Alleviating effect of *Bauhinia variegata* leaves extract on altered serum adipokines and impaired kidney function in male rats with experimentally induced obesity

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Abstract: Obesity has emerged as independent risk factor for chronic kidney disease (CKD). The aim of this study was to investigate the possible alleviating effect of *Bauhinia variegata* leaves extract (BEX) on kidney disease in the obese rats. Male Wister rats (170±5g) were fed high fat diet (HFD) for 12 weeks for induction of obesity, while BEX (40 mg/100g b.wt) was given orally for the same duration. The obese rats showed marked increase in the body weight gain and adiposity index, with corresponding rise in serum and kidney lipids [total lipids (TLs), total cholesterol (TC), phospholipids (PLs) and triglycerides (TGs)]. This goes with significant increase in serum glucose, glycated hemoglobin (HbA1c), leptin, insulin, insulin resistance, LDL-C, vLDL-C, and decrease in adiponectin and HDL-C levels. Meanwhile, a reduction of serum Na^+, total protein and albumin, with elevation in their levels in the urine of obese rats was demonstrated. Results also showed high serum concentration of creatinine, uric acid, urea and K^+, concomitant with decline in their urinary levels, as well as in creatinine clearance. Histopathological changes, characterized by atrophy of glomerular tufts, focal glomerulosclerosis, renal tubular degeneration and interstitial cellular inflammation were also demonstrated. However, administration of BEX to HFD-fed rats was effective in preventing altered serum adipokines (adiponectin and leptin), hyperinsulinemia, insulin resistance and associated histopathological changes relevant to development of kidney disease. Thus, BEX can be considered as a natural plant product for preventing kidney dysfunction associated with obesity.
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Key words: Obesity, Adipokines, Insulin resistance, *Bauhinia variegata,* Kidney dysfunction.

1. Introduction:

Obesity is a major health problem characterized by an imbalance between energy intake and energy expenditure, with increased fat accumulation in adipose tissue **(Aronne and Isoldi, 2007).** Multiple factors including, sedentary life style, lack of exercise and intake of energy rich diets are thought to explain most cases of obesity **(Rang *et al.*, 2003).** Currently, obesity is known to be associated with different diseases**,** including coronary heart disease, osteoarthritis, type 2 diabetes, stroke and different types of cancers **(Johnson *et al.*, 2010).** In addition, obesity has considered as a major risk factor for developing chronic kidney disease (CKD) **(Ritz *et al.*, 2011).** Several mechanisms have been suggested as responsible factors for deterioration of kidney disease in obese populations. However, the alteration in endocrine features of adipose tissue is more likely to play a central role **(Schlondorff, 2008).**

Adipose tissue produces hormone-like peptides, named adipocytokines or adipokines. Among such hormones, adiponectin, and leptin represent a link between obesity and CKD **(Abbate *et al.*, 2008).** The majority of obese subjects have higher plasma leptin, with decreasing adiponectin levels **(Baratta *et al.*, 2004).** A decrease of adiponectin levels has shown to be associated with dyslipidemia, insulin resistance, endothelial dysfunction and kidney damage **(Shankar *et al.*, 2011).** Meanwhile, higher leptin levels have described to cause hyperglycemia, adrenergic activation, increased blood pressure and renal dysfunction **(Carlyle *et al.*, 2002).** As such, altered serum adiponectin and leptin levels can be considered as a major cause initiating interrelated sequence of events, resulting in renal dysfunction in the obese subjects.

Nowadays, there is widespread interest in establishing alternative non pharmacological ways, based on medicinal plants to manage obesity and related health problems **(Aggarwal, 2010).** Among these, *Bauhinia variegata* is a flowering plant from the family Leguminosae, which is commonly planted in gardens, parks and roadsides as ornamental plant in many warm temperate and subtropical regions. Almost all parts of the plant (seeds, root steam bark, flowers and leaves) are used effectively for healing skin diseases, ulcers and diarrhea **(Nadkarni, 1996).** Other studies revealed their ability to produce antitumor, anti-inflammatory, anti-bacteria and hepatoprotective properties **(Rajani and Purnima, 2009).** Traditionally,

Bauhinia variegata extracts are used as remedy for obesity. However, the claim for the utility of this plant extract in treatment of obesity and related disorders has not been scientifically evaluated. Hence, this study was carried out to investigate whether prolonged intake of *Bauhinia variegata* leaves extract (BEX) could prevent the risk of developing kidney disease in male rats with dietary-induced obesity and further to evaluate the contributing mechanisms.

2. Materials and methods:

2.1. Animals

This study was performed on male Wister albino rats initially weighing 170±5 g. Rats were obtained from the Institute of ophthalmic Disease Research, Cairo, Egypt. They were housed in stainless steel cages under controlled conditions of temperature (25 ± 2 ^{0}C), humidity (55 ± 5%) and 12 hr light/dark cycle. Animals were permitted adequate standard diet purchased from Meladico Feed Company (Auber city, Cairo, Egypt) and given water *ad libitum* for one week of acclimation before the beginning the experimental work. All animals were treated in accordance with Mansoura University guidelines for the care and use of laboratory animals.

2.2. Plant material and preparation of the extract

Fresh leaves of *Bauhinia variegat*a were collected during November, 2013 from the local gardens of Mansoura University, Mansoura, Egypt. Plant identity was confirmed by Dr. Ibrahim Mashali, Professor of Botany, Botany Department, Faculty of Science, Mansoura University. The fresh leaves were washed with clean water, dried in shade and pulverized to get a coarse powder. Dried powdered leaves (5 kg) were exhaustively extracted using 95% ethanol at room temperature for 72 hr. Obtained extract was evaporated to dryness in a rotatory vacuum evaporator under reduced pressure to yield 400g of *Bauhinia variegata* leaves extract (BEX). The extract was suspended in 1% Tween-80 for oral administration.

2.3. Research design

After the acclimation period, rats were randomly divided into five groups (n=6 rats)**.** The first was fed a standard diet and served as normal control group. The second was fed a standard diet and received 1% Tween-80 orally as vehicle at dose (0.1 ml/100g b.wt). In the third group, rats were fed a standard diet and received freshly prepared BEX orally at dose (40 mg/100g b.wt) dissolved in 1% Tween-80 **(Santanu Saha *et al.*, 2011).** Rats of the fourth group were fed high fat diet (HFD) consisted of standard diet in powder form mixed with melted animal abdominal fat (30%) and extra pure cholesterol (2%) **(EL-wakf *et al.*, 2013)** while rats of the fifth group were fed HFD and received BEX orally, as described in the above groups. All animal groups were received their respective treatments daily for 12 weeks. Animal's body weight (g) was recorded at the start and end of the experiment for obtaining body weight gain.

