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Preventive Effect of *Bridelia ferruginea* Against High-fructose Diet Induced Glucose Intolerance, Oxidative Stress and Hyperlipidemia in Male Wistar Rats

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ABSTRACT

The root of *B. ferruginea* is traditionally used as a treatment for type 2 diabetes. The present study was investigated to evaluate the preventive effect of *B. ferruginea* on some markers of metabolic syndrome in type 2 diabetes (hyperlipidemia, glucose intolerance, obesity and oxidative stress) induced by high fructose and fat diet in male Wistar rat. The rats received fructose diet (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received distilled water for high fructose diet group, metformine 100 mg kg⁻¹ per day or extract 125 and 250 mg kg⁻¹ per day for treatment group. The control group received only distilled water during the experiment. After 6 weeks of experiment, fasting blood glucose, liver MDA level, body weight gain, intra abdominal grease, serum triglyceride (TG) and total cholesterol levels in treated groups were significant lower than that of high- fructose diet group. However, rats in treated group were not found to have a significant change of blood HDL Cholesterol level. In the oral glucose tolerance test, rats in treated group had a significantly reduced blood glucose concentration during 180 min after glucose load, indicating that *B. ferruginea* root improved glucose tolerance. In conclusion, our plant can prevent metabolic syndrome induced by high fructose diet.

Key words: *Bridelia ferruginea*, fructose, diabetes, metabolic syndrome

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative lack of/for resistance to insulin and is characterized by hyperglycemia in the postprandial and/or fasting state (Leila *et al.*, 2007). At the present time it is estimated that 150 million people worldwide have diabetes and that the number will increase to 220 million by 2010 and 300 million by 2025. Globally, type-2 diabetes (non insulin dependent diabetes mellitus) accounts for more than ninety percent of all cases of diabetes is caused by a combination of resistance to insulin action and impaired insulin secretion, resulting glucose intolerance (Li *et al.*, 2005).

The type 2 diabetes is often associated with metabolic syndrome, witch is characterized by intolerance of glucose, dyslipidemia, obesity and coronary heart disease, resulting in reduced quality of life and increased risk of mortality and morbidity (Reddy *et al.*, 2008). The

prevalence of metabolic syndrome has dramatically increased worldwide due to a modern lifestyle (Hydrie *et al.*, 2010) and an increase of consumption of high-sugar diets especially fructose (Misra and Khurana, 2008).

Many oral antidiabetic agents, such as biguanides are available for the treatment of type 2 diabetes (Li and Yin, 2005), but these synthetic agents are associated with drawbacks such as rigid and multiple dosing regimen, high-cost, inaccessibility and untoward effects (De Melo *et al.*, 2002). These factors have contributed to the recent increase in the use of folkloric plant products (Inanc *et al.*, 2007).

This way, *Bridelia ferruginea* (Euphorbiaceae) is used for the treatment of diabetes in Togolese traditional medicine according to an ethnobotanical survey.

It is known that, excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus, non-alcoholic fatty liver disease and cardiovascular diseases (Jurgens *et al.*, 2005).

The aim of this study is to evaluate the preventive effect of the hydroalcoholic extract of *B. ferruginea* root on metabolic syndrome like anti-hyperglycemic, anti-hyperlipidemic and antioxidant properties on rats fed with high-fructose diet. These rats form a model of diet-induced insulin resistance, associated with glucose intolerance, hepatic oxidative stress, hyperlipidemia and high risk of coronary heart diseases.

MATERIALS AND METHODS

Chemicals: Thiobarbituric acid, fructose, cholesterol and glucose were purchased from sigma-aldrich.

Plant material: The roots of *Bridelia ferruginea* were collected in the month of August 2008 from the Bagbé district in the south of Togo. Botanical authentication was confirmed at the Department of Botany, Lomé University, Lomé, Togo.

Preparation of crude extract: The root bark of *B. ferruginea* were sliced, shade dried, coarsely powdered and cold macerated with ethanol (80%, v/v) to obtain ethanolic extract. The solvent extract was evaporated *in vacuo* (45°C). The yield recorded was 31.5% (w/w). The extract was dissolved in distilled water for oral administration to experimental animals.

