Anti-obesity, antiatherogenic, anti-diabetic and antioxidant activities of *J. montana* ethanolic formulation in obese diabetic rats fed high-fat diet

Mohammed Abdalla Hussein

Biochemistry department, Faculty of Pharmacy, October 6th University, October 6th City, Egypt.

ABSTRACT

J. montana extract in the form of ethanolic formulation is rich in polyphenols, mono- and sesquiterpenes, Essential oils, flavonoids, tri- tetra- and pentamethoxy quercetin derivatives. The present study was designed to investigate the anti-obesity, antiatherogenic, anti-diabetic and antioxidant activities of *J. montana* using obese diabetic rats' model. Rats received either regular diet, high-fat diet or high-fat diet with additional *J. montana* (150 and 300 mg/kg bw) for 8 weeks. In the preventive experiment, *J. montana* co-administered with a high fat diet significantly inhibited body weight gain, blood glucose, triglyceride, total cholesterol, LDL-C, vLDL-C, HDL-C, free fatty acid and atherogenic index levels in a dose dependent manner. *J. montana*-treated rats at doses of 150 and 300 mg/kg improved the insulin resistance index when compared to the high fat diet (HFD) control. Finally, the present study is designed to evaluate the effect of ethanolic extract of *J. montana* on figh fat diet induce obesity in rats. *J. montana* treatment (150 and 300 mg/kg bw) for 8 consecutive weeks prior to obese rats administration significantly prevented the decrease in the levels of hepatic oxidative stress biomarkers reduced Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT). The *J. montana* extract, also exhibited its capacity to prevent the elevated thiobarbituric acid reactive substances (TBARS) in the liver tissue. In conclusion, the anti-obesity actions of *J. montana* are considered attributable to increased expression of energy expenditure-related fatty liver degradation, and decreased fatty acid synthesis and fat intake in the liver. Taken together, *J. montana* has potential as a preventive agent for type 2 diabetes mellitus (and possibly obesity) and deserves clinical trial in the near future.

Keywords: *J. montana*, anti-obesity, type 2 diabetes mellitus, glucose, insulin resistance index, atherogenic index.

Correspondence : Tel.: +2 (012) 4832580; fax: +2 (02) 38353270.

E-mail: abdallamohammed_304@yahoo.com; abdallamohammed_405@hotmail.com

INTRODUCTION

There is a worldwide epidemic of obesity, which is associated with a number of pathologies including dyslipidemia, glucose intolerance, insulin resistance and diabetes mellitus, all of which are risk factors for cardiovascular disease and mortality.[1-3] Diabetes is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. The aim of therapy in diabetes is to achieve normoglycemia to prevent later microvascular complications (retinopathy, nephropathy, neuropathy and microangiopathy), and intensive therapy to achieve glycemic control has been shown to significantly diminish the risk of long-term complications.[4] Since lipid abnormalities, leading to premature atherosclerosis, are the major cause of cardiovascular diseases in diabetic patients, ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. The five types of oral anti-diabetic drugs, currently approved for the treatment of type 2 diabetes do not have a favorable effect on cardiovascular disease[5], and some of these drugs are associated with serious adverse effects. Thus, new, relatively non-toxic, therapeutic agents are needed to treat hyperglycemia, which also would correct dyslipidemia to reduce the risk of cardiovascular complications of diabetes. Several FDA-approved drugs for conditions other than obesity have been investigated as treatment of excess body weight.[6,7] Metformin is one such drug. Metformin, the biguanide most widely used for the treatment of type 2 diabetes mellitus[8], may be useful in aiding weight loss. In diabetic patients, it suppresses endogenous glucose production and may also act as an

insulin sensitizer. It also helps diabetic patients lose weight or at least keep their weight stable.[9] In recent years, polyphenols have been reported to possess various pharmacological actions, including anti-obesity[10, 11], anti-diabetic [12, 13] and anti-cancer.[14] Many plant extracts and plant products have been shown to have significant anti-obesity and anti-diabetic activities,[15,16] which may be an important property of medicinal plants associated with the treatment of several ill fated diseases including obesity, diabetes and atherosclerosis. Plant-derived polyphenols minimize obesity induced diabetes.[17] Among these herbal resources, the plant *Jasonia montana* occurs in the Mediterranean and adjacent areas,[18] including the Sinai Peninsula.[19] The herb has a strong aromatic odor and is used in traditional medicine for diarrhea, stomachache, and chest diseases.[20] *Jasonia montana* is one of the most common medicinal plants. The *Jasonia montana* owes its therapeutical activity to different groups of effective substances, which make up the complex effect of the drug. A literature survey indicated that some mono- and sesquiterpenes,[21-24] flavonoids,[25] Essential oils are of greatest importance among all effective substances.[26] Poly-phenols exist in many plants and are especially abundant in *Jasonia montana*[27], whose dried leaves are used as antioxidant. *Jasonia montana* and polyphenol-enriched plant extracts have no known toxicity. Thus polyphenols from *Jasonia montana* and possibly other plant sources represent a promising potential species.[28] These polyphenols are more potent antioxidants than vitamins C and E.[29] Polyphenol rich extracts from *Jasonia montana* inhibit lipid peroxidation in experimental animals.[28-30] Not surprisingly, plants such as *Jasonia montana* contain high levels of polyphenols,[29] which are excellent scavengers of reactive and represent a promising antiobesity effect. The different extracts of the plant were also tested for hypoglycemic, antidiabetic and anticholestatic activities.[28, 30] Recently, Hussein and Farghaly[31] studied the protective activity of *Jasonia* ethanolic extract against liver and kidney damage induced by iron-overloaded in adult rats and suggest that the aerial parts of *J. montana* extract may effectively normalize the impaired antioxidant status in iron-overloaded rats model experiment. *In vivo* tests have been conducted with *Jasonia montana* to determine, for example, its, hypoglycemic,[28] antioxidant, anticholestatic[30] and antihaemostatic[31] activities. But there are no reports of the anti-obesity effects of *Jasonia montana* in obese diabetic rats fed high-fat diet.

In continuation of my interested research program in the extraction and therapeutic evaluation of *Jasonia montana* extract[28,30,31], I report herein, a facile route to explain the anti-obesity, antiatherogenic anti-diabetic and antioxidant effects of *Jasonia montana* extract in the form of ethanolic formulation in obese diabetic rats fed high-fat diet, which may pave the way for possible therapeutic application.

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *J. montana* were collected from the Sinai Peninsula.