2.4. Samples collection

At the end of the study period, all rats were placed individually in metabolic cages for 24 hr. Urine was collected, centrifuged and stored at -20^{0}c until analysis. Animals were overnight fasted and blood was collected from each rat under ether anesthesia via retro-orbital puncture method to obtain serum for hormonal and biochemical analysis. Immediately after collecting blood, the animals were sacrificed by cervical dislocation, dissected and the two kidneys were removed. The right kidney was weighted, homogenized in ice cold saline solution and the homogenate was kept frozen at -20^{0}C until being analyzed, while the left kidney was fixed in 10% formalin for histological examination. Adiposity index was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight x100, and expressed as adiposity percentage **(Taylor and Phillips 1996).**

2.5. Biochemical analysis

Different biochemical parameters, including total lipids (TLs)**,** total cholesterol (TC)**,** triglycerides (TGs)**,** phospholipids (PLs)**,** high-density lipoprotein-cholesterol (HDL-C) and glucose,were estimated using kits supplied by Biodiagnostic Co. Mansoura, Egypt. LDL-C and vLDL-C were calculated according to the equations applied by **Ahmedi *et al.*, (2008)**. Glycated hemoglobin (HbA 1c) was estimated using kit of Bio Systems S.A Costa Brava (Spain). Kidney function test was determined by evaluating serum and urine levels of sodium (Na^+)**,** potassium (K^+)**,** total protein (TP)**,** albumin (alb)**,** urea**,** uric acid**,** and creatinine, using kits of Biodiognostic Co. Mansoura, Egypt. Creatinine clearance (Cr-clearance), was estimated, as described by **Schirmeister, (1964).** Adiponectin and leptin were determined using ELISA kits according to the methods of **Schlottmann *et al.* (2013)** and **Considine *et al.* (1996)**, respectively. Insulin was measured by ELISA kit as described by **Flier *et al*. (1976),** while insulin resistance (IR) was calculated using homeostasis model assessment of insulin resistance (HOMA-IR) based on the equation of **Matthews *et al.(* 1985).**

2.6. Statistical analysis

Data were analyzed by one way analysis of variance (One–way ANOVA), followed by Least Significant Difference (LSD) test, using SPSS statistical package, version 17.00 software. The results were expressed as means ± S.E and values were considered statistically significant at $P < 0.05$ **(Snedecor and Cochran, 1982).**

3. Results:

As shown from Tables (1 , 2, 3) administration of BEX to normal rats did not produce any significant changes in all tested parameters in comparison to

normal rats, indicating its non toxic effect at applied dose. Feeding rats on high fat diet (HFD) tended to exhibit obesity features, as evidenced by a significant increase in the body weight gain and adiposity index compared to control rats. On the other hand, administration of BEX to animals fed on HFD showed significant reduction in the body weight gain and adiposity index compared to obese rats (Table 1).

The present obesity model also showed significant increase in serum levels of glucose, glycated hemoglobine (HbAlc%), insulin, insulin resistance (IR), leptin, LDL-C and vLDL-C, with decrease in HDL-C level and adiponectin levels. This goes along with elevation in serum and kidney total lipids (TLs), total cholesterol (TC), triglycerides (TGs), and phospholipids (PLs). Administration of HFD-fed rats with BEX significantly decreased serum glucose, HbAlc%, insulin, IR, leptin and various lipid constituents compared to obese rats, however the results revealed significant increase in serum levels of adiponectin and HDL-C (Table 2).

Meanwhile, a reduction of serum Na^+, TP and alb, with elevation in their levels in the urine of obese rats were demonstrated. Data also showed significant increase in the serum levels of creatinine, urea, uric acid and K^+, concomitantly with a decline in their urinary levels, as well as in creatinine clearance. However, administration of BEX tended to reverse these biochemical changes near to normal values compared to obese rats (Table 3).

Table 1. Changes in body weight gain and adiposity index (adiposity %) for all experimental groups.

Parameters	Animal groups				
	Control	Vehicle	BEX	HFD	HFD+BEX
Body weight gain(g)	106.50±4.32	106.55±5.23	104.33 ±4.98	170.33 ±6. 03[a]	118.40±3.80 [abc]
Adiposity %	5.93±0.42	5.81±0.38	5.74±0.56	10. 22 ± 1.18 [a]	7.43 ± 0.18 [abc]
ANOVA	$P < 0.05$				

Values are means± SE of six animals for each group. Vehicle = Tween-80, BEX= *Bauhinia variegata* leaves extract, HFD = high fat diet. a: significant when compared with control. b: significant when compared (HFD+BEX) with HFD. c: significant when compared (HFD+BEX) with BEX.

Table 2. Changes in serum and kidney biochemical parameters for all experimental groups.

Parameters		Animal groups				
		Control	Vehicle	BEX	HFD	HFD+BEX
Serum	Adiponectin (ng/mL)	22.50±0.30	22.22±0.31	22.26±0.34	16.13 ±0.18 [a]	18.27±0.20 [abc]
	Leptin (ng/mL)	18.23±0.09	18.14±0.07	18.13±0.07	29.33 ±0.17 [a]	23.04±0.10 [abc]
	Insulin (µlU/mL)	1.81 ±0.07	1.82 ±0.01	1.80±0.01	3.33 ± 0.09 [a]	2.28 ±0.03 [abc]
	HOMA-IR	7.21 ±0.08	7.26 ±0.02	7.12±0.12	23.30 ±0.22 [a]	12.50±0.19 [abc]
	Glucose (mg/dL)	87.40±0.92	87.16±1.24	86.6±1.40	175.16±2.90 [a]	102.16±2.65 [abc]
	Hb Alc %	4.42 ±0.13	4.43 ±0.12	4.31 ±0.10	6.13 ±0.02 [a]	5.o5±0.16 [abc]
	TLs (mg/dL)	414.44±1.8	413.70±26	413.33±0.9	666.29±1.31 [a]	551.47±3.32 [abc]
	TC (mg/dL)	100.49±0.4	100.53±0.2	99.96 ±0.61	139.88±0.74 [a]	116.81±1.02 [abc]
	TGs (mg/dL)	97.29±1.66	97.39±1.98	96.21 ±1.68	138.96±2.70 [a]	117.73±1.29 [abc]
	PLs (mg/dL)	108.81±1.0	108.50±2.6	108.09±2.22	148.36±3.08 [a]	125.38±1.37 [abc]
	HDL-C (mg/dL)	41.62±0.59	41.12±0.30	41.51 ±0.11	33.88 ±1.39 [a]	37.88 ±0.40 [abc]
	LDL-C (mg/dL)	39.50±0.31	39.47±0.33	39.79 ±0.74	65.44 ±2.6 [a]	52.30 ±0.99 [abc]
	vLDL-C (mg/dL)	19.69±0.15	19.57±0.16	19.58 ±0.11	29.25±1.05 [a]	24.68 ±0.69 [abc]
Kidney	TLs (mg/dL)	414.44±1.8	413.70±26	413.33±0.9	666.29±1.3 [a]	551.47±3.32 [abc]
	TC (mg/dL)	100.49±0.4	100.53±0.2	99.96 ±0.61	139.88±0.7 [a]	116.81±1.02[abc]
	TGs (mg/dL)	97.29±1.66	97.39±1.98	96.21 ±1.68	138.96±2.7 [a]	117.73±1.29 [abc]
	PLs (mg/dL)	108.81±1.0	108.50±2.6	108.09±2.2	148.36±3.0 [a]	125.38±1.37 [abc]
ANOVA		$P < 0.05$				