Preparation of high Fructose diet (HF): For 100 mL of liquid diet : We dissolve 6 g of fructose with 125 mL of tween 80 in distilled water to have 50 mL of a solution noted A; We dissolve 0.4 g of cholesterol in lard to have 50 mL of oily solution noted B. The two solutions A and B were mixed to have an emulsion which is administered at a dose of 10 mL kg⁻¹.

Animals: Male Wistar rats weighing between 160 and 200 g were used in the study. The animals were housed with a 12 h light/dark cycle at the Animal House of University of Lomé, Togo. The animals had free access to standard diet and water.

Experimental design: A protocol of 42 days was used; animals were randomly assigned into five groups of 6 each as given below:

- **Group I as control (C):** The rats received distilled water (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received distilled water again (5 mL kg⁻¹ per day)
- **Group II (HF):** The rats received fructose diet (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received distilled water (5 mL kg⁻¹ per day)
- **Group III (D1):** The rats received fructose diet (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received extract 250 mg kg⁻¹ per day in a volume of 5 mL kg⁻¹ per day
- **Group IV (D2):** The rats received fructose diet (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received extract 125 mg kg⁻¹ per day in a volume of 5 mL kg⁻¹ per day
- **Group V (Met):** The rats received fructose diet (10 mL kg⁻¹ per day) during 42 days; at the 15th day to the 42nd day 15 min before, they received metformine 100 mg kg⁻¹ per day in a volume of 5 mL kg⁻¹ per day

The substances were administered by gavages, during the experimentation animals body weight was measured at the first day, 7 th, 14 th, 21st, 28th, 35th and 42nd and blood glucose level the first day, 14 th, 28th and 42nd.

Oral Glucose Tolerance Test (OGTT): At the end of experimental period (42 days), the 12 h fasted animals were subjected to oral glucose tolerance test. For this, a 80% glucose solution was introduced directly into the stomach by gavages at a dose of 4 g kg⁻¹ b.wt. to conscious rats. Blood glucose level was determined at 0 (before glucose administration), 30, 60, 120 and 180 min after glucose administration; using One Touch Ultra 2 glucometer.

Sample collection: Blood was collected from rats with capillary tube from retino-orbital plexus of the animals in dry tube. The samples were centrifuged at 3000 g for 15 min and the serum obtained was aliquoted and frozen for serum total Cholesterol (Ch), HDL-Cholesterol (HDL-Ch), triglycerides (TG), Aspartate aminotransferase (ASAT), Alanine Aminotransferase (ALAT), Creatin-Kinase (CK), Alkaline phosphatase (ALP) determined using Human Diagnostic Kit.

After the experimental period the animals were killed by cervical decapitation. The body was cut open and liver, heart and intra abdominal grease were dissected and were weighed. The liver was frozen during 48 h maximum for evaluation of lipid peroxidation.

Malondialdehyde (MDA) assay: Liver tissues were homogenized with cold 1.5% KCl to make a 10% homogenate. Three milliliters of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid (TBA) aqueous solution were added to 0.5 mL of 10% homogenate. The mixture was heated for 45 min and after cooling, 4 mL of *n*-butanol was added and mixed. Absorbance of butanol phase was measured at 535 and 520 nm. The difference of the two measurements was used as the MDA value (nmol g⁻¹ tissue) (Sepici-Dincel *et al.*, 2007).

Statistical analysis: The results are expressed as Means±SEM. The effect on each parameter was examined using one-way analysis of variance. Individual differences among groups were analyzed employing Tukey's test $p < 0.05$ was considered significant.

RESULTS

Effect of the extract on the body weight and the intra abdominal grease in control, high fructose fed and treatment groups: As shown in Fig. 1, the rats fed a high-fructose diet showed a slight increase in body weight, as compared to the control (C) group at day 42 ($p < 0.001$). It was found that the variation of the body weight was significantly reduced by the extract (D1, D2) and the reference drug (Met) as compared to the rats fed high-fructose alone (HF) $p < 0.01$ and $p < 0.001$, respectively. After 6 weeks of the experiment, the rat fed with a high fructose diet showed also a significant increase of intra abdominal grease, as compared to the control ($p < 0.001$). Only the extract at the dose of 250 mg/kg/day and metformine at the dose of 100 mg/kg/day reduced significantly the intra abdominal grease compared to the high-fructose diet group, $p < 0.001$ (Fig. 2).