Preparation of ethanolic extract

Air-dried aerial parts of the plant (1.5 kg) was crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield viscous mass. The ethanolic extract was kept in airtight containers in a deep freeze maintained at 4 °C until the time of further use.

Phytochemical screening

A phytochemical analysis of aerial parts of *J. montana* was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, coumarins and triterpenes.[32]

Experimental set up

This experiment was carried out to examine the anti-obesity and anti-diabetic affects of *J. montana* ethanolic extract in obese diabetic rats fed high-fat diet. Adult albino rats weighing around 150 ± 5 gms were purchased from Faculty of Veterinary Medicine, Cairo University. The animals were housed in individual cages with free access to water in a temperature-controlled facility with a 12:12-h light-dark cycle, and the animals were weighed periodically. During the acclimatization period, each animal was raised on a regular diet (Dyets Inc., Bethlehem, PA) *ad libitum*.

Ethanolic extract of *J. montana* air-dried aerial parts, given repeatedly for an 8-week period *in vivo*. A suspended solution of 3g % was prepared for intragastric intubation of rats. The animals were randomly divided into five groups 6 rats in each, two controls groups and three treatment groups.

Control group-I (was received a regular diet + 1 ml tween 80 for an 8-week period).

Control group-II (was received a high-fat diet + 1ml tween 80 for an 8-week period).

Group III: Was fed a high-fat diet with *J. montana* ethanolic extract (150 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period.[30]

Group IV: Was fed a high-fat diet with *J. montana* ethanolic extract (300 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period.[30]

Group V: Was fed a high-fat diet with metformin (500 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period.[33]

Hyperglycemia induction diet was purchased from Dyets Inc. (AIN-76 diet #101772, Bethlehem, PA, USA). The nutrition contents of the high fat diet were similar to those of the regular diet except the addition of beef tallow (Table 1).[34] Body weights were measured weekly, and every other week, blood was collected for blood glucose analysis. At the end of the study, blood was also collected for the determination of plasma insulin, insulin resistance index, atherogenic index and lipid levels. In addition, *in vivo* antioxidant and lipid peroxidation parameters in tissue of liver after which they were killed.

Table 1. Composition of the diets

Ingredients	Regular diet (g/kg diet)	High fat diet (g/kg diet)
Casein	200	200
DL-Methionine	3.0	3
Corn Starch	150	150
Sucrose	500	150
Cellulose	50	-
Corn oil	50	-
Beef tallow	-	400
Mineral mixture[a]	35	35
Vitamin mixture[a]	10	10
Choline bitartrate	2	2
Energy, kJ/g	0.9	1.30
Protein, % kcal/kg	13.3	13.3
Carbohydrate, % kcal/kg	47.4	19.8
Fat, % kcal/kg	8.0	65.7
Fiber, % kcal/kg	8.0	-
Salt mixture	13	13
Cotton seed oil	10.3	10.3

[a]AIN 76A Rodent Purified Diet (34)

Blood sampling and plasma assay

Blood was withdrawn from the orbital venous plexus every other week, using a heparinized capillary tube without anesthesia. The blood samples were placed on ice, centrifuged, and plasma stored at -20°C until assayed. The plasma glucose concentration was determined using the glucose oxidase method (Youngdong Pharmaceutical Co, Korea). The plasma insulin concentration was measured according to the protocol described by the manufacturer of the insulin ELISA kit (Shibayagi Co., Japan). The insulin resistance index, calculated by insulin (mU/ml) X glucose (mM)/22.5 (35). Plasma triglyceride, total cholesterol, HDL- cholesterol and free fatty acid concentrations were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea). Plasma LDL-cholesterol level was calculated from Friedewald[36] formula (LDLcholesterol = total cholesterol – triglycerides/5 – HDLcholesterol). Plasma vLDL-cholesterol concentration was calculated according to Nobert (47) formula (vLDL-cholesterol = triglycerides/5). The atherogenic index [log (TG/HDL-C)] was also calculated.[37] On the other hand, liver triglyceride and cholesterol content were measured as described previously.[38,39] Briefly, a portion (100 mg) of liver tissue was homogenized in phosphate buffer saline (pH 7.4, 1 ml). The homogenate (0.2 ml) was extracted with isopropyl alcohol (1 ml), and the extract was analyzed using a Triglyceride E-Test (Wako Pure Chemical Industries) to determine liver triglyceride content. The homogenate (0.2 ml) was extracted with chloroform-methanol (2: 1, 1 ml), and the extract was concentrated under a nitrogen stream. The residue was dissolved in isopropyl alcohol and analyzed using a Cholesterol E-Test (Wako Pure Chemical Industries). Finally, another portion from liver was blotted, weighed and homogenized with methanol (3 volumes). The lipid extract obtained by the method of Folch et al.[40] It was used for the estimation of thiobarbituric acid reactive substances (TBARS).[41] Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced Glutathione (GSH),[42] Glutathione peroxidase (GPx)[43], Glutathione reductase (GR)[44], Superoxide dismutase (SOD) [45] and Catalase (CAT).[46]

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA)[47] followed by least significant difference (LSD) test to compare various groups with each other. Results were expressed as mean ± standard

error (SE) and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significant and highly significant, respectively. F-probability expresses the general effect between groups. The means that were not significantly different are followed by the same superscript symbol(s).

RESULTS

Body weight and food intake were determined once every 2 weeks. The body weight of the normal rats in the regular diet group gradually increased as the rats grew during the 8- week trial. In contrast, the body weight of animals on the high fat diet showed rapid increases during the course of the trial (Table 2). Weight gains in regular diet and high fat diet control groups during the 8-week period were 17.7±2.4g and 56.8±4.1g, respectively (Table 3). Subjects fed the high fat diet and *J. montana* ethanolic extract showed a gradual increase in body weight, but the increase was significantly less than that detected for the high fat diet control group in spite of continued and prolonged access to the high fat diet (Table 2). *J. montana* 150 and 300 mg/kg bw prevented the weight gain by 31.19 % and 48.96 %, respectively, compared to the body weight of the high fat diet control group. Feed efficiency, calculated by weight gain divided by total food intake during the 8-week period, was compared in order to figure out the relationship between food intake and weight gain. As shown in Table 3, weight gain of the high fat diet control rats was actually due to the increased food intake. However, body weights of *J. montana* extract fed rats were significantly reduced despite the even larger increase in food intake compared to the high fat diet control rats. Feed efficiency of the *J. montana* ethanolic extract (300 mg/kg) fed group was 1.9, which is lower than the value shown for the high fat diet control group and similar to that of regular diet fed group mainly due to a significant reduction of food intake compared to the high fat diet control group, indicating that *J. montana* extract could be a fascinating drug that allows patients to slim down despite an increase in food intake or reduction of physical activity. On the other hand, body weight of metformin fed group was near to that of regular diet fed group mainly due to a significant reduction of food intake compared to the high fat diet control group.

