.05$ was considered to be statistically significant).

Table (5) showed significant increases in cholesterol, triglycerides, AST and ALT levels with marked decline in HDL level in HCHFD group when compared with control group, improving these results after treatment with *Bombax ceiba* extract compared to HCHFD group and the prophylactic group showed remarkable results by returning lipid profile and liver enzymes (AST ,ALT) to normal levels.

Table 6 : Serum Adropin, IL-6, RBP-4, Salusin-α and Salusin-β in different studied groups:

	Control (STD) n=12	**STD+ Bombax n=12**	**HCHFD n=12**	**Bombax+ HCHFD (Prophylactic) n=12**	**HCHFD+ Bombax (Treatment) n=12**
Adropin (pg/ml)	857 ± 74.5 [a]	929 ± 73.5 [b]	628.1 ± 43.8 [c]	884 ± 107[d]	800± 63.4[e]
IL-6 (pg/ml)	72.3 ± 9.3 [a]	58.4 ± 6.1 [b]	96.6 ± 7.9[c]	45.6 ± 3.2[d]	59.3 ± 10.8[b]
RBP-4 (pg/ml)	141.6 ± 9.7 [a]	124.2 ± 22.85[b]	496.3 ± 62.6[c]	135.5 ± 18.8[a]	157.5 ± 16.1[d]
Salusin-α (pg/ml)	174.6 ±13.9 [a]	292.4 ± 14.4[b]	127.8 ± 10.5[c]	197.5 ± 11.0[d]	174.5 ± 10.7[a]
Salusin-β (pg/ml)	3.24 ± 1.06 [a]	1.7± 0.3 [b]	4.6± 1.8[c]	1.94± 0.38[b]	1.83 ± 0.01[b]

The values are expressed as mean ± standard error (SE). In each group, n denotes the number of rats. Different letters (superscripts a, b, c, d) indicate statistical differences (One-way ANOVA with post hoc test. a value of $p<0.05$ was considered to be statistically significant).

Table (6) showed significant increases in IL-6, RBP-4 and salusin-β levels with significant decrease in adropin and salusin-α level in HCHFD group as compared to the control group, such findings have improved in prophylactic and treated groups after treatment with *Bombax ceiba* extract when compared with HCHFD group. Furthermore, there was a marked improvement in all markers in the prophylactic group compared to the treated group.

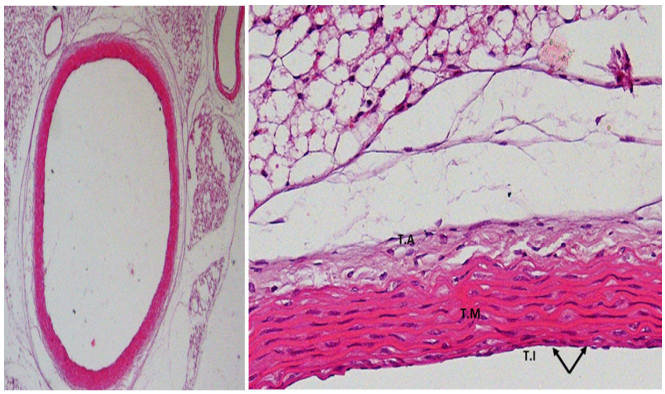

Fig. 2 : A photomicrography of aorta tissue for standard diet group (control group) showing in left photo complete and average thickness of aorta and in right one with higher magnification showing the layers of elastic aortic artery as the inner most tunica intima (T.I) which formed of endothelial cells, then the tunica media (T.M) formed of collagenous fibers, elastic lamellae and smooth muscle cells and the outermost layer tunica adventitia (T.A) formed of connective tissue (H&E 100x,200x).