Effect of the extract on the evolution of 12 h fed rats blood glycemia during the experiment: There was no significant variation in the blood glucose concentrations of control group (C) throughout the experimental period. Group HF, showed a gradual and significant increase in blood glucose levels from 14 days onwards till the end of experimental period. The blood glucose concentrations of the treated groups showed no significant variation throughout the experimental period except at 14 days $p < 0.001$ compared to group HF (Fig. 3).

Effect of the extract on the glucose tolerance in control, high fructose fed and treatment groups: The analysis of the glucose tolerance test and the comparison between areas under the curve (AUC) of glycemia during 180 min from control and experimental groups show that fructose fed rats developed glucose intolerance (Fig. 4a, b). The AUC of glucose during OGTT of group HF was elevated by 32% ($p < 0.001$) when compared with Group Control. The AUC of glucose in treated groups (D1, D2 and Met) was significantly lower than group HF showing improved glucose tolerance.

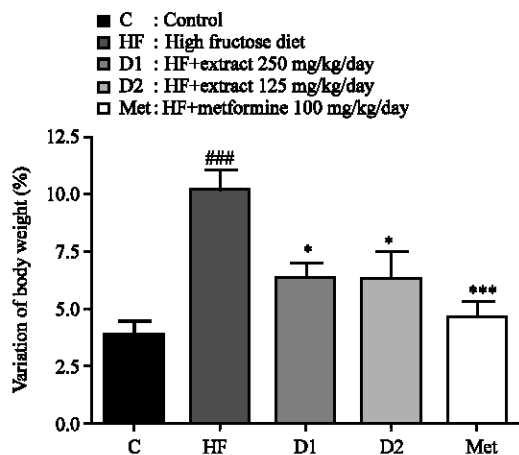


Fig. 1: Effect of the extract on the variation of body weight. The high fructose diet was administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd. The body weight of animals were measured at the first day, 7th, 14th 21st 28th 35 and 42nd to evaluate animals weight gain during the experimentation. The data were expressed as Mean \pm SEM ($n = 6$) and evaluated by ANOVA followed by Tukey's test at 5% * $p < 0.05$ (vs. HF) ### $p < 0.001$ (vs. C) *** $p < 0.001$ (vs. HF)

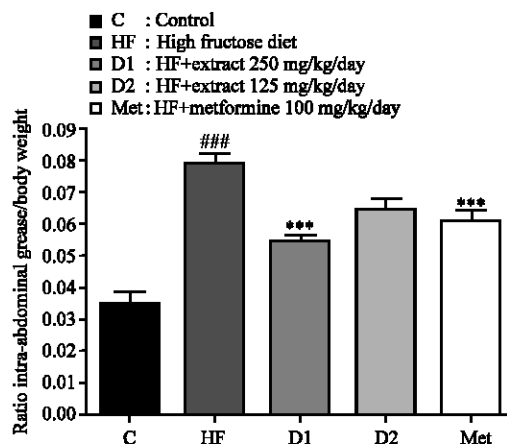


Fig. 2: Effect of the extract on the ratio of intrabdominal grease/body weight. The high fructose diet was administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd. At the end of the experiment, the intra abdominal grease were collected and reported to the body weight for each animal . The data were expressed as Mean±SEM (n = 6) and evaluated by ANOVA followed by Tukey's test at 5%. ###p<0.001 (vs. C) ***p<0.001 (vs. HF)