Values are means± SE of six animals for each group. Vehicle = Tween-80, BEX= *Bauhinia variegata* leaves extract, HFD = high fat diet. a: significant when compared with control. b: significant when compared (HFD+BEX) with HFD. c: significant when compared (HFD+BEX) with BEX.

Table 3. Changes in serum and urine biomarkers of kidney function for all experimental groups.

Parameters		Animal groups				
		Control	Vehicle	BEX	HFD	HFD+BEX
Serum	Creatinine(mg/dl)	0.64 ±0.06	0.64 ±0.07	0.63 ±0.09	2.13 ±0.08 [a]	1.05±0.08 [abc]
	Urea (mg/dl)	44.82 ±0.65	44.63 ±1.27	44.21 ±1.38	54.34 ±0.59 [a]	48.17 ±0.8 [abc]
	Uric acid (mg/dl)	1.32 ±0.06	1.37 ±0.07	1.23 ±0.19	2.63±0.14 [a]	1.80 ±0.15 [abc]
	Na^+ (mg/dl)	141.40±0.29	141.29±0.26	141.09±0.26	123.85±1.70 [a]	135.82±1.02 [abc]
	K^+ (mg/dl)	2.78 ±0.14	2.76 ±0.17	2.75 ±0.20	5.36 ±0.22 [a]	3.23 ±0.26 [abc]
	TP (g/dl)	6.50 ±0.18	6.60 ±0.22	6.37 ±0.19	4.98 ±0.21 [a]	5.84 ±0.15 [abc]
	Alb (g/dl)	2.97 ±0.14	2.91 ±0.22	2.96 ±0.18	1.55 ±0.13 [a]	2.03 ±0.14 [abc]
Urine	Creatinine(mg/dl)	41.33 ±0.64	41.85 ±1.27	41.16 ±1.03	29.93 ±0.52 [a]	35.45 ±0.86 [abc]
	Urea (g/dl)	1.54 ±0.09	1.52 ±0.01	1.55 ±0.08	0.43 ±0.06 [a]	1.15 ±0.07 [abc]
	Uric acid (mg/dl)	44.82 ±0.65	44.63 ±2.38	44.21 ±1.30	30.68 ±0.20 [a]	39.84 ±1.19 [abc]
	Cr-clearance (ml/min)	0.66 ±0.08	0.66 ±0.10	0.65 ±0.11	0.21 ±0.01 [a]	0.53 ±0.04 [abc]
	K^+ (mg/dl)	125.93±1.13	125.01±1.33	125.84±1.55	75.62±0.23 [a]	92.61 ±0.83 [abc]
	Na^+ (mg/dl)	133.64±0.46	133.14±0.21	133.61±0.59	146.51±0.24 [a]	139.74±0.55 [abc]
	TP (g/dl)	0.42 ±0.05	0.46 ± 0.05	0.50 ±0.08	3.11 ±0.39 [a]	2.26 ±0.31 [abc]
	Alb (g/dl)	0.38 ±0.04	0.38 ±0.05	0.37 ±0.05	1.69 ±0.06 [a]	0.92 ±0.04 [abc]
ANOVA		$P < 0.05$				

Values are means± SE of six animals for each group. Vehicle = Tween-80, BEX= *Bauhinia variegata* leaves extract, HFD = high fat diet. a: significant when compared with control. b: significant when compared (HFD+BEX) with HFD. c: significant when compared (HFD+BEX) with BEX.

Kidney histopathological examination

Histopathological examination of kidney from control group (**Fig.A**) showed normal structural organization of renal corpuscles with well-defined glomeruli **(G)** surrounded by narrow Bowman's space **(BC)**. Normal proximal **(PT)** and distal tubules **(DT)**, as well as collecting ducts **(CD)** were also observed. The same normal structure was exhibited by the vehicle **(Fig.B)** and *Bauhinia variegata* **(Fig.C)** groups. HFD-fed group **(Fig.D&E)** showed atrophy of glomerular tufts, accompanied by focal glomerulosclerosis **(GS)**,vacuolar degeneration in proximal convoluted tubules **(DPT)** and focal interstitial cellular inflammation **(star)**. Degenerated renal tubules **(arrow head)** and marked brush border loss **(thick arrow)** were also observed. Co-administration of BEX and HFD **(Fig.F)** showed almost normal appearance of renal corpuscles and renal tubules.