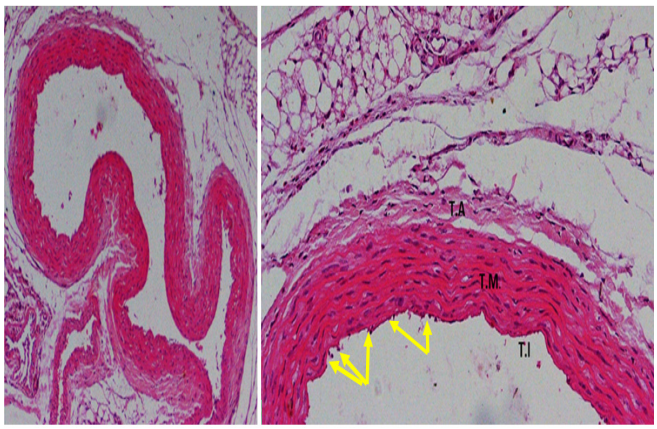

Fig. 3 : A photomicrography of aorta tissue for high-carbohydrate high-fat diet (HCHFD) showing in left photo mildly thickened and tortuous aorta and in right one with higher magnification showing the layers of elastic aortic artery as the inner most tunica intima (T.I) which formed of endothelial cells, which also showing deposition of many foam cells (yellow arrows) then the tunica media (T.M) formed of collagenous fibers, elastic lamellae and smooth muscle cells and the outermost layer tunica adventitia (T.A) formed of connective tissue (H&E100x,200x).

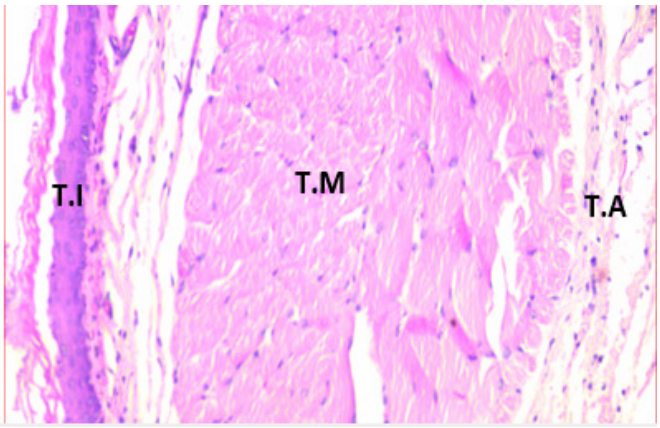

Fig. 4 : A photomicrography of aorta tissue for standard diet group; *Bombax ceiba* extract showing the layers of elastic aortic artery as the inner most tunica intima (T.I) which formed of endothelial cells, then the tunica media (T.M) formed of collagenous fibers, elastic lamellae and smooth muscle cells and the outermost layer tunica adventitia (T.A) formed of connective tissue, there is some sort widening between T.I and T.M due to mild edema (H&E200x).

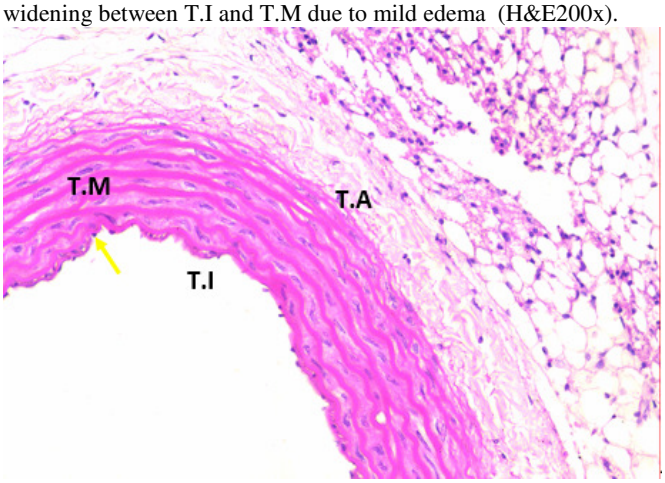

Fig. 5 : A photomicrography of aorta tissue for HCHF; *Bombax ceiba* extract (treatment) showing the layers of elastic aortic artery as the inner most tunica intima (T.I) which formed of endothelial cells with very few foam laden cells (yellow arrows), then the tunica media (T.M) formed of collagenous fibers, elastic and smooth muscle cells and the outermost layer tunica adventitia (T.A) formed of connective tissue are looking normal (H&E 200x).