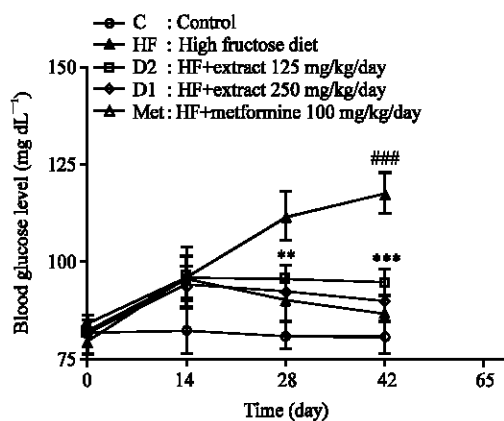


Fig. 3: Effect of the extract on the evolution of 12 h fed rats blood glycemia during the experiment. The high fructose diet was administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd . Blood glucose level were estimated at the first day, 14 th, 28th and 42nd. The data were expressed as Mean±SEM (n = 6) and evaluated by ANOVA followed by Tukey's test at 5% **p<0.01, ***p<0.001 (vs. HF); ###p<0.001 (vs. C)

Effects on hepatic MDA levels in control, high fructose fed and treatment groups: As showing Fig. 5, in high fructose fed group (HF), liver tissue MDA levels were significantly increased compared to control groups (p<0.05). Administration of extract (250 and 125 mg kg⁻¹) in acute study significantly decreased the tissue MDA levels (p<0.05) after 28 days (15th to 42nd) of treatment compared to high fructose fed group (p<0.05). We have the same thing with the administration of metformine at the dose of 100 mg kg⁻¹ during 28 days.

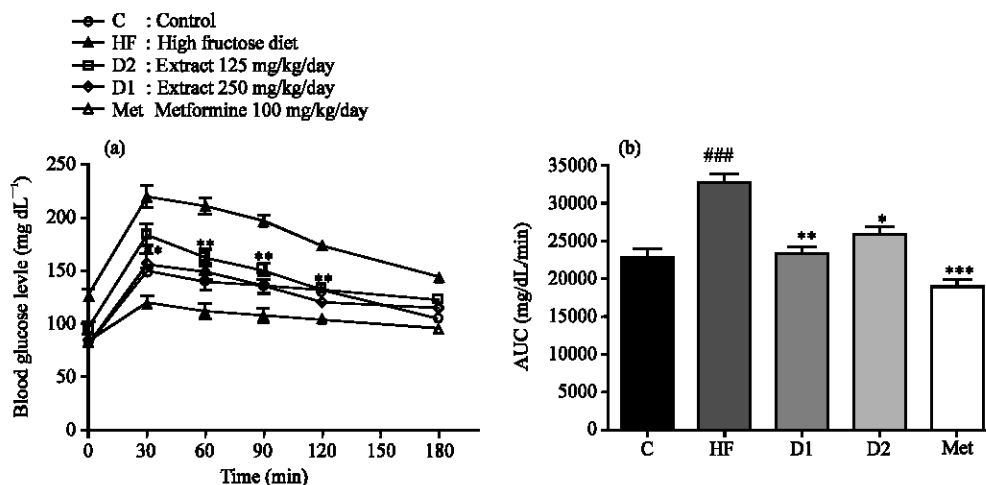


Fig. 4: Effect of the extract on the glucose tolerance test on rats. (a) Blood glucose level and (b) AUC (area under the curve). The high fructose diet was administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd. A 80% glucose solution was introduced directly into the stomach by gavages at a dose of 4 g kg⁻¹ b.wt. to conscious rats. Blood glucose level was determined at 0 (before glucose administration), 30, 60, 120 and 180 min after glucose administration; using One Touch Ultra 2 glucometer. The data were expressed as Mean±SEM (n = 6) and evaluated by ANOVA followed by Tukey's test at 5%. *p<0.05, **p<0.01, ***p<0.001 (vs. HF); #p<0.05; ###p<0.01; ####p<0.001 (vs. C)

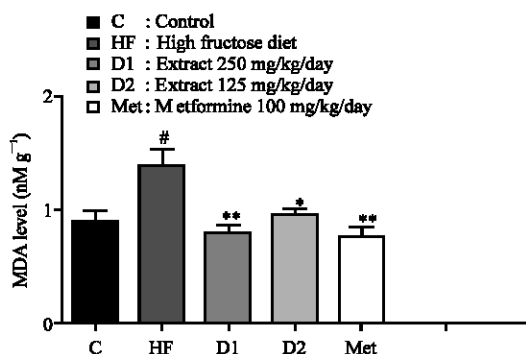


Fig. 5: Effects on hepatic MDA levels in control, high fructose diet and treatment groups. The high fat diet were administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd. At the end of the experiment liver were collected for MDA assay. The data were expressed as Mean±SEM (n = 6) and evaluated by ANOVA followed by Tukey's test at 5%. #p<0.05 (vs. C) *p<0.05 (vs. HF) **p<0.01 (vs. HF)