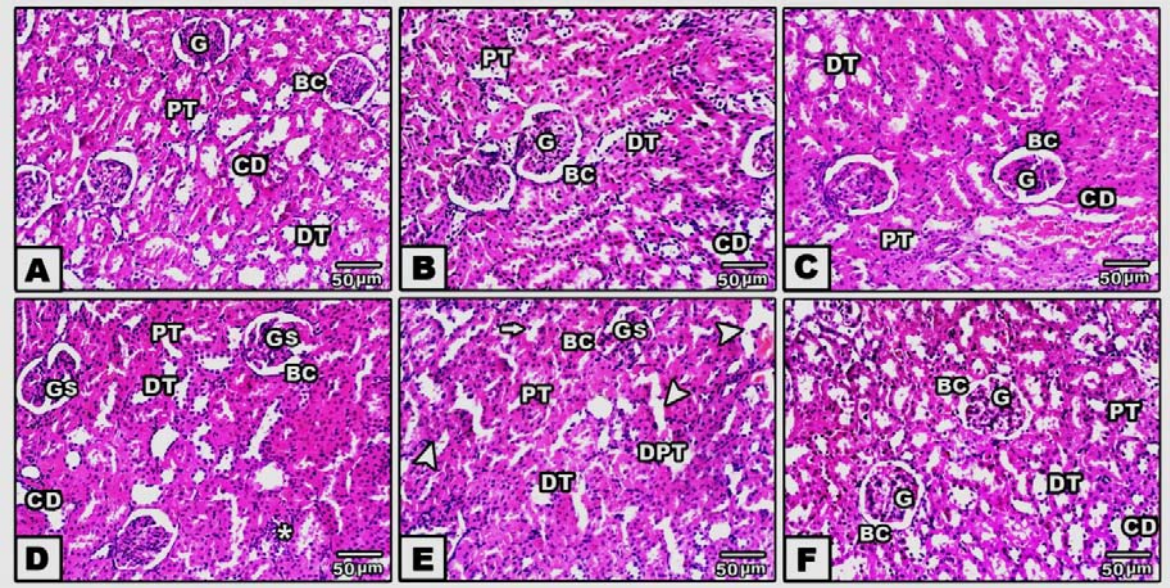

Plate1.Photomicrograph showing transverse sections of kidney from different experimental groups**.**

4. Discussion:

Obesity constitutes a major health problem of increasing prevalence. Although the etiology of obesity is complex, dietary factors particularly, increased consumption of high fat diet (HFD) causes excessive fat accumulation and obesity **(Thaler *et al.*, 2012).** As frequent, experimental models of HFD-fed animals were used to produce obese conditions bearing close resemblance to human obesity **(Wang et *al.*, 2008)**. Similar pattern was demonstrated in the present study, where rats fed on HFD for 12 weeks displayed an increase in the body weight gain and adiposity index, indicating development of obesity.

Recently, obesity is considered as an independent risk factor for incidence of chronic kidney disease (CKD) **(Wang *et al.*, 2008).** Kidney is the primary organ for regulating electrolytes and removing nitrogenous waste products, such as urea, creatinine and uric acid **(Afzali *et al.*, 2010),** thereby helping in maintaining optimum composition of body fluids. In this context, several researchers indicated that feeding on HFD leads to kidney dysfunction, characterized by changes in both serum and urine electrolytes (Na^+ and K^+), urea, creatinine and uric acid in an opposite direction. A decline in creatinine clearance with increase in the urinary protein concentration was also observed **(Takahashi *et al.*, 2011).**

Creatinine and serum urea are considered to be glomerular function markers and are directly related to uric acid concentrations, particularly due to the urate excretion relationship **(Choe, *et al.*, 2008).** With progression of kidney disease, the process of accumulation and decreased urinary excretion plays a crucial role and leads to retention of metabolites (e.g. creatinine, urea and uric acid)**.** Hyperuricemia causes deposition of uric acid crystals in glomeruli and renal tubule lumen, which in turn causes decreased glomerular filtration rate (GFR), inflammation of the renal parenchyma and impaired urinary flow, thereby promotes progression of renal dysfunction **(Saito *et al.*, 2010)**. In the present study, increased serum creatinine, urea and uric acid, with decrease in their urinary concentrations, as well as in levels of creatinine clearance, may therefore represent independent predictors for progression towards CKD. Other consequences include, increased amounts of proteinurea,with changes in serum and urine electrolytes (Na^+ and K^+) were also demonstrated, which may occur as a result of degeneration in the glomerular basement membrane **(Appuhamy *et al.*, 2012),** and renal tubular injury, characterized by tubular flattening and loss of tubule brush border (**Cardenas and Gonzalez, (2013).** Results of the present study also showed marked histopathological changes, as evident by atrophy of glomerular tufts, focal glomerulosclerosis, renal tubular degeneration and interstitial cellular inflammation, thereby, providing further evidence for development of kidney disease with obesity.

In this line, several studies indicated that obesity is associated with multiple changes in the hormonal features of adipose tissue which in turn may be considered as a major cause for developing kidney disease. Adipose tissue has considered as an endocrine organ having the ability to produce a number of hormones, named adipokines. Most abundant adipokines includes, adiponectin and leptin which appear to play critical role in the genesis of kidney disease in the obese populations **(Ogura *et al.*, 2004)**. Adiponectin is a peptide hormone produced exclusively by adipose tissue**.** Adiponectin increases insulin sensitivity, stimulates fatty acid oxidation, glucose uptake and lactate production in muscle cells **(Méndez, *et al.*, 2006).** Reduction of adiponectin levels often occurs with obesity, as that exhibited in the current study, which is mainly related to inhibition of adiponectin gene expression **(Benedix, *et al.*, 2011)**.

An association between reduced adiponectin level and kidney disease has been established. Such association was initially raised in a clinical study of men with hypertension in which serum adiponectin and albuminuria levels were inversely correlated **(Tsioufis *et al.*, 2005).** Adiponectin null mice exhibited albuminuria, with pathologic findings of podocytes foot process hypertrophy **(Sharma *et al.*, 2008)**. Podocytes are specialized epithelial cells with prominent foot processes, which line the glomerular capillaries and play a major role in the selective permeability of glomerular filtration barrier **(Serradeil *et al.*, 1997).** Podocytes express the adiponectin receptor-1 (AdipoR1) and treatment of null mice with adiponectin normalizes albuminuria and restores foot process architecture. The mechanism for this effect is mediated through adiponectin activation of 5-AMP protein kinase (AMPK), which is known to exert potent anti-proliferative effects. AMPK via suppression of NADPH-oxidase may protect against increased reactive oxygen species (ROS) and oxidative stress, with improvement in podocyte cytostructure in adiponectin treated animals **(Zhou *et al.*, 2013).** Additional support for the renal protective effect of adiponectin is provided by a study using 5/6 nephrectomy animal model in which adiponectin treatment inhibited albuminuria and renal fibrosis **(Ohashi *et al.*, 2007).**