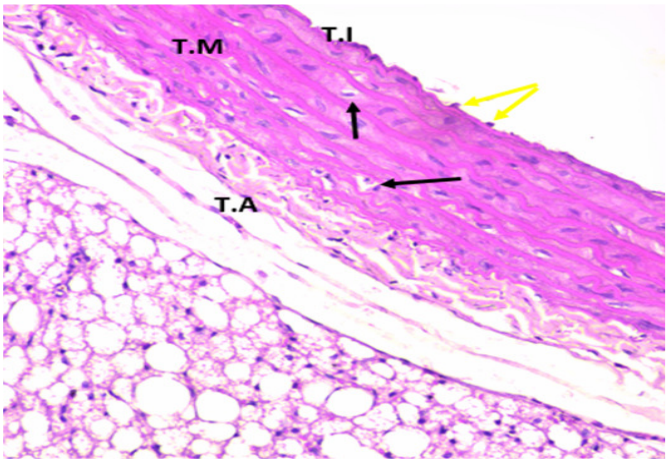

Fig. 6 : A photomicrography of aorta tissue for HCHF; *Bombax ceiba* extract (prophylactic) showing the layers of elastic aortic artery as the inner most tunica intima (T.I) which formed of endothelial cells interrupted by foam laden cells (yellow arrows), then the tunica media (T.M) formed of collagenous fibers, elastic and smooth muscle cells with some cells have signs of degeneration with pyknotic nuclei (black arrows) and the outermost layer tunica adventitia (T.A) formed of connective tissue (H&E 200x).

Discussion

High-carbohydrate high-fat diets that induced diabetes in rats were recognized by hyperglycemia, compensatory hyperinsulinemia, elevated triglycerides and hypertriglyceridemia analogous to those of the later stage of type 2 diabetes (Guang-Kai *et al.,* 2017).

Hyperglycemia is the most important feature of diabetes mellitus. The significant decreases in the blood glucose level of HCHF diet induced by diabetic rats following administration of Bombax ceiba extract have hypoglycemic activity. As showed in Table (3), this can be attributed to the phytochemical components of the extract, such as flavonoids, alkaloids, tannins and phenolic compounds. Rani *et al.* (2012) observed that flavonoids significantly decrease level of glucose by stopping the action of α-glucosidase enzyme. Generally, alkaloids stop the action of the α-glucosidase enzyme and reduce glucose transport via the intestinal epithelium cell (Mishra *et al*., 2010). Our study was in agree with the previous study of Khurshid *et al*. (2018) which postulated that phytochemicals detected in *Bombax ceiba* extract decreased the level of glucose by stopping the action of α-glucosidase enzyme leading to reduction in glucose transport via the intestinal epithelium and increase in insulin level as showed in Table (4).

Insulinogenic effects of different medicinal plant extracts has been reported, resulting in β-cells inactivation. The potential mechanism by which its anti-hyperglycemic activity is exerted could have been due to the increased secretion of insulin from β-cells and regenerated β-cells. In this condition, several other plants have been reported to have antihyperglycemic activity with a stimulating effect on insulin secretion (Bhavsar and Talele, 2013).

High-carbohydrate high-fat diets induced diabetic rats' also demonstrated dyslipidemia as evidenced from the increased TC and TG levels with consequent decreased HDL, as in type 2 diabetes. It was found that *Bombax ceiba* extract upregulates protein pivotal for mitochondrial bioenergetics and down regulates proteins controlling *de novo* lipogenesis. This provides a molecular basis that supports *Bombax ceiba* extract ability to manage diabetic complications by improving dyslipidemia (Guang-Kai *et al.,* 2017).