Insulin resistance index (IRI)

Plasma glucose was determined every other week and was compared between groups in table 4. Plasma glucose levels were barely increased in the regular diet fed control group, while a marked increase after the 8 weeks was observed for rats only fed with the high fat diet. *J. montana* ethanolic extract fed rats, however, showed a significant decrease in blood glucose levels in a dose dependent manner when compared to the high fat diet control group

Table 2. Changes in body weight of control and experimental groups of rats

Groups	Number of weeks Body weight of rats(g)				
	0	2	4	6	8
Control group-I Regular diet (RD)	150.7 ±4.2	153.6 ±6.8[a]	160.5 ±5.4[a]	166.9 ±7.3[a]	168.4 ±9.4[a]
Control group-II High-fat diet (HFD)	150.6 ±3.9	172.5 ±6.4[d]	191.2 ±2.7[d]	204.6 ±3.9[c]	206.9 ±5.81[d]
HFD+ *J. montana* extract 150 mg/kg bw	152.6 ±5.3	157.4 ±6.2[b]	170.6 ±4.8[c]	184.5 ± 6.1[b]	191.7 ±5.4[d]
HFD + *J. montana* extract 300 mg/kg bw	154.2 ± 4.3	154.3 ± 4.9	162.7 ±5.7[b]	174.5 ±5.2[a]	183.2 ±4.4[c]
HFD+ Metformin 500 mg/kg bw	150.2 ±7.2	162.4 ±5.3[a]	166.2 ±6.8[a]	173.1 ±4.9[a]	176.2 ±5.2[a]
F-probability	NS	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01
LSD at 5% level	--	0.77	2.15	2.23	2.23
LSD at 1% level	--	0.78	2.90	2.95	2.45

Body weight of rats consuming regular diet, high fat diet, high fat diet plus *J. montana* ethanolic extract or fed with high fat diet plus 500 mg/kg of metformin during the 8-week period. Values are given as mean ± SD for groups of six animals each. High-fat diet control rats were compared with regular diet control rats. Experimental groups were compared with the high-fat diet control rats.

Table 3. Effect of *J. montana* ethanolic extract on weight gain, food intake, and feed efficiency

Groups	Initial	Final	Weight gain (g/8 wk)	Food intake (g/8 wk)	Feed efficiency ($\times 10^{-3}$)
Control group-I Regular diet (RD)	150.7 ±4.2	168.4 ±9.4[a]	17.7 ±2.4[a]	9567[a]	1.9[a]
Control group-II High-fat diet (HFD)	150.6 ±3.9	206.9 ±5.81[d]	56.82 ±4.1[c]	10989[a]	5.17[d]
HFD+ *J. montana* extract 150 mg/kg bw	152.6 ±5.3	191.7 ±5.4[c]	39.1 ±5.4[b]	13393[b]	2.92[b]
HFD + *J. montana* extract 300 mg/kg bw	154.2 ± 4.3	183.2 ±4.4[b]	29.0 ±3.8[d]	15246[c]	1.9[a]
HFD+ Metformin 500 mg/kg bw	150.2 ±7.2	176.2 ±5.2[a]	26.0 ±2.9[d]	9320[b]	2.79[b]
F-probability	NS	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01
LSD at 5% level	NS	11.44	2.13	2.15	0.57
LSD at 1% level	NS	15.59	2.29	2.56	0.77

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

*Significantly different from control group at *p< 0.05*.

®Significantly different from control group at *p< 0.01*.

Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

Table 4. Effect of *J. montana* ethanolic extract on plasma insulin, plasma glucose and insulin resistance index (IRI)

Groups	Plasma insulin (mU/ml)	Plasma glucose (mM)	insulin resistance index (IRI)
Control group-I Regular diet (RD)	93.7± 6.11[a]	6.4 ± 0.8[a]	26.6[b]
Control group-II High-fat diet (HFD)	173.6 ± 8.32[f]	12.2 ± 2.1[f]	94.13[f]
HFD+ *J. montana* extract 150 mg/kg bw	113.5 ± 6.2[d]	8.15 ± 1.6[b]	41.11[c]
HFD + *J. montana* extract 300 mg/kg bw	107.2 ± 7.4[c]	7.24 ± 1.4[a]	34.49[b]
HFD+ Metformin 500 mg/kg bw	97.3 ± 5.81[b]	7.4 ± 1.8[a]	25.125[a]
F-probability	*P*<0.01	*P*<0.01	*P*<0.01
LSD at 5% level	24.58	0.187	4.59
LSD at 1% level	33.48	0.225	6.26

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats. Insulin Resistance Index = insulin (mU/ml) x glucose (mM) / 22.5.

(Table 4). Plasma insulin levels in *J. montana* 150 and 300 mg/kg bw treated groups were also markedly decreased by 34.62% and 38.25%, respectively when compared to the high fat diet control group (Table 4). Also, plasma glucose levels in *J. montana* 150 and 300 mg/kg bw treated groups were also markedly decreased by 33.19% and 40.65%, respectively when compared to the high fat diet control group (Table 4). The insulin resistance index, calculated by [insulin (mU/ml) X glucose (mM)]/22.5, of the high fat diet control group was 3.5 times higher than that of the regular diet group, while insulin resistance indices of *J. montana* 150 and 300 mg/kg bw were

significantly reduced by 56.33% and 63.36%, respectively, when compared to the high fat diet control group (Table 4). Improvement of insulin resistance in the *J. montana* ethanolic extract (300 mg/kg bw) fed group was significant and comparable to the metformin (500 mg/kg bw) fed group. This result suggests that wild *J. montana* extract was able to lower the blood glucose level partially due to the improvement of insulin resistance.