Besides the above effects of adiponectin, other studies have focused on the role of leptin **((Ouchi *et al.*, 2003; Ogura *et al.*, 2004)**. Leptin is a peptide hormone abundantly produced by adipocytes and is positively correlated with adiposity**.** Positive link between body fat and serum leptin is probably explained by the increased release of leptin from large

fat mass, thus leptin can serve as an indicator of body fat content **(Masoud and Adel, 2006).** In agreement, the present findings showed an association between elevated serum leptin and increased tissue lipid accumulation in the obese rats, which is well corroborated with data from the study of **Bjorbaek and Hollenberg (2002).** Leptin causes a decrease in food consumption by decreasing appetite, where it acts on leptin receptors presenting in the hypothalamus and other tissues **(Silva and Hall, 2004)**. Leptin receptors have also been isolated on endothelial and smooth muscle cells (SMC**).** These observations suggest a role for this adipokine in controlling proliferation and migration of SMC and endothelial cells, thus influencing blood vessel tone and thickness of its wall **(Yamagishi *et al*., 2001).** Increased leptin has shown to induce proliferation of glomerular endothelial cells, expression of glomerular transforming growth factor beta1 (TGFB1) and production of collagen type IV-mRNA, resulting in focal glomerulosclerosis. Usually, obese patients show increased risk of developing glomerulosclerosis, with increased serum leptin concentration**.** Consequently, a correlation has been described between increased leptin levels and augmented albuminuria, while a negative correlation was established between high leptin levels and GFR which is a leading cause for kidney disease **(Ouchi *et al.*, 2003).**

Further research in the relation between obesity and kidney disease provided evidence for the role of insulin resistance (IR). Hyperinsulinemia and IR are established metabolic features of obesity, as evidenced in the current study and in other investigations **(Lonardo *et al.*, 2006).** IR is a state in which higher concentration of insulin is required to maintain normoglycemia. In obese states, IR is closely related to overproduction of free fatty acids (FFAs) from the lipolytically active intra-abdominal adipocytes, which in turn leads to decreased insulin sensitivity **(Lang and Andersson, 2005).** Other mechanisms may be related to the altered production of both adiponectin and leptin with increasing adiposity. Given the fact that leptin and adiponectin respond in reciprocal manner with obesity, the higher leptin/ adiponectin ratio is thought to be strongly associated with IR, characterized by increased glucose levels and hyperlipidemia **(Parving, *et al.*, 2000)**.

Increased blood glucose levels induces injury in the kidney through various pathways, including increases in the formation of advanced glycation end products, (AGEs) which play a critical role in the development of kidney disease **(Twigg *et al.*, 2002).** AGEs are heterogeneous groups of macromolecules that are normally formed non-enzymatically by the interaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids, but their formation increases under high glucose levels **(Thornalley and Ahmed, 2007).** Engagement of AGEs to their receptors (AGERs) has been shown to play a critical role in renal complications, probably through production of a variety of cytokines, including tumor necrosis factor-β (TNF-β), which in turn increases production of mesangial matrix, leading to glomerulosclerosis and renal damage **(Yan *et al.*, 2007)**. Among the most investigated AGEs is glycated hemoglobin (HbA1c) that occurs through non enzymatic glycation of hemoglobin. Increased blood glucose concentration and HbA1c, can predict elevated urinary proteins and renal dysfunction **(Preshaw *et al.*, 2012).** In agreement, the present study showed increased serum glucose and HbA1c, in association with increased levels of proteinuria in the obese rats, indicating decline of kidney function.

In this context, other studies provided evidence that hyperlipidemia contributes mainly to the progression of kidney disease. Alteration and / or abnormality in the metabolism of lipids and lipoproteins are very common conditions that taken place with obese populations **(Prabu *et al.*, 2009).** Lipid abnormality related to obesity include an elevated serum TC, LDL-C, vLDL-C and TGs with a reduction in serum HDL-C. Generally the prevalence of hyperlipidemia increases with deteriorating renal function, where the degree of hypertriglyceridemia and elevation of LDL-C being proportional to the severity of renal impairment **(Choudhary *et al.*,2005).** The cause is that obesity is characterized by a different composition and distribution of LDL-C, resulting in an increased concentration of the more atherogenic small dense LDL-C, which in turn can move through endothelial fenestrations, entering the subendothelial space where transformation into plaque can occur, leading to atherosclerosis and progression of kidney disease **(Houstis *et al*., 2006).** Accordingly, the present data showing increased serum and kidney lipids (TLs, TC, TGs, and PLs), with increased serum LDL-C, vLDL-C and decreased HDL-C in HFD-fed rats, may represent a key event for developing kidney disease associated with obesity.

Recently, several studies recommended the benefits of using medicinal plants for reducing the risk of obesity and associated complications **(Kishino *et al.*, 2006).** Among which, *Bauhinia variegata* is one of the traditionally used plants in this concern. In the present study, administration of BEX to HFD-feed rats tended to exert marked anti-obesity activity and to prevent functional and structural alterations of the kidney, as evident by normalized biochemical parameters (urea, creatinine, uric acid, and total protein) and histological features of the kidney. Alterations of adiponectin, leptin, insulin and related metabolic abnormalities were also prevented. In this context, prior studies provided

evidence that *Bauhinia variegata* leaves are rich source of active phytochemicals, in particular, flavonoids **(Brahmachari *et al.*, 2011).** Flavonoids are a group of natural substances having various phenolic structures and are known for their potential effects in prevention or treatment of several diseases **(Mink *et al.*, 2007).** One of the best described flavonoids is quercetin. Previous study by **Birari and Bhutani (2007)** indicated the efficacy of quercetin to inhibit lipid absorption, transport and accumulation in adipose tissue, via stimulating lipogenesis and lipid mobilization. This may occur through activating protein kinase A (PKA), which in turn activates the hormone sensitive lipase being responsible for enhanced lipolysis **(Warwar *et al.*, 2006).** As such, the presently observed anti-obesity effect of BEX could be attributed to the beneficial effect of its flavonoid constituent, quercetin as reported by **Jin *et al.* (2011).** The therapeutic effect of BEX could be also due to presence of the other flavonoid compound, Kaempferol **(Calderon-Montano *et al.*, 2011).** In agreement, **Tomoko *et al.* (2011)** suggested that kaempferol abundantly present in *Kaempferia parviflora* reduced the accumulation of visceral fat in diet- induced obese rats by escalating lipid metabolism through down regulation of sterol regulatory element binding protein (SREBP) **(Sato, 2010).** In this regard, it is well known that obesity-induced hyperleptinemia is positively correlated with increased body weight and visceral fat **(Van Dielen *et al.*, 2002).** Lowered serum leptin,as seen herein could therefore occurs by means of reducing animals body weight gain following BEX administration**.**

Results of the present study also showed marked elevation of serum adiponectin after administration of BEX. A finding that may be related to the high quercetin content of this plant extract. Querciten has shown to exert anti-inflammatory effects, probably through inhibiting nuclear factor-kB (NF-kB) which regulates the gene expression of the pro-inflammatory cytokine, TNF-α **(Nair *et al.*, 2006).** This phenomenon is, therefore may improve the inflammatory status of visceral adipose tissue in the obese populations and thus increasing plasma levels of adiponectin **(Rivera *et al.*, 2008).** Considering this effect and the fact that adiponectin is essential for facilitating insulin sensitivity **(Albert *et al.*, 2005)** it can suggest that increased adiponectin level by BEX may contribute to improved IR and related metabolic abnormalities, as that reported in the current study.