Effects on serum biochemical parameters in control, high fructose fed and treatment groups: The rats fed a high-fructose diet showed a significant increase of TG (29.83±1.9 to 74.17±4.01) with (p<0.001) and cholesterol (39.67±3.28 to 68.33±3.62) with p<0.01, as compared to the control group at day 42.

Interestingly, the level of APL, Ch and TG were decreased respectively from 332.12±79.6 to 189.7±37.4 U L⁻¹ (p<0.05); 68.33±3.62 to 53.6±3.4 mg dL⁻¹ (p<0.05); 74.17±4 .01

Table 1: Effect on serum biochemical markers on control, high fat diet and treated groups

Biochemical markers	C	HF	D1	D2	Met
PAL (U L ⁻¹)	65.78±14.1	332.12±79.6 [#]	189.70±37.4 [*]	251.18±63.21	104.30±19.7 [*]
ASAT (U L ⁻¹)	124.60±10.63	119.20±8.14	131.30±13.9	128.30±9.9	129.30±11.5
ALAT (U L ⁻¹)	42.00±2.73	44.00±2.57	47.00±5.52	44.00±3.5	51.00±6.62
CK (U L ⁻¹)	433.64±41.83	580.00±54	737.00±150	657.00±91	528.00±83
Ch (mg dL ⁻¹)	39.67±3.28	68.33±3.62 ^{##}	53.60±3.4 [*]	57.95±3.2	37.86±4.62 ^{**}
HDL Ch (mg dL ⁻¹)	26.50±1.67	33.67±1.4	45.67±3.36	43.33±4.6	42.56±3.09
TG (mg dL ⁻¹)	29.83±1.9	74.17±4.01 ^{###}	47.00±6.48 ^{***}	54.67±5.6 ^{**}	31.67±2 ^{***}

C: Control, HF: High fructose diet, D1: High fructose diet +extract 250 mg/kg/day, D2: High fructose diet +extract 125 mg/kg/day, Met: High fructose diet +Metformine 100 mg/kg/day. The high fructose diet was administered for 42 days to rats and drugs daily for 28 days at the 15th to 42nd. Serum biochemical markers were estimated at the end of the experiment. The data were expressed as mean±S.E.M. (n = 6) and evaluated by ANOVA followed by Tukey's test at 5%. *p<0.05; **p<0.01; ***p<0.001 (vs. HF); #p<0.05 ##p<0.01; ###p<0.001 (vs. C)

to 47.01±6.48 mg dL⁻¹ (p<0.001) in the extract (250 mg/kg/day) treated group. Similarly, the levels of PAL, Ch and TG have been decreased significantly by the administration of metformine in the experimental groups. The extract (125-250 mg/kg/day) as well as Metformine (100 mg/kg/day) administered orally at the doses did not alter significantly (p>0.05) the levels of ASAT, ALAT, CK and HDL-Ch (Table 1).

DISCUSSION

Dietary fructose is a monosaccharide which can induce metabolic disorders including glucose intolerance, hypertension and dyslipidemia which is of pathophysiologic importance for the development of diabetes and atherosclerosis (Schaefer *et al.*, 2009). There are many reports in the literature describing an increase in body weight and glycemia with the consumption of high-fructose diets in both humans and animal models (Rizkalla *et al.*, 1993; Tordoff and Alleva, 1990). Present results are consistent with previous studies (Aguilera *et al.*, 2004; Xi *et al.*, 2007; Suwannaphet *et al.*, 2010) which found that consumption of high-fructose diets markedly induces an increase in glycemia associated with dyslipidemia and, consequently, a reduction of insulin sensitivity. A significant increase in the incremental AUC of glucose concentrations after glucose loading during OGTT are seen in rats fed HF diets, indicating that the ability of insulin to stimulate glucose disposal is markedly impaired in peripheral tissues.