Plasma lipid levels

The effects of *J. montana* ethanolic extract on plasma lipid levels were examined at the end of the treatment. The plasma lipid levels in high fat diet fed rats were dramatically increased compared to the levels in regular diet fed rats except for the HDL-cholesterol (HDL-C) level, which is supposed to be higher than 40 mg/dl (Table 5). In the high fat diet control group, plasma triglyceride (TG) was increased by 1.69-fold (115 to 195 mg/dl), total cholesterol was increased by 2.15-fold, LDL-cholesterol increased by 6.78-fold, vLDL-cholesterol increased by 1.69-fold, free fatty acid increased by 1.94-fold, and total cholesterol (TC) increased as reflected in the increase in LDL-cholesterol (LDL-C) concentration compared to those in the regular diet group. *J. montana* 150 mg/kg bw-treated group, however, showed considerably reduced levels of TG, TC, LDL-C, v LDL-C and free fatty acid by 20.32%, 31.18%, 52.33%, 20.32% and 29.09%, while they showed an increased level of HDL-C by 81.02% with compared to that in high fat diet fed control group. On the other hand, *J. montana* 300 mg/kg bw-treated group, however, showed considerably reduced levels of TG, TC, LDL-C, v LDL-C and free fatty acid by 35.03%, 42.73%, 67.20%, 35.02% and 99.93%, while they showed an increased level of HDL-C by 91.63% with compared to that in high fat diet fed control group. The atherogenic index, calculated by log (TG/HDL-C), of the high fat diet control group was 6.93 times higher than that of the regular diet group, while atherogenic index of *J. montana* 150 and 300 mg/kg bw were significantly reduced by 53.98% and 70.68%, respectively, when compared to the high fat diet control group (Table 5). Metformin remarkably improved high fat diet induced dyslipidemia, and all lipid related plasma parameters in metformin fed rats were comparable to those in regular diet fed rats.

Liver triglyceride and cholesterol content

Table 6 shows liver lipid content. At the end of administration, liver triglyceride and cholesterol levels were significantly higher for the high fat diet fed group when compared to the regular fed group. For high fat diet/*J. montana* 150 and 300 mg/kg bw groups, triglyceride and cholesterol accumulation were significantly

Table 5. Effect of *J. montana* ethanolic extract on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL- cholesterol (LDLC), vLDL- cholesterol (vLDLC), nonesterified fatty acid (NEFA) and atherogenic index.

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	vLDL-C (mg/dl)	NEFA (mEq/dl)	Atherogenic index
Control group-I Regular diet (RD)	115.62 ±5.1[a]	151.64 ±6.71[a]	92.63 ±6.19[a]	35.97 ±3.88[a]	23.12 ± 2.75[a]	723.6 ±23.09[a]	0.096 ±0.008[a]
Control group-II High-fat diet (HFD)	195.3 ±9.34[d]	325.42 ±21.7[f]	42.22 ±5.76[d]	244.14 ±17.5[f]	39.06 ± 4.70[c]	1406 ±31.68[f]	0.665 ±0.021[f]
HFD+ *J. montana* extract 150 mg/kg bw	155.6 ±4.72[c]	223.94 ±13.62d	76.43 ±5.11[a]	116.39 ±7.45[f]	31.12 ± 3.66[b]	997 ±11.48[d]	0.309 ±0.011[c]
HFD + *J. montana* extract 300 mg/kg bw	126.88 ± 8.30[b]	186.36 ±10.73[c]	80.91 ±6.46[a]	80.07 ±4.53[d]	25.38 ± 4.18[a]	878 ±21.70[b]	0.195 ±0.013[d]
HFD+ Metformin 500 mg/kg bw	122.4 ±9.44[b]	180.71 ±10.43[c]	80.43 ±5.66[a]	75.8 ±5.37[d]	24.48 ± 2.97[a]	816 ±13.47[d]	0.182 ±0.014[d]
F-probability	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001
LSD at 5% level	2.36	1.17	3.88	13.46	0.96	19.47	4.53
LSD at 1% level	2.08	1.75	4.25	11.28	0.82	15.46	4.78

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

LDL-C (mg/dl) = TC-HDL-[TG / 5]

vLDL-C(mg/dl) = [Triglycerides/5]

Atherogenic index = log (TG/HDL-C)

suppressed. Liver triglyceride levels in *J. montana* 150 and 300 mg/kg bw treated groups were markedly decreased by 47.68% and 55.16%, respectively when compared to the high fat diet control group (Table 6). Liver total cholesterol levels in *J. montana* 150 and 300 mg/kg bw treated groups were also markedly decreased by 32.55% and 42.21%, respectively when compared to the high fat diet control group (Table 6).

Table 6. Effect of *J. montana* ethanolic extract on liver triglyceride and cholesterol content.

Groups	Liver triglyceride (mg/g liver)	Liver cholesterol (mg/ g liver)
Control group-I Regular diet (RD)	85.73 ± 5.11[a]	3.7 ± 0.82[h]
Control group-II High-fat diet (HFD)	178.92 ± 13.55[f]	6.42 ± 1.37[d]
HFD+ *J. Montana* extract 150 mg/kg bw	93.60 ± 4.15[b]	4.33 ± 1.25[a]
HFD + *J. Montana* extract 300 mg/kg bw	80.22 ± 4.18[a]	3.71 ± 0.45[a]
HFD+ Metformin 500 mg/kg bw	84.09 ± 7.46[b]	4.09 ± 5.24[c]
F-probability	*P*<0.001	*P*<0.001
LSD at 5% level	1.27	0.78
LSD at 1% level	2.10	0.36

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

Liver thiobarbituric acid reactive substances (TBARS), reduced Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT) content.

Tables 7 and 8 shows liver TBARS, GSH, GPx, GR, SOD and CAT content. At the end of administration, liver TBARS level was significantly higher for the high fat diet fed group when compared to the regular fed group. For both high fat diet/*J. montana* 150 and 300 mg/kg bw groups TBARS accumulation was significantly suppressed. Liver TBARS level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly decreased by 44.03% and 55.96%, respectively when compared to the high fat diet control group (Table 7). Also, liver GSH, GPx, GR, SOD and CAT were significantly lower for the high fat diet fed group when compared to the regular fed group (Tables 7&8). Liver GSH level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly increased by 91.42 % and 142.85%, respectively when compared to the high fat diet control group (Table 7). Liver GPx level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly increased by 47.28% and 77.11%, respectively when compared to the high fat diet control group (Table 7). On the other hand, Liver GR level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly increased by 55.29% and 120%, respectively when compared to the

Table 7. Effect of *J. montana* ethanolic extract on liver thiobarbituric acid reactive substances (TBARS), reduced Glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione reductase (GR)