In this regard, different extracts from *Bauhinia variegata* leaves have been used for their hypoglycemic and hypolipidemic effects **(Rajkapoor *et al.*, 2009).** Evidence is provided for the presence of insulin like protein molecules in *Bauhinia variegat*a leaves that has partial amino acids sequences identical to bovine insulin **(Gupta *et al.*, 2009)**. Plant insulin is protected from hydrolysis in the digestive tract, crosses the intestinal barrier and promotes several metabolic activities as those of animal insulin, through glucose transportation into the cells and by phosphorylating proteins regulating carbohydrate metabolism (**Arain *et al.*, 2012**). Other specific compounds such as, ß-sitosterol was identified in *Bauhinia variegata* leaves. ß-sitosterol is one of phytosteroles present in the plant that is widely used in treating hypercholesterolemia, through inhibiting absorption of intestinal cholesterol and blocking cholesterol movement from the liver to the blood **(Maruthappan and Shree, 2010).** Further investigation regarding metabolic effects of *Bauhinia variegata* leaves showed marked lowering of serum glucose, TGs, and LDL-C with increasing HDL-C, which may be related to flavonoid compounds abundantly present in the plant **(Veerapur *et al.*, 2010).** Flavonoids may affect blood glucose and lipid profile by insulin-enhancing activity and may regulate the expression of gene involved in glucose uptake and insulin signaling in rats fed on HFD **(Rivera *et al.*, 2008).** The findings of the present study have similarly established marked hypoglycemic effects and lipid improving capacity following administration of BEX to HFD-fed rats, probably due to the presence of flavonoids and other active constituents such as ß-sitosterol and insulin-like protein molecules. However, further investigations are essential for separation and identification of these active constituents to elucidate precise mechanisms contributing for BEX health benefits**.**

In conclusion, the present data demonstrated that long term feeding on HFD causes marked obesity, in parallel with disrupted functional and structural integrity of the kidney, which appear to occur directly through alteration of adipokines (adiponectin and leptin) production and /or indirectly through developing insulin resistance and related metabolic abnormalities. However, administration of BEX to HFD-fed rats showed potential activity to prevent obesity and associated alterations relevant to incidence of kidney injury. Thus, BEX could be considered as a natural product of benefits for people at increased risk of developing kidney disease, particularly the obese subjects.

References:

1. Abbate, M.; Zoja, C. and Remuzzi, G. (2008): How does proteinuria cause progressive renal damage. Journal of the American Society of Nephrology, 17 (11): 2974–2984.
2. Afzali A, Weiss, NS, Boyk EJ and Ioannou GN (2010): Association between serum uric acid level and chronic liver disease in United States. Hepatology 52:578-589.