Hypertriglyceridemia and hypercholesterolemia are the most prevalent lipid abnormalities in diabetes. Hypertriglyceridemia is also correlated with the metabolic effects of hyperinsulinemia, insulin resistance glucose intolerance and hypercoagulability. Management of the *Bombax ceiba* extract significantly decreased the levels of TG and TC (Table 5) resulting in a hypolipidemic effect due to reduced cholesterolgenesis and synthesis of fatty acid. Furthermore, the use of *Bombax ceiba* extract as a treatment has led to a significant increased in the level of HDL (Table 5). These values are a very desirable in the biochemical state for prevention of atherosclerosis and ischemic conditions (Khurshid *et al*., 2018).

Previous researches have shown that photochemicals have the ability to reduce lipid. Flavonoids prevent LDL oxidation; lower levels of TC and TG lead to lower risk of atherosclerosis (Salvamani *et al*., 2014).

Histopathological results indicated that in the control group, there were neither fatty deposition nor increased the

wall thickness (Figure 2). On the other hand, foamy laden cells were seen to be deposited in the inner layer of aortic wall in HCHFD group (Figure 3) which markedly decreased after treatment with *Bombax ceiba* extract (Figure 5), also when used as a prophylactic treatment this may derive the efficacy of *B. ceiba*'s to reduce circulating lipids, free fatty acids (Figure 6) (Gupta *et al*., 2013).

Simran *et al*. (2013) clarified that ALT is more specific marker than AST to determine the hepatic toxicity, since AST is also found in kidney and cardiac muscles. The *Bombax ceiba* extract showed high levels of flavonoids and phenolic compounds acting as natural antioxidants. They could scavenge free radicals. Anti-oxidant principles can therefore participate in the hepatoprotective activities. In our research, *Bombax ceiba* extract decreased AST and ALT levels (Table 5) suggest that *Bombax ceiba* extract may be used as a liver toxicity treatment agent in agreement with Khurshid *et al*. (2018).

LDL cholesterol activates endothelial cells, macrophages lymphocytes, fibroblasts, and vascular smooth muscle to secrete IL-6 which act as soluble mediator of the inflammatory response and will diffuse from sub mucous lining into the vascular lumen, so that the high levels of IL-6 were detected in the blood of high-fat diet rats (Darwin *et al*., 2017). Our results showed significant increase in IL-6 levels in HCHFD group when compared with control group, such findings have improved after treatment with *Bombax ceiba* extractin compare to HCHFD group (Table 6).

Adropin has been recognized as a fixed secretary protein containing 76 amino acids encoded by a protein by a gene associated with the protein energy balance. Adropin is expressed in liver, brain, umbilical vein and endothelial cells of the coronary artery (Kumar *et al*., 2008). The mechanism of the beneficial effects of adropin on the endothelial function has shown that serum adropin promotes production and improves the bioavailability of nitric oxide, which improves arterial stiffness. These results indicated that serum adropin has a possible mechanism that underlies enhanced endothelial function (Badawy *et al*., 2020).

Our study showed marked decrease in the level of adropin in HCHFD group when compared to the control, these outcomes improved after treatment with *Bombax ceiba* extractin compared to HCHFD group.

Retinol binding protein 4 is the only circulating vitamin A specific transport protein whose function is to deliver vitamins to target tissues. Glucose transporter type 4 (GLUT 4) expressions in obesity and T2D is marked decline in adipocytes. The rate-limiting step for glucose use by muscle and adipose tissue is glucose transport via GLUT4 (Jin *et al*., 2020).

Yang *et al*. (2005) suggested that adipocyte-specific deletion of GLUTS resulted in a significant increase of RBP4 causing systemic insulin resistance, and that decreased of RBP4 improved insulin resistance. This has identified a new role for RBP4 in regulating the insulin action and it is documented as an adipocyte-derived hormone. Measuring serum RBP4 is therefore a useful means for understanding metabolic disorders and the previous postulate help us to interpret our results which showed significant increase in the level of RBP-4 in HCHFD group when compared with the control group, such results improved in prophylactic and treated groups after administration with *Bombax ceiba* extract compared to HCHFD group (Table 6).