Groups	TBARS (n mol of MDA formed/g tissue)	GSH (mg/g tissue)	GPx (mg of GSH consumed/min/ mg protein)	GR (mg of GSH consumed/min/ mg protein)
Control group-I Regular diet (RD)	25.45 ± 3.18[d]	5.73 ± 1.08[g]	10.45 ± 2.07[h]	2.11 ± 0.08[a]
Control group-II High-fat diet (HFD)	76.42 ± 6.11[f]	1.75 ± 0.07[a]	5.33 ± 0.19[a]	0.85 ± 0.04[d]
HFD+ *J. montana* extract 150 mg/kg bw	42.77 ± 5.25[c]	3.35 ± 0.18[c]	7.85 ± 1.48[b]	1.32 ± 0.07[c]
HFD + *J. montana* extract 300 mg/kg bw	33.65 ± 4.46[a]	4.25 ± 0.12[d]	9.44 ±1.35[d]	1.87 ± 0.06[c]
HFD+ Metformin 500 mg/kg bw	61.8 ± 5.27[d]	2.90 ± 0.08[a]	6.17 ±1.43[f]	1.06 ± 0.05[b]
F-probability	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.01
LSD at 5% level	10.83	2.062	1.62	0.470
LSD at 1% level	14.78	2.812	2.22	0.642

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

high fat diet control group (Table 7). In addition, Liver SOD level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly increased by 82.95% and 129.55%, respectively when compared to the high fat diet control group (Table 8). Finally, Liver CAT level in *J. montana* 150 and 300 mg/kg bw treated groups were markedly increased by 30.70% and 64.31%, respectively when compared to the high fat diet control group (Table 8).

DISCUSSION

Obesity is the most common nutritional disorder in the developed world and it is considered a risk factor associated with the development of major human diseases, including cardiovascular disease, diabetes, and cancer. A daily consumption of diets high in fat tends to promote obesity.

Increased intake of high caloric (energy and fat) food promotes body fat storage and greater body weight and adiposity in humans[48] and animals.[49] Over-the-counter remedies based on nutritional supplements are extremely popular, especially with respect to obesity and body composition. Inhibition of the digestion and absorption of dietary fat has been used as targets in obesity treatment.[50] The anti-obesity effects of *J. montana* ethanolic extract was investigated using obese diabetic rats fed high-fat diet as a model of obese type-II diabetes. When fed a high-fat diet, rats develop obesity and type-II diabetes by 12-weeks old,[51] and these rats are thus widely used for research into obesity and diabetes.[52] In the present study, high-fat diet was administered to 8-week-old to induce severe obesity and diabetes and the effects of *J. montana* ethanolic extract were evaluated. High-fat diet is widely used in studies on obesity and diabetes.[53] *J. montana* was found to significantly suppress increases in body weight, showing anti-obesity actions (Table 2). Plasma glucose and insulin levels were significantly higher for the high-fat diet group than for the regular diet group, and severe type II diabetes was induced. *J. montana* suppressed these increases in plasma glucose and insulin levels. The insulin resistance index, a simpler method to measure insulin sensitivity usually used in clinical and animal studies,[54] was significantly decreased in *J. montana* -treated groups compared to the high-fat diet group, indicating that *J. montana* reduced hyperglycemia[28] and hyperinsulinemia (Table 4). These findings clarify that *J. montana* suppresses obesity and diabetes caused by a high-fat diet. Obesity is caused by low energy expenditure and increased fatty acid synthesis from carbohydrates and fat intake by organs. *J. montana* ethanolic extract was found to significantly suppress increases in plasma lipids content, showing anti-obesity actions (Table 5). High-fat diet also increased liver fat accumulation and induced fatty liver, but *J. montana* administration lowered fat accumulation, clarifying that *J. montana* ethanolic extract suppresses TG, TC, HDL-C, LDL-C, vLDL-C and free fatty acid (Table 5). The elevation of TG, TC, LDL-C, v LDL-C and free fatty acid up on high fat diet feeding is not surprising and is in agreement with several studies.[30] The

Table 8. Effect of *J. montana* ethanolic extract on liver Superoxide dismutase (SOD) and Catalase (CAT).

Groups	SOD (unit min/mg/protein)	CAT (μ moles of H_2O_2 consumed min/mg/protein)
Control group-I Regular diet (RD)	4.25 ± 0.12^{g}	36.44 ± 3.45^{a}
Control group-II High-fat diet (HFD)	1.76 ± 0.11^{d}	19.25 ± 3.45^{d}
HFD+ *J. montana* extract 150 mg/kg bw	3.22 ±0.06^{c}	25.16± 2.74^{d}
HFD + *J. montana* extract 300 mg/kg bw	4.04 ±1.64^{g}	31.63 ± 4.06^{f}
HFD+ Metformin 500 mg/kg bw	2.38 ± 0.22^{a}	23.47 ± 3.55^{c}
F-probability	$P < 0.001$	$P < 0.001$
LSD at 5% level	3.65	13.54
LSD at 1% level	3.94	17.63

Values represent the mean ± SE (n=6). High-fat diet (HFD) control rats were compared with regular diet (RD) control rats. Experimental groups were compared with the high-fat diet control rats.