There is now much emerging evidence that chronic consumption of high-fructose diets contributes to excessive formation of Reactive Oxygen Species (ROS). This leads to induced oxidative stress and mediated insulin resistance (Houstis *et al.*, 2006).

Moreover, an increase in cellular ROS accumulation directly triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase and nuclear factor-kappa B that, in turn, phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) proteins (Evans *et al.*, 2005). Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerates the degradation of IRS-1, causing impaired glucose uptake in muscle, liver and adipose tissues (Evans *et al.*, 2005).

Fructose-induced hyperglycemia is one of the important factors to increase ROS, lipid peroxidation causing the depletion of the antioxidant defense status in various tissues (Reddy *et al.*, 2009). The findings of this study are in agreement with other investigations that reported a significant increase in lipid peroxidation (Reddy *et al.*, 2009).

In this study, the rats fed a high-fructose diet showed a significant increase of blood TG and cholesterol level, body weight, intra abdominal grease, other research reported the same things (Ghule *et al.*, 2009). It is Known that when the free fatty acid supply exceeds utilization, non-adipose tissues start accumulating TG, which is aggravated by the simultaneous presence of hyperglycemia. Subsequently, the formation of reactive, long-chain fatty acyl-CoAs and toxic metabolites such as ceramide, the activation of protein kinase C, an increase of oxidative stress, may all contribute to apoptosis and the decline of cells (Poitout and Robertson, 2002; Tushuizen *et al.*, 2007). Thus, the regulation of hyperlipidemia would play an important role in the etiology of diabetes and the complication of hyperglycemia. The elevated serum TG and total cholesterol levels were significantly reduced by the oral administration of the extract (250 mg kg⁻¹), whereas it did not affect the HDL-Cholesterol concentration significantly. These results imply that *B. ferruginea* root extract can prevent diabetic pathological conditions induced by hyperlipidemia through lowering TG and total cholesterol under type 2 diabetes.

The present findings show that extract of *B. ferruginea* prevents body-weight gain, intra abdominal grease accumulation and hyperglycemia in high fructose fed rats. *B. ferruginea* can be useful in the treatment of diabetes like others plants which were explored as antidiabetic on rats (Ene *et al.*, 2008; Ekor *et al.*, 2010).

CONCLUSIONS

The current study indicates that the *Bridelia ferruginea* root in rats fed a high-fructose fat diet can prevent the development of hyperglycemia and hyperlipidemia as well as reduce an oxidative stress.

The present study also provides additional evidence in support the use of this plant in the treatment of diabetes mellitus traditionally in Togo.

REFERENCES

- Aguilera, A.A., G.H. Diaz, M.L. Barceleta, O.A. Guerreoro and R.M.O. Ros, 2004. Effect of fish oil on hypertension, plasma lipids and tumornecrosis factor- α in rats sucrose metabolic syndrome. Effect of fish oil on hypertension, plasma lipids and tumornecrosis factor- α in rats sucrose metabolic syndrome. *J. Nutr. Biochem.*, 15: 350-357.
- De Melo, E.J.M.Jr., M.J. Raposo, J.A.L. Neto, M.F.A. Diniz, C.A.C. Marcelino and A.E.G. Santana, 2002. Medicinal plants in the healing of dry socket in rats: Microbiological and microscopical analysis. *Phytomedicine*, 9: 109-116.
- Ekor, M., A.O. Odewabi, A.G. Bakre, K.S. Oritogun, T.E. Ajayi and O.V. Sanwo, 2010. Comparative evaluation of the protective effect of the ethanolic and methanolic leaf extracts of *Sida acuta* against hyperglycaemia and alterations of biochemical and haematological indices in alloxan diabetic rats. *J. Pharmacol. Toxicol.*, 5: 1-12.
- Ene, A.C., E.A. Nwankwo and L.M. Samdi, 2008. Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi* L.) oil on their body weights. *J. Pharmacol. Toxicol.*, 3: 141-146.
- Evans, J.L., B.A. Maddux and I.D. Goldfine, 2005. The molecular basis for oxidative stress induced insulin resistance. *Antioxid. Redox. Signal.*, 7: 1040-1052.
- Ghule, B.V., M.H. Ghante, A.N. Saoji and P.G. Yeole, 2009. Antihyperlipidemic effect of the methanolic extract from *Lagenaria siceraria* stand. fruit in hyperlipidemic rats. *J. Ethnopharmacol.*, 124: 333-337.
- Houstis, N., E.D. Rosen and E.S. Lander, 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nat.*, 440: 944-948.