Salusin-α reduces the formation of foam cells and decreases the development of atherosclerosis, while salusin-β increases the formation of foam cells and atherosclerosis (Watanabe *et al*., 2008). The serum level of salusin-β is correlated positively with the incidence of coronary artery disease (Liu *et al*. 2015). The serum level of salusin-α is lower while the level of salusin-β is higher in patients with diabetes mellitus, coronary artery disease and cerebrovascular disease than in healthy persons. Cakir *et al*. (2017) stated that lower levels of salusin-α have been reported in the heart, aorta, liver and brain of rats with metabolic syndrome, this finding was in agreement with our result as showed in table (6).

Our results revealed a marked increase in salusin-β with significant decrease in salusin-α levels in HCHFD group as compared with the control group, such outcomes improved after treatment with *Bombax ceiba* extractin compared to HCHFD group.

Conclusion

The current study clearly shows that *Bombax ceiba* extract has prolonged and potential hypoglycemic, hypolipidemic and hepatoprotective activities and confirms the traditional uses of this plant in the treatment of diabetes, lipid profile and its associated liver toxicity. The pharmacological actions that have been observed may account for the phytochemicals present in this plant. Further study is needed to characterize *Bombax's* potential compounds and their role in the control and management of diabetes and hepatotoxicity.

Abbreviations

ALT	Alanine aminotransferase
Apo B	Apolipoprotein B
AST	Aspartate aminotransferase
B. ceiba	*Bombax ceiba*
CVD	Cardiovascular disease
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
eNOS	Endothelial Nitric Oxide Synthase
FPG	Fasting plasma glucose
GLUT 4	Glucose transporter type 4
HCHF	High-carbohydrate high-fat diets
HDL	High density lipoprotein
HFD	High-fat diet
HOMA-IR	Homeostatic model assessment for insulin resistance
IL-6	Interleukin-6
MetS	Metabolic syndrome
RBP4	Retinol-binding protein 4
Sal- α	Salusin-alpha
Sal- β	Salusin-beta
SD	Standard Diet
SE	Standard error
SkM	Skeletal muscle
SPSS	Statistical package for the social science
T.A	Tunica adventitia
T.I	Tunica intima
T.M	Tunica media
T2D	Type 2 diabetes mellitus
TC	Total Cholesterol
TG	Triglycerides

References

Alim A and Sharmin R, Hossain MS, Ray DN, Khan MRI, Islam MA, *et al*. (2012). Evaluation of hypoglycemic effect of compound(s) from petroleum ether fraction of ethanol extract of Mangifera indica red leaves. *J Pharm*., 2(6):14-19.

Aydin S. (2014). Three new players in energy regulation: preptin, adropin, irisin. Peptides, 56: 94–110.

Badawy E, El-laithy NA, Morsy SM, Ashour AN, Elias T, Masoud M *et al.* (2020). Role of swimming on muscle PGC-1α, FNDC5 mRNA, and assessment of serum omentin, adropin, and irisin in high carbohydrate high fat (HCHF) diet induced obesity in rats. *Egypt J Med Hum Gene,*; 21, 37.

Bhavsar CJ, Talele GS. (2013). Potential anti-diabetic activity of Bombax ceiba. *Bang J Pharmacol*. 8(2):102-106.

Butler AA, Tam ChS, Stanhope KL, Wolfe BM, Ali MR, O'Keeffe M, *et al*. (2012). Low circulating adropin concentrations with obesity and aging correlate with risk factor for metabolic disease and increase after gastric bypass surgery in humans. *Endocrinol Metab,* 97(10):3783–91.

Cakir M, Duzova H, Taslidere A, Orhan G & Ozyalin F. (2017). Protective effects of salusin-α and salusin-β on renal ischemia/reperfusion damage and their levels in ischemic acute renal failure. *Biotechnic & Histochemistry*. 92:2, 122-133.

Christopher RW, Mai KT, Katrina LS, Martin EY, Heinrich A. (2007). Western diet, but not high fat diet, causes derangements of fatty acid metabolism and contractile dysfunction in the heart of Wistar rats: *Biochem. J.* 406: 457–67.