results showed that upon administration *J. montana* ethanolic extract for 8 weeks significantly decreased the plasma TG, TC, LDL-C, vLDL-C, and free fatty acid (Table 5). Most of the reduction in plasma cholesterol occurred in the fraction of LDL. Because of apo B containing lipoprotein fractions are through to be responsible for cholesterol deposition in atherosclerotic plaques,[55] a reduction in LDL would be advantageous clinically extract had an improving effect on the hypercholesterolemia induced by a high fat diet. *J. montana* treated animals also showed a decrease in the atherogenic index with hypercholesterolemic groups (Table 5), which is generally believed to be beneficial since the HDL level inversely correlated heart disease and reduction in this ratio is considered as an anti-atherosclerotic factor. Not surprisingly, plants such as *Jasonia montana* contain high amount of polyphenols[28], mono- and sesquiterpenes[21-24], Essential oils and flavonoids[25]; 3,6,7,3`,4`-pentamethoxy quercetin (artemitin), 3,6,7,3`-tetramethoxy quercetin (chrysosplenetin), 3,6,3`,4`-tetramethoxy quercetin, 3,6,7-trimethoxy kaempferol, 3,6,3`-trimethoxy quercetin (jaceidin), 3,6,4`-trimethoxy quercetin (centaureidin), 3,3`,4`-trimethoxy quercetin, 3,6-dimethoxy quercetin, 3,3`-dimethoxy quercetin, 7,4`-dimethoxy quercetin, quercetin, quercetin-3-O-â-D-4C1-glucopyranoside, 3,5-dicaffeoyl-quinic acid, caffeic acid, quercetin-3-O-L-1C4- rhamnopyranoside (Quercitrin) and quercetin-3-O-â- D-4C1 glucuronopyranoside[56] which are excellent hypocholesterolemic effect. The ability of quercetin to reduce plasma lipids in diabetic animals could be explained by the insulin releasing capacity of quercetin in isolated rat islets of Langerhans.[57] The reduction of plasma lipids (Table 5) and liver cholesterol and triglycerides (Table 6) might have a role in the mechanism of action to augment the hypolipidemic activity of the *J. montana* ethanolic extract containing flavonoids, especially quercetin derivatives. This assumption is supported by three mechanisms; the first is indicated that *J. montana* extract successfully decreased the intestinal cholesterol absorption *in situ*.[58] The second mechanism indicated that the hypolipidemic activity of *J. montana* flavonoids treatments may also be mediated *via* inactivation of hepatic HMG-CoA reductase, a key enzyme, in cholesterol synthesis. In concurrence with this attribution, Raz *et al*.[59] state that inhibitors of hepatic HMG-CoA reductase are well established drugs for the treatment of hypercholesterolemia and decrease the incidence of dyslipidemia in diabetic subjects. This also coincides well with the work of Jung *et al*.[60], who state that flavonoids decrease liver HMG-CoA reductase activity in type 2 diabetic mice. Moreover, quercetin has been reported to lower hepatic and blood cholesterol levels, as stated by Park *et al*.[61]. Taken together, it can be concluded that the ameliorative effect of *J. montana* extract on plasma lipids variables may be attributed to their insulin releasing capacity and insulin binding affinity and decreasing intestinal cholesterol absorption and activity of hepatic HMG-CoA reductase. In the third mechanism, amelioration of the glycemic, lipidemic and antioxidant states of diabetic rats in response to treatment with *J. montana* flavonoids may also be attributed to the increased expression of PPARγ. The effect of PPARγ on lipid and glucose control may be explained according to Lee *et al*.[62], Staels and Fruchart[63], Feige *et al*.[64] and Lefebvre *et al*.[65], who state that PPARγ promotes pre-adipocyte differentiation, stimulates the storage of fatty acids (FAs) in adipocytes and enhances insulin sensitivity. The action of PPARγ on insulin sensitivity results from its ability to channel FAs into adipose tissue, thus decreasing plasma FA concentration and alleviating lipotoxicity in skeletal muscle, liver and pancreas. Also, PPARγ activation has been reported to improve insulin resistance by lowering the hepatic triglyceride content[66,67], activating hepatic glucokinase expression[68] and exhibiting an antiatherogenic effect synergistic with an HMG-CoA reductase inhibitory effect in rabbits.[69] In addition, PPARγ can affect insulin sensitivity by regulating adipocyte hormones, cytokines and proteins that are involved in insulin resistance. Indeed, PPARγ down regulates the expression of genes encoding resistin and TNFα, whereas it induces adiponectin expression, which increases FA oxidation by activation of the AMP-activated protein kinase pathway.[70,71] Also , Willson *et al*.[72] demonstrated the activation of PPARγ to improve insulin sensitivity and lower circulating levels of glucose, triglycerides and FFAs without stimulating insulin secretion in rodent models of type 2 diabetes. Jung *et al*.[60], who found an increased expression of PPARγ by the flavonoids and that of Anandharajan *et al*.[70], who showed an increased PPARγ expression by *Pterocarpus marsupium* isoflavone on L6 myoblasts and myotubes. Moreover, Hussein and Abdel-Gawad[30] reported that the *J. montana* ethanolic extract decrease serum TNFα level in rats with liver cholestasis induced by ethinylestradiol. The presence of these compounds in *J. montana* ethanolic extract play a role in the observed hypocholesterolemic effects (Tables 5 and 6). These compounds may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic β-cell, modulation of glucose release from liver, activation of

insulin receptors and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output. My study suggests that *J. montana* ethanolic extract containing polyphenols may act on liver to increase energy expenditure related fatty liver degradation. Furthermore, *J. montana* polyphenols may decrease mRNA expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)], the rate-limiting enzymes of fatty acid synthesis in the liver, and mRNA expression of [sterol regulatory element-binding protein (SREBP)-1c[73], which controls the expression of these enzymes.[74] These findings indicate that the anti-obesity actions of *J. montana* ethanolic extract are may due to increased expression of energy expenditure-related genes in liver and decreased fatty acid synthesis and fat intake in the liver. On the other hand, Insulin resistance can be generated by decreased adiponectin secretion and decreased TNF-α secretion.[75,76,30] Tables 7 and 8 illustrate the activities of liver TBARS, GSH, GPx, GR, SOD and CAT. TBARS levels were increased in liver in group II rats are a clear manifestation of excessive formation of free radical and activation of lipid peroxidation. The significantly reduced the levels of TBARS, in rats administered with *J. montana* ethanolic extract along with HFD. Glutathione, an endogenous antioxidant defense, is found in liver at high concentration. It plays a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens.[77] Table 7 and 8 demonstrates the levels of oxidative stress biomarkers; GSH, GPx, GR, SOD and CAT in HFD rats. The significant fall in the levels of tissues oxidative stress biomarkers were observed in high fat diet rats (Group II) as compared to the control rats (Group I). Administration of ethanolic extract of *J. montana* along with HFD rats substantially enhanced the levels of these enzymes when compared with HFD rats (Group II). Not surprisingly, plants such as *Jasonia montana* contain high amount of polyphenols[28], mono- and sesquiterpenes[21-24], Essential oils, flavonoids[25] and other tri- tetra and tetraquercetin derivatives[56] which are excellent antioxidant effect. My results also coincides with Hussein[29], Hussein and Abdel-Gawad[30] and Hussein and Farghaly[31] who proved that the antioxidant activity of *J. montana* ethanolic extract using different mechanisms. Various pharmacological actions of *J. montana* have been reported. The *J. montana* used in the present study acted on fatty liver and was shown to possess anti-obesity and anti-diabetic actions. While many studies have described plant extracts exhibiting antioxidant and anti-diabetic actions, to the best of our knowledge, none have demonstrated anti-obesity and anti-diabetic actions *via* reduction of insulin resistance and atherogenic index. The preliminary phytochemical screening of *J. montana* revealed the presence of flavonoids. Flavonoids (or bioflavonoids) are natural products that are capable of modulating of insulin resistance and atherogenic index. Anti-obesity, antiatherogenic, anti-diabetic and antioxidant activities of *J. montana* ethanolic formulation in Obese diabetic rats fed high-fat diet has not been reported earlier to my knowledge, and this study is might be the first observation of that kind.