- Hydrie, M.Z.I., A. Basit, A.S. Shera, R. Hakeem and A. Hussain, 2010. Dietary patterns associated with risk for metabolic syndrome in urban community of Karachi defined by cluster analysis. *Pak. J. Nutr.*, 9: 93-99.
- Inanc, N., C. Betul, S. Habibe, B. Meral and T. Sultan, 2007. Use of herbs by the patients with diabetes in kayseri, Turkey. *Pak. J. Nutr.*, 6: 310-312.
- Jurgens, H., W. Haass, T.R. Castaneda, A. Schurmann and C. Koebnick *et al.*, 2005. Consuming fructose-sweetened beverages increases body adiposity in mice. *Obes. Res.*, 13: 1146-1156.
- Leila, Z., D.S. Eliandra, H.C. Luisa, C.J. Anildo, G.P. Moacir, S. Bruno, R. Fatima and B.S. Mena, 2007. Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. *J. Ethnopharmacol.*, 109: 151-155.
- Li, R. and M. Yin, 2005. *Pharmacology*. People's Medical Publishing House, Beijing, pp: 317-319.
- Li, Y., S. Wen, B.P. Kota, G. Peng and G.Q. Li *et al.*, 2005. Punica granatum flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J. Ethnopharmacol.*, 99: 239-244.
- Misra, A. and L. Khurana, 2008. Obesity and the metabolic syndrome in developing countries. *J. Clin. Endocrinol. Metab.*, 93: 9-30.
- Poitout, V. and R.P. Robertson, 2002. Mini review: Secondary beta cell failure in type 2 diabetes-A convergence of glucotoxicity and lipotoxicity. *Endocrinology*, 143: 339-342.
- Reddy, S.S., R. Karuna, R. Baskar and D. Saralakupari, 2008. Prevention of insulin resistance by ingesting aqueous extract of *Ocimum sanctum* to fructose-fed rats. *Horm. Metab. Res.*, 40: 44-49.
- Reddy, S.S., P. Ramatholisamma, R. Karuna and D. Saralakupari, 2009. Preventive effect of *Tinospora cordifolia* against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. *Food. Chem. Toxicol.*, 47: 2224-2229.
- Rizkalla, S.W., J. Boillot, V. Tricottet, A.M. Fontvieille and J. Luo *et al.*, 1993. Effects of chronic dietary fructose with and without copper supplementation on glycemic control, adiposity, insulin binding to adipocytes and glomerular basement membrane thickness in normal rats. *Br. J. Nutr.*, 70: 199-209.
- Schaefer, E.J., J.A. Gleason and M.L. Dansinger, 2009. Dietary fructose and glucose differentially affect lipid and glucose homeostasis. *J. Nutr.*, 139: 1257S-1262S.
- Sepici-Dincel, A., S. Ereften, C. Cemal, M. Sengelenc and E. Yesiladad, 2007. Effects of *in vivo* antioxidant enzyme activities of myrtle oil in normoglycemic and alloxan diabetic rabbits. *J. Ethnopharmacol.*, 110: 498-503.
- Suwannaphet, W., A. Meeptom, S. Yibchok-Aun and S. Adisakwattana, 2010. Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. *Food Chem. Toxicol.*, 48: 1853-1857.
- Tordoff, M.G. and A.M. Alleva, 1990. Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight. *Am. J. Clin. Nutr.*, 51: 963-969.
- Tushuizen, M.E., M.C. Bunck, P.J. Pouwels, S. Bontemps and J.H. van Waesberghe *et al.*, 2007. Pancreatic fat content and beta cell function in men with and without type 2 diabetes. *Diabetes Care*, 30: 2916-2921.
- Xi, L., Z. Quian, G. Xu, S. Zheng and S. Sun *et al.*, 2007. Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. *J. Nutr. Biochem.*, 18: 64-67.