Cole TG, Kuisk I, Patsch W, Schonfeld G. (1984). Effects of high cholesterol diets on rat plasma lipoproteins and lipoprotein cell interactions: *Lipid Res*., 25: 593-603.

Darwin E, Elfi E F, Dachriyanus (2017). Effect of Arginine on IL-6, IL-17 and TGF-ß levels in high-fat Diet-Induced Hypercholesterolemia Rat. *J Young Pharm*, 9(1): 83-86.

Drury RA, Wallington EA. (1980). Carleton's Histological Techniques. 4th Edition 1980, *Oxford University Press*, *New York*, 195.

Guang-Kai XU, Xiao-Ying QIN, Guo-Kai WANG, Guo-Yong XIE, Xu-Sen LI, Chen-Yu SUN, *et al*. (2017). Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of *Bombax ceiba* leaves on high-fat diet and streptozotocin induced Type 2 diabetic rats. Chin J Nat Med., 15(3): 168-177.

Gupta P, Goyal R, Chauhan Y, Sharma PL. (2013). Possible modulation of FAS and PTP-1B signaling in ameliorative potential of Bombax ceiba against high fat diet induced obesity. *BMC Complement Altern Med*.; 25(13):281.

Hemant P, Sameer SK, Balvant J, Kusum J. (2009). Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.). *BMC Complement Altern Med*., 9: 48.

Jalapure SS, Gadge NB. (2011). Diuretic Effects of Young Fruit Extracts of Bombax ceiba lin Rats. *Indian J Pharm Sci*., 73(3):306-311.

Jin C, Lin L, Han N, Zhao Z, Liu Z, Luo S, *et al*. (2020). Plasma retinol-binding protein 4 in the first and second trimester and risk of gestational diabetes mellitus in Chinese women: a nested case-control study. *Nutrition & Metabolism*.; 17:1.

Khurshid Alam AHM, Sharmin R, Maruf I, Joarder HH, Alamgir M, Mostofa G (2018). Antidiabetic and Hepatoprotective Activities of *Bombax ceiba* Young Roots in Alloxan-Induced Diabetic Mice. *J Nutrition Health Food Sci.* 6(5):1-7.

Kimura A, Kishimoto T. (2010). IL-6: Regulator of Treg/Th17 balance. *European Journal of Immunology*; 40: 1830-1835.

Kristófa E, Klusóczkia Á, Veressa R, Shawa A, Combia ZS, Vargaa K, (2019). Interleukin-6 released from differentiating human beige adipocytes improves browning. *Experimental Cell Research.*; 377: 47–55.

Kumar GK, Zhong J, Gao S, Rossi J, McGuinness OP, Halmen HH (2012). Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity,* 20(7): 1394–402.

Kumar J, Kumar M. (2011). Medicinal plants for diabetes mellitus: A traditional approach. *Int Archive Appl Sci Tech*.; 2(1):37-46.

Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN (2008). Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism: *Cell Metab*. 8(6):468–81.

Kwang O, Young A, Hyesung L. (2007). Isoflavone rich bean sprout cookie improves lipid metabolism in hyperlipidemic rat: *The FASEB Journal*.; l (21):846 –8.

Lee MK, Han K, Kim MK, Koh ES, Kim ES, Nam GE, Kwon HS (2020). Changes in metabolic syndrome and its components and the risk of type 2 diabetes: a nationwide cohort study. *Scientific Reports.* 11; 10(1):2313.

Liu J, Ren YG, Zhang LH, Tong YW, Kang L. (2015). Serum salusin-β levels are associated with the presence and severity of coronary artery disease. *J. Invest. Med*.; 63: 632–635.

Lopez-Virella MF, Stone P, Ellis S, Colwell JA. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem*. 23(5):882-884.

Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M (2010). Adropin is a novel regulator of endothelial function. *Circulation*. 122(11): 185–92.

Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985). Homeostasis model assessment: insulin resistance and β-cell function fromfasting plasma glucose and insulin concentrations in man. *Diabetologia*. 28(7):412-9.