In conclusion, the present study showed that the effects of Anti-obesity, antiatherogenic, anti-diabetic and antioxidative activities of *J. montana* depend on the presence of a high amount of polyphenols, mono- and sesquiterpenes, Essential oils, flavonoids and other di, tri and tetraquercetin derivatives in *J. montana* ethanolic extract.

REFERENCES

1. Eckel RH, York DA, Rossner S, Hubbard V, Caterson I, Jeor ST. Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: executive summary. *Circulation.* 2004**;** 110:2968-75.
2. Barr ELM, Zimmet PZ, Timothy A, Welborn TA, Jolley D, Magliano DJ. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation*. 2007;116:151-7.
3. Chu NF, Pan WH. Prevalence of obesity and its comorbidities among schoolchildren in Taiwan. *Asia Pacific Journal of Clinical Nutrition*. 2007;16:601-7.
4. DCCT. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complication Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA*, 2002;287:2563-9.
5. Fisman EZ, Motro M, Tenenbaum A. Noninsulin antidiabetic therapy in cardiac patients: current problems and furure prospects. *Advance in Cardiology*. 2008;45:154-70.
6. Shapira NA, Goldsmith TD, McElroy SL. Treatment of binge-eating disorder with topiramate: a clinical case series. *J Clin Psychiatr.* 2000;61:368-72.
7. Anderson JW, Greenway FL, Fujioka K. Bupropion SR enhances weight loss: a 48-week double-blind, placebo- controlled trial. *Obes Res.* 2002;10:633-41.
8. Bailey CJ. Biguanides and NIDDM. *Diabetes Care*. 1992;15:755-72.
9. Kara M, Elizabeth S, Allen L, Julie Y, Jonathan F, Frank D, Stephen A. Metformin as Treatment for Overweight and Obese Adults: A Systematic Review. *Annals of Family Medicine.* 2005;3:457-61.
10. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disor.* 2002;26: 1459-64.
11. Ohta Y, Sami M, Kanda T, Saito K, Osada K, Kata H. Gene expression analysis of the anti-obesity effect by apple polyphenols in rats fed a high fat diet or a normal diet. *J Oleo Sci* 2006;55: 305-14.

12. Ruzaidi A, Amin I, Nawalyah AG, Hamid M, Faizul HA. The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. *J Ethnopharmacol*. 2005;98:55-60.
13. Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antidiabetes and anti-obesity activity of Lagestroemia speciosa. *Evid Based Complement Alternat Med*. 2007;4:401-7.
14. Peng G, Dixon DA, Muga SJ, Smith TJ, Wargovich MJ. Green tea polyphenols -epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. *Mol Carcinog*. 2006;45:309-19.
15. Nagao T, Komine Y, Soga S. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr*. 2005;81:122-9.
16. Tomonori N, Tadashi H, Ichiro T. A Green Tea Extract High in Catechins Reduces Body Fat and Cardiovascular Risks in Humans. *Obesity.* 2007;6:1473-83.
17. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Hakulinen T, Aromaa A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr*. 2002;76:560-8.
18. Merxmuller H, Liens P, Roesseler H. In: J.B. Heywood, J.B. Harborne and B.L. Turner (Eds.), The Biology and Chemistry of Compositae, 1977; p. 590. Academic Press, New York.
19. Tackholm V. Students Flora of Egypt, 1974; p. 562. Cairo University, Beirut.
20. Tackholm V. Alfred Kaiser's Sinai-Herbarium, 1969; p. 562. Cairo University Herbium, Cairo.
21. Ahmed AA, Jakupovic. Sesqui and monoterpenes from *Jasonia montana*. *J. Phytochemistry*. 1990;29:3656-61.
22. Eid F, El-Dahmy S, Gupta PK. Constituents from *Jasonia montana* and *Allagopuppus dicotoma Pharmazie*. 1987;42:423-24.
23. Ahmed,, AA, Jakupovic, Eid JF, Ali AA. 11-Hydrojasionone; A new sesquiterpene type from *Jasonia montana*, *Phytochemistry*. 1988; 27:3875-77.
24. Ahmed AA. Two geraniol derivatives from *Jasonia montana*, *Pharmazie*. 1991;46:362-63.
25. Ahmed AA, Ali AA, Mabry TJ. Flavonoid aglycones from *Jasonia montana*, *Phytochemistry*. 1989;28:665-7.
26. Hammerschmidt FJ, Clark AM, Soliman FM, El- Kashoury ESA, Abd El-Kawy MM, El-Fishawy AM. Chemical composition and antimicrobial activity of essential oils of *Jasonia candicans* and *Jasonia montana*, *Planta Medica*.1993;59:68-70.
27. Tawfeq A, Adnan J, AL-Rehailyb, Joanna R, Polsc, John R, Porter, Jaber S, Mossab, Bahar Ahmed. Three new diterpenes and the biological activity of different extracts of *Jasonia Montana*. *Natural Product Research.* 2005;19:253-65.
28. Hussein MA. Antidiabetic and antioxidant activity of *Jasonia Montana* extract in streptozotocin-induced diabetic rats. *Saudi Pharmaceutical Journal.* 2008;16:214-21.
29. Zhao B, Li X, He R, Cheng S, Xin W. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys*. 1989;14:175-85.
30. Hussein MA, Abdel-Gawad SM. Protective effect of *Jasonia montana* against ethinylestradiol induced cholestasis in rats. *Saudi Pharmaceutical Journal.* 2010;18:35-45.
31. Hussein MA, Farghaly HS. Protective Effects of *Jasonia montana* Against Lipid Peroxidation in Liver and Kidney of Iron-overloaded Rats. *Australian Journal of Basic and Applied Sciences*. 2010;4:2004-12.
32. Hammerschmidt FJ, Clark AM, Soliman FM, El-Kashoury ESA, Abd El-Kawy, MM, El-Fishawy AM. Chemical composition and antimicrobial activity of essential oils of Jasonia candicans and Jasonia montana. *Planta Med*. 1993;59:68-70.
33. Lee A, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulindependent diabetes. *Obes Res.* 1998;6:47-53.
34. Assinewe VA, Baum BR, Gagnon D, Arnason JT. Phytochemistry of Wild Populations of Panax quinquefolius L. (North American Ginseng). *J. Agric. Food. Chem.* 2003;51:4549-53.
35. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologi.*1985;28:412-19.
36. Friedewald WT. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499-502.
37. Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Clin Biochem*. 2001;34:583-8.
38. Han LK, Kimura Y, Okuda H. Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. *Int J Obes Relat Metab Disord.* 1999;23:174-9.
39. Park SH, Ko SK and Chung SH. Euonymus alatus prevents the hyperglycemia and hyperlipidemia induced by high-fat diet in ICR mice. *J Ethnopharmacol.* 2005;102:326-35.
40. Folch J, Lees M, Sloane GH. A simple method for the isolation and purification of total lipids from animals tissues. *J Biol Chem*. 1957**;** 226:497-504.
41. Nichans WH, Samulelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur *J Biochem.* 1968;6:126-30.
42. Ellman GL. Tissue sulfhydroyl groups. Arch.Biochem.Biophy, 1959;82:70-6.
43. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hatman DG, W.G. Hoekstra WG selenium; Biochemical roles as a component of glutathione peroxidise. *Science,* 1973**,** 179:588-92.
44. Horn D. In: Bergmayer, H. (Ed.), Methods in Enzymatic Analysis. Academic Press, London, 1965;pp. 75–879.
45. Kakkar P, Das B, Visvanathan PN. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys*, 1984;21:130-2.
46. Sinha AK. Colorimetric assay of catalase. *Anal.Biochem,* 1972;47:389-392.
47. Roa M, Blane K, Zonneberg M. One way analysis of variance. Version IA (C). PC-STAT, Program coded by University of Georgia, USA, 1985.
48. Bray GA, Lovejoy JC, Smith SR, DeLany JP, Lefevre M, Hwang D, Ryan. The influence of different fats and fatty acids on obesity, insulin resistance and inflammation. *J Nutr*. 2002;132:2488-91.
49. Estadella D, Oyama LM, Damaso AR, Ribeiro EB, Oller do Nascimento CM. Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. J. *Nutrition*. 2004;20:218-24.
50. Weigle DS. Pharmacological therapy of obesity: past, present, and future. *J Clin Endocrin & Metab.* 2003;88(6):2462-69.
51. Hayashi K, Ito M. Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KKAy mice. *Biol Pharm Bull*. 2002;25:188-92.
52. Tsuchida A, Yamauchi T, Takekawa S, Hada Y, Ito Y, Maki T. Peroxisome proliferator-activated receptor (PPAR)α activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPAR α, PPARγ, and their combination. *Diabetes.* 2005;54:3358-70.