3. Aggarawal, B. B. (2010):Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu. Rev.Nutr. 30: 173–199.
4. Ahmedi, S. A.; Borou-Mand, M. A.; Moghddam, K. G.; Tajik, P. and Dibaj, S. M. (2008): The impact of low serum triglyceride on LDL-cholesterol estimation. Archives of Iranian Med., 11: 318–321.
5. Albert, CM.; Oh, K.; Whang W.; Manson, JE.; Chae, CU. and Stampfer, MJ. (2005): Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary disease. Heart Circulation., 112:3232–3238.
6. Appuhamy, J. A., Knoebel, N. A.; Nayananjalie, W. A.; Escobar, J. and Hanigan, M. D. (2012): Isoleucine and leucine independently regulate mTOR signaling and protein synthesisin MAC-T cells and bovine mammary tissue slices. J Nut.,142:484491.
7. Arain, S.; Memon, N.; Rajput, MT.; Sherazi, S. TH.; Bhanger, MI. and Mahesar,SA. (2012): Physico-chemical characteristics of oil and seed residues of *Bauhinia variegata* and *Bauhinia linnaei*. Pak J Anal Environ Chem., 13: 16-21.
8. Aronne, L. J. and Isoldi, K. K. (2007): Overweight and obesity: Key components of cardiometabolic risk. Clin. Cornerstone. 8: 29-37.
9. Baratta, R.; Amato, S.; Degano, C.; Farina, MG.; Patanè, G. and Vigneri, R. (2004): Adiponectin relationship with lipid metabolism is independent of body fat mass: Evidence from both crosssectional and intervention studies. J Clin Endocrinol Metab.; 89: 2665-2671.
10. Benedix, F.; Sabine, W.; Robert, P.; Dennis, G.; Claus, L.; Hans, L. and Stephanie, W. (2011): Weight loss and changes in salivary ghrelin and adiponectin: Comparison between sleeve gastrectomy and Roux-en-Y Gastric Bypass and Gastric Banding. Obes Surg. 21:616–624.
11. Birari, R.B. and Bhutani, K.K. (2007): Pancreatic lipase inhibitions from natural sources: Unexplored potential. Drug Discovery Today, 12: 879-889.
12. Bjorbaek, C. and Hollenberg, AN. (2002): Leptin and melanocortin signaling in the hypothalamus. Vitam Horm 65:288.
13. Brahmachari, G.; Mandal, LC.; Roy, R.; Jash, SK.; Gorai, D.; Mondal, A.; Majhi, S. and Lupeol, (2011): A pharmaceutically potent triterpenoid, from the ripe fruits of *Rauvolfia tetraphylla* L. (Apocynaceae).J Ind Chem Soc. 88(2):303-305.
14. Calderon-Montano, JM.; Burgos-Moron, E.; Perez-Guerrero, C. and Lopez- Lazaro, M. (2011): A review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 11(4):298-344.
15. Cárdenas-García, M. and González-Pérez, PP (2013): Applying the tuple space- based approach to the simulation of the caspases, an essential signalling pathway. J Integr Bioinform, 10: 225.
16. Carlyle, M.; Jones, O.; B. Kuo, J. J. and Hall, J. E. (2002): Chronic cardiovascular and renal actions of leptin: role of adrenergic activity, Hypertension, 39,(2):496–501.
17. Choe, JY.; Park, SH.; Kim, JY.; Shin, IH. And Kim, SK. (2008): Change in serum uric acid between baseline and 1-year follow-up and its associated factors in male subjects. Clin Rheumatol, 27:483–489.
18. Choudhary, MI.; Rahman, AU.; Alam, JM.; Jalel, S. and Naheed, S. (2005): Effects of ethanolic extract of Iris germanica on lipid profile of rats fed on high fat diet. Journal of Ethnopharmacology, 98: 217-220.
19. Considine, RV.; Sinha, MK.; Heiman,ML.; Kriauciunas, A.; Stephens, TW.; Nyce, MR. and Ohannesian, JP. (1996): Serum immunoreactive-leptin concentrations in normal weight and obese humans. N. Engl. J. Med., 334: 292-295.
20. El-Wakf, A.M.; El-habiby, El. and Mogalli, A. (2013): Curcumin acts as cardiovascular protector via improving leptin and insulin resistance in obese male rats. J Am Sci. 9 (3):397-405.
21. Flier, J. S.; Kahn, C. R.; Jarrctt, D. B. and Roth, J. (1976): Characterization of antibodies to the insulin receptor. J. Clin. Invest. 58:1442.
22. Gupta, R.; Paarakh, MP. and Gavani, U. (2009): Isolation of Phytoconstituents from the leaves of *Bauhinia variegata* Linn. Journal of Pharmacy Research; 2 :1315-1316.
23. Jin, D.; Xu, Y.; Mei, X.; Meng, Q.; Gao, Y.; Li, B. and Tu, Y. (2011): Antiobesity and lipid lowering effects of theaflavins on high-fat diet induced obese rats. Journal of Functional Foods, 5, 1142-1150.
24. Johnson, RJ.; Sanchez-Lozada, LG. and Nakagawa, T. (2010):The effect of fructose on renal biology and disease. J Am Soc Nephrol; 21:2036-2039.
25. Kishino, E.; Ito, T.; Fujita, K. and Kiuchi, Y.A. (2006): Mixture of the *Salacia reticulate* (*Kotala himbutu*). Aqueous extract and cyclodextrin reduces the accumulation of visceral fat mass in mice and rats with high-fat diet-induced obesity. Journal of Nutrition, 136: 433-439.
26. Lang, P. and Andersson, G. (2005): Differential expression of monomeric and proteolytically processed forms of tartrate-resistant acid phosphatase in rat tissues. Cell Mol Life Sci., 62: 905–918.
27. Lonardo, A.; Lombardini, S.; Scaglioni. F.; Carulli, L.; Ricchi, M. and Ganazzi, D. (2006):

Hepatic steatosis and insulin resistance: does etiology make a difference? J Hepatol, 44:190–196.
28. Maruthappan, V. and Shree, K.S. (2010): Effects of *Phyllanthus reticulatus* on lipid profile and oxidative stress in hypercholesterolemic albino rats. Indian J. Pharmacol, 42: 388–391.
29. Masoud, AY. And Adel, AA. (2006): Correlation between serum leptin levels, body mass index and obesity in Omanis. Sultan Qaboos Med J., 6: 28-31.
30. Matthews, DR.; Hosker, JP.; Rudenski, AS.; Naylor, BA.; Treacher, DF. And Turner, RC. (1985): Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 : 412–419.
31. Méndez-Sánchez, N.; Chavez-Tapia, NC.; Zamora-Valdés, D. and Uribe, M. (2006): Adiponectin, structure, function and pathophysiological implications in non-alcoholic fatty liver disease. Mini Rev Med Chem. 66:651-656.
32. Mink, PJ.; Scrafford, CG.; Barraj, LM.; Harnack, L.; Hong, C.; Nettleton, JA.; Jacobs, DR. Jr.; Mink, PJ.; Scrafford, CG and Barraj, LM. (2007): Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am. J. Clin. Nutr., 85:895–909.
33. Nadkarni, AK. (1996): Indian Materia Medica, vol 1. Mumbai: Popular Prakashan Ltd. 184-185.
34. Nair, MP.; Mahajan, S.; Reynolds, JL.; Aalinkeel, R.; Nair, H. and Schwartz, SA. (2006): The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor a) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kB system. Clin. Vaccine Immunol., 13:319–328.
35. Ogura, T.; Matsuura, K. and Matsumoto, Y. (2004): Recent trends of hyperuricemia and obesity in Japanese male adolescents, 1991 through 2002. Metabolism. 53(4):448–453.
36. Ohashi, K.; Iwatani, H.; Kihara, S.; Nakagawa, Y.; Komura, N.; Fujita, K.; Maeda, N.; Nishida, M. and Katsube, F. (2007): Exacerbation of albuminuria and renal fibrosis in subtotal renal ablation model of adiponectin-knockout mice. ArteriosclerThromb Vasc Biol 27: 1910–1917.
37. Ouchi, N.; Kihara, S. and Funahashi T. (2003): Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation.; 107: 67- 674.
38. Parving, H.; Osterby, R. and Ritz, E. (2000): Diabetic Nephropathy." In Brenner B. (ed): Brenner & Rector's The Kidney" 6th Edition. 1731-1773. W.B. Saunders Co. Philadelphia.
39. Prabu, VA.; Rajkumar, M. and Perumal, P. (2009): Seasonal variations in physicochemical characteristics of *Pichavaram mangroves*, southeast coast of India, Journal of Environmental Biology, 29(6):945-950.
40. Preshaw, PM.; Alba, AL.; Herrera, D.; Jepsen, S.; Konstantinidis, A.; Makrilakis, K. and Taylor R. (2012): Periodontitis and diabetes: A two- way relationship. Diabetologia 55: 21-31.
41. Rajani, GP. and Purnima, A. (2009): In vitro antioxidant and antihyperlipidemic activity of *Bauhinia variegata* Linn. Indian J Pharmacol oct., 41(5)227-232.
42. Rajkapoor, B.; Jayakar, B.; Murugesh, N. and Sakkthisekaran D. (2009): Chemoprevention and cytotoxic effect of *Bauhinia variegata* against N-nitrosodiethylamine induced liver tumours and human cancer cell lines. Journal of Ethnopharmacology.104: 407-409.
43. Rang, HP.; Dale, MM.; Ritter, JM. and Moore, PK. (2003):Obesity. Pharmacology 5th ed. Churchill livingstone. An imprint of Elsevier Limited., 394-402.
44. Ritz, E.; Koleganova, N. and Piecha, G. (2011): Is there an obesity-metabolic syndrome-related glomerulopathy? Curr Opin Nephrol Hypertens; 20:44-49.
45. Rivera, L.; Morón, R.; Sánchez, M.; Zarzuelo, A. and Galisteo, M. (2008): Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity 16:2081–2087.
46. Saito, J.; Matsuzawa, Y. and Ito. H. (2010): The alkalizer citrate reduces serum uric Acid levels and improves renal function in hyperuricemic patients treated with the xanthine oxidase inhibitor allopurinol. Endocr Res. 35:145–154.
47. Santanu Sahaa, E.V.S. ; Subrahmanyamb, K.S.; Chandrashekarc, and Shashidhara, C. (2011): Shastryd *in vivo* study for anti-inflammatory activity of *Bauhinia variegata* L. Leaves. Pharmaceutical Crops, 2: 70-73.
48. Sato, R. (2010): Sterol metabolism and SREBP activation. Archives of Biochemistry and Biophysics, 501: 177-181.
49. Schirmeister, J. (1964):determination of creatinine in serum. DSH. Med. Wschr.,89: 1940.
50. Schlondorff, D.O. (2008):Overview of factors contributing to the pathophysiology of progressive renal disease, Kidney International, 74: 860–866.
51. Schlottmann, I.; Dees, C. and Funke, R. (2013): The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. Ann Rheum Dis doi: 10.1136/2012-203194.