Mishra SB, Rao CHV, Ojha SK, Vijayakumar M, Verma A, Alok S. (2010). An analytical review of plants for anti-diabetic activity with their phytoconstituent & mechanism of action. *Inter J Pharma Sci*., 1(1):29-46.

Niepolski L, Sowinska A, Grzegorzewska AE (2015). Plasma adropin level is negatively associated with residual diuresis and protein-energy wasting in maintenance hemodialysis patients. Abstr. Congr. XLII Eur. Soc. Artif. Organs, Leuven, 2–5.09.2015. *Int J Artif Organs,* 38(7): 381.

Passing H, Bablok W. (1983). New biochemical procedure for testing the equality of measurements from two different analytical methods. *J Clin Chem Clin Biochem*. 21(11):709-20.

Rani N, Vasudeva N, Sharma SK. (2012). Quality assessment and anti-obesity activity of Stellaria media (Linn.) Vill. BMC Complement. *Altern Med*., 12:145.

Reeves PG (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr.* 27(5): 838-41.

Rehman M, Akhtar N, Mustafa R. (2017). Antibacterial and antioxidant potential of stem bark extract of *Bombax ceiba* collected locally from South Punjab area of Pakistan. *Afr J Tradit Complement Altern Med*.; 14(2):9-15.

Reitman S, Frankel S. .(1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. clin. Pathol*.; 28(1):56-63.

Rosales FJ, Ritter SJ , Zolfaghari R, Smith JE, Ross AC (1996). Effects of acute inflammation on plasma retinol, retinol-binding protein, and its mRNA in the liver and kidneys of vitamin A-sufficient rats. *Journal of Lipid Research*, 37, 962-971.

Sahin I, Aydin S. (2013). Serum concentration and kidney expression of salusin-α and salusin-β in rats with metabolic syndrome induced by fructose. *Biotechnic & Histochemistry*. 88(3-4): 153-160.

Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. (2014). Antiartherosclerotic effects of plant flavonoids. *BioMed Res. Int*., 1-11.

Sato K, Koyama T, Tateno T, Hirata Y, Shichiri M. (2006). Presence of immune reactive salusins a in human serum and urine. *Peptides*, 27(11):2561–6.

Simran A, Manisha V, Sushma A, Satish S. (2013). Phytochemistry and hepatoprotective activity of aqueous extract of Amaranthus tricolor Linn. Roots. *J. Ayurveda Integr. Med*., 4(4):211–15.

Topuz M, Celik A, Aslantas T, Demir AK, Aydin S, Aydin S. (2013). Plasma adropin levels predict endothelial dysfunction like flow-mediated dilatation in patients with type 2 diabetes mellitus. *J Investig Med*., 61(8):1161-4.

Watanabe T, Nishio K, Kanome T, Matsuyama TA, Koba S, Sakai T (2008). Impact of salusin-alpha and -beta on human macrophage foam cell formation and coronary atherosclerosis. *Circulation*, 117: 638–48.

Wu L, Fang J, Chen L, Zhao Z, Lou Y, Lin C (2014). Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. *Clin Chem Lab Med*., 52(5):751–8.

Yalow R, Bauman WA. (1983). Insulin in health and disease. In: Ellenberg, M, Rifkin, H (eds) Diabetes Mellitus: Theory and Practice. New York: *ExcerptaMedica*., 119–50.

Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*. 21; 436 (7049):356-62.

Zhou CH, Liu LL, Wu YQ, Song Z, Xing SH. (2012). Enhanced expression of salusin-b contributes to progression of atherosclerosis in LDL receptor deficient mice. *Can J Physiol Pharmacol*, 90(4):463–71.

Zhou CH, Pan J, Huang H, Zhu Y, Zhang M. (2014). Salusin-b, but Not Salusin-a, Promotes Human Umbilical Vein Endothelial Cell Inflammation via the p38 MAPK/JNK-NF-kB Pathway. *PLoS ONE*, 9(9): e107555.