53. Hildebrandt AL, Kelly-Sullivan DM, Black SC. Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet induced obese mice. *Eur J Pharmacol.* 2003;462:125-32.
54. Sasaki Y, Suzuki W, Shimada T, Iizuka S, Nakamura S, Nagata M. Dose dependent development of diabetes mellitus and nonalcoholic steatohepatitis in monosodium glutamate-induced obese mice. *Life Sci*. 2009;85:490-8.
55. Schaefer E, Asztalos B. Cholesteryl ester transfer protein inhibition, high-density lipoprotein metabolism and heart disease risk reduction. *Curr. Opin. Lipidol.* 2006;17:394-8.
56. Fathy MS, Mohamed YM, Hossam MA, Samir MO.Cytotoxic activity of flavonoids of *Jasonia montana* Vahl. (Botsch). (Astraceae) growing in Egypt. *Australian Journal of Basic and Applied Sciences.* 2009;3:148-52.
57. Ovaskainen ML, Torronen R, Koponen JM, Sinkko H, Hellstrom J, Reinivuo H, Mattila P. Dietary intake and major food sources of polyphenols in Finnish adults. *J. Nutr.* 2008;138:562-6.
58. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: Chemistry, bioavailability and effects on health. *Nat. Prod. Rep*. 2009;26: 1001-43.
59. Raz I, Eldor R, Cernea S, Shafrir E. Diabetes, insulin resistance, derangements in lipid metabolism. Cure through intervention in fat transport and storage. *Diabetes Metab Res Rev*. 2005;21:3-14.
60. Jung UJ, Lee MK, Park YB, Kang MA, Choia MS. Effect of citrus favonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *Int J Biochem Cell Biol.* 2006; 38:1134-45.
61. Park SY, Bok SH, Jeon SM, Park YB, Lee SJ, Jeong TS, Choi MS. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. *Nutr Res*. 2002;22:283-95.
62. Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. *Endocrinology.* 2003;144:2201-07.
63. Staels B, Fruchart JC. Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes*. 2005;54:2460-70.
64. Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. *Prog Lipid Res*. 2006;45:120-59.
65. Lefebvre P, Chinetti G, Fruchart JC, Staels B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J Clin Invest.* 2006;116:571-80.
66. Fulgencio JP, Kohl C, Girard J, Pegorier JP. Troglitazone inhibits fatty acid oxidation and esterification, and gluconeogenesis in isolated hepatocytes from starved rats. *Diabetes*. 1995;45:1556-62.
67. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K. The mechanisms by which both heterozygous peroxisome proliferatoractivated receptor gamma (PPARγ) deficiency and PPARγ agonist improve insulin resistance. *J Biol Chem*. 2001; 276:41245-54.
68. Kim SY, Kim HI, Park SK, Im SS, Li T, Cheon HG. Liver glucokinase can be activated by peroxisome proliferator-activated receptor-γ. *Diabetes*. 2004;53:S66-S70.
69. Shiomi M, Ito T, Tsukada T, Tsujita Y, Horikoshi H. Combination treatment with troglitazone, an insulin action enhancer, and pravastatin, an inhibitor of HMG-CoA reductase, shows a synergistic effect on atherosclerosis in WHHL rabbits. *Atherosclerosis* 1999;142:345-53.
70. Hevener AL, He W, Barak Y, Le J, Bandyopadhyay G, Olson P, Wilkes J, Evans RM, Olefsky J. Muscle-specific PPARγ deletion causes insulin resistance. *Nat Med.* 2003;9:1491-97.
71. Anandharajan R, Pathmanathan K, Shankernarayanan NP, Ram AV, Arun B. Upregulation of GLUT-4 and PPARγ by an isoflavone from *Pterocarpus marsupium* on L6 myotubes: a possible mechanism of action. *J Ethnopharmacol.* 2005;97:253-60.
72. Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. *J Med Chem*. 2000; 43:527-50.
73. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M.Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci.* 2001;98:7522-27.
74. Guillou H, Martin PG, Pineau T. Transcriptional regulation of hepatic fatty acid metabolism. *Subcell Bioche.* 2008;49:3-47.
75. Borst SE. The role of TNF-alpha in insulin resistance. *Endocrin.* 2004;23:177-82.
76. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest.* 2006;116: 1784-92.
77. Abou Ghalia AH, Fouad IM. Glutathione and its metabolizing enzymes in patients with different beningn and malignant diseases. *Clin. Biochem.*2000;33:657-62.