52. Serradeil-Le Gal, C.; Raufaste, D. Brossard, G.; Pouzet, B.; Marty, E.; Maffrand, JP. and Le Fur, G. (1997): Characterization and localization of leptin receptors in the rat kidney. FEBS Lett 404: 185–191.
53. Shankar, A.; Sun, L. and Klein, B. (2011): "Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study, Kidney International; 11:1231–1238.
54. Sharma, S. and RamachandraRao, G. (2008):Adiponectin regulates albuminuria and podocyte function in mice. Journal of Clinical Investigation, 118 :1645–1656.
55. Silva, J. J. and Hall, J. E. (2004): Role of hypothalamic melanocortin 3/4-receptors in mediating chronic cardiovascular, renal, and metabolic actions of leptin," Hypertension, 43 : 1312–1317.
56. Snedecor, C.W. and Cochran, W.C. (1982): Statistical Methods. 7th ed. The State University Press American, Iowa.P.593.
57. Takahashi, MM.: Oliveira, EP.; Carvalho, AL.; Dantas, LA. and Burini, FH. (2011): Metabolic syndrome and dietary components are associated with coronary artery disease risk score in free-living adults: a cross-sectional study. Diabetol Metab Syndr, 3:7.
58. Taylor, BA. And Phillips, SJ. (1996): Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics, 34:389-398.
59. Thaler, J. P.; Schur, E. A.; Guyenet, S. J.; Hwang, B. H. Dietrich, M. O.; Zhao, X. and Sarruf, D. A. (2012): Obesity is associated with hypothalamic injury in rodents and humans. J. Clin. Invest.,122: 153–162.
60. Thornalley, PJ. and Ahmed, N. (2007): Advanced glycation endproducts: what is their relevance to diabetic complications? Diabetes Obese Metab.,9:233-245.
61. Tomoko, A., Tsutomu, S. and Susumu, T. (2011): Antiobesity Effects of *Kaempferia parviflora* in spontaneously obese type 2 diabetic mice. Journal of Natural Medicines, 65, 73-80.
62. Tsioufis, C.; Dimitriadis, K.; Chatzis, D.; Vasiliadou, C.; Tousoulis, D.; Papademetriou, V; Toutouzas, P.; Stefanadis, C. and Kallikazaros, I.(2005): Relation of microalbuminuria to adiponectin and augmented C-reactive protein levels in men with essential hypertension. Am J Cardiol 96: 946.
63. Twigg, SM.; Joly, AH.; Chen, MM.; Tsubaki, J.; Kim, HS and Rosenfeld, RG. (2002):Protein-connective tissue growth factor/IGF-binding protein-2 is a mediator in the induction of fibronectin by advanced glycosylation end-products in human dermal fibroblasts. Endocrinology; 143:1260-1269.
64. Van Dielen, F.M.; Veer, C.; Buurman, W.A. and Greve, J.W.M. (2002): Leptin and soluble leptin receptor levels in obese and weight-losing individuals. Journal of Clinical Endocrinology & Metabolism, 87: 1708-1716.
65. Veerapur, VP.; Prabhakar, KR.; Thippeswamy, BS.; Bansal, P.; Srinivasan, KK. and Unnikrishnan, MK. (2010): Antidiabetic effect of *Dodonaea viscosa* (L). Lacq, aerial parts in high fructose-fed insulin resistant rats: A mechanism based study. Ind. J. Exp. Biol. 48:800-810.
66. Wang, Y.; Chen, X. and Song, Y. (2008): Association between obesity and kidney disease: A systemic review and meta-analysis. Kidney Int., 73:19-33.
67. Warwar, N.; Efendic, S. O. and Stenson, CG. (2006): Dynamics of glucose-induced localization of PKC isoenzymes in pancreatic β -cells. Diabetes-related changes in the GKrat. Diabetes, 55: 590–599.
68. Yamagishi, D.; Edelstein, X. L.; Du, Y.; Kaneda, M.; Guzm´an, and Brownlee, M. (2001):Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A, The Journal of Biological Chemistry, 276 : 25096–25100.
69. Yan, HD.; Li, XZ.; Xie, JM. and Li, M. (2007): Effects of advanced glycation end products on renal fibrosis and oxidative stress in cultured NRK-49F cells. Chin Med J (Engl).;120:787-93.
70. Zhou, C.J.; Huang, S.; Liu, J.Q.; Qiu, S.Q.; Xie, F.Y. and Song, H.P. (2013): Sweet tea leaves extract improves leptin resistance in diet-induced obese rats. J of Ethnopharmacology, 145: 386-392.

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