Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 9, 2015

Original Article

AMELIORATIVE EFFECTS OF *ARTEMISIA JUDAICA* L. EXTRACT AGAINST ALLOXAN-INDUCED BIOCHEMICAL ALTERATIONS IN MALE WISTAR RATS

EMAN G.E. HELAL[a], NOURAN ABOU-AOUF[a], HANY NADY YOUSEF[b], FATIMAH M YOUSEF[c], AL SAYEDA MOHAMMAD KHATTAB[a]

[a]Department of Zoology, Faculty of Science, Al-Azhar University (Girls), Nasr City, Cairo, Egypt, [b]Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt, [c]Department of Food and Nutrition, Faculty of Home Economics, King Abdulaziz, University, Jeddah, Saudi Arabia.
Email: hany_barsoum@edu.asu.edu.eg

Received: 24 May 2015 Revised and Accepted: 06 Jul 2015

ABSTRACT

Objective: The current study aims to evaluate the potential of crude leaf extract of *Artemisia judaica* L. (AJE) in reducing the biochemical abnormalities accompanied to alloxan-induced diabetes in male Wistar rats.

Methods: Thirty male albino rats (100-110 g) were divided equally into three groups including control, diabetic and diabetic+AJE. Diabetes was induced by using a single dose of alloxan (120 mg/kg of body weight). Serum biochemical parameters, including insulin, glucose, triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL), total proteins, albumin, globulin, renal markers (creatinine, urea, uric acid), activities of aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyltransferase (ɤGT) were measured in all groups. Also, values of homeostasis model assessment of insulin resistance (HOMA-IR) and ratios of albumin: globulin (A: G), TC/HDL (risk factor-1), LDL/HDL (risk factor-2) were calculated for each group.

Results: Diabetic rats showed reduction in body weight and marked decline in the values of serum insulin, protein profile indices and HDL accompanied with marked elevation in values of glucose, HOMA_IR, triglycerides, TC, LDL, VLDL, TC/HDL, LDL/HDL, renal markers and activities of the estimated enzymes. Supplementation of diabetic rats with AJE, twice daily for 30 days, significantly ameliorated most of the estimated biochemical parameters.

Conclusion: The current results demonstrate that AJE possesses a hypoglycemic effect and acts as a protective factor against metabolic abnormalities induced by diabetes mellitus.

Keywords: *Artemisia judaica*, Alloxan, Diabetes, Lipid profile, Renal markers, Hyperglycemia.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia resulting from deficiency of insulin production, insulin action, or both [1, 2]. The chronic hyperglycemia of diabetes is associated with disturbances in carbohydrate, protein, and fat metabolisms, in addition to long-term complications affecting the eyes, kidneys, nerves, heart and blood vessels [3-6]. According to WHO, about 143 million people worldwide suffering from diabetes and the number may likely to double by the year 2030 [7]. The use of plant materials for medicinal purposes is an ancient practice which has become even more relevant in modern perspective for general health and for specific diseases [8]. Recently there is a growing interest in herbal remedies due to the side effects associated with the available oral hypoglycemic agents for the treatment of diabetes mellitus [9]. Also the search for improved, safe and natural antidiabetic agents has been recommended by World Health Organization [10].

Artemisia judaica (family Asteraceae), also known as " Shih kharasani" in Arabic, is a perennial fragrant shrub which grows widely in Sinai Peninsula of Egypt. It is widely used in folk medicine and is recommended as a healer plant by Bedouins there [11]. Al-Mustafa and Al-Thunibat [12] reported that *A. judaica* is one of medicinal plants which has potential of antioxidant activity and used as a traditional anti-diabetic agent. The current study was designed to evaluate the efficacy of AJE in reducing the metabolic abnormalities accompanied to alloxan-induced diabetes in male albino rats.

MATERIALS AND METHODS

Plant materials

Samples of mature fresh green leaves of *A. judaica* L. were collected from the Southern Sinai, Egypt. The plant was identified and authenticated by a botanist at the Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt.

Preparation of aqueous extract of *A. judaica* leaves

After being thoroughly rinsed with sterile distilled water, leaves of shih were ground dried in the shade, and then powdered with a blender. Crude hot water extract of the plant leaves was prepared by boiling 2g of the plant powder with 200 ml distilled water for 15 min. The obtained extract was allowed to cool at room temperature then filtered through Whatman No.2 filter Paper. The resultant extract was stored in a glass container in refrigerator. This extract was freshly prepared each two days.

Experimental animals

Thirty adult male albino rats of the Wistar strain weighing 100-110g and of similar age (8-10 weeks) were obtained from the animal house of Theodor Bilharz Research Institute (TBRI), El-Giza, Egypt. They were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given a standard pellet rodent diet, in addition of water ad libitum. The rats were maintained under standard laboratory conditions at 25±2 °C, relative humidity 55±5% and normal photoperiod (12h light/dark cycle). All animal experiments were performed under protocols approved by the local Institutional Animal Ethics Committee of Ain Shams University.

Induction of diabetes

Diabetes mellitus was induced in animals by a single intraperitoneal injection of alloxan (120 mg/kg body weight) dissolved in freshly prepared physiological saline. After three days of alloxan injection, rats were deprived of food overnight and they were then given glucose (3 g/kg body weight) by gastric intubation. After 2h of oral glucose administration, blood samples were taken from tail vein and the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer and compatible blood glucose strips. Animals with fasting blood glucose levels ≥ 300 mg/dl were considered as mild diabetic animals and included in the experiment. The control rats were injected with physiological saline alone as placebo.

Experimental protocol

Experimental animals were divided into three groups (ten for each) as follows:

Group I: (Control group): Untreated non-diabetic rats.

Group II: (Diabetic group): Rats were injected intraperitoneally with a single dose of alloxan (120 mg/kg dissolved in saline solution).

Group III: Diabetic rats treated orally with AJE (28.5 mg/kg twice/day) for 30 days.

Blood collection

At the end of the experiment and under diethyl ether anaethesia, blood samples were taken from the retro-orbital plexus of the overnight fasted animals. Blood samples were centrifuged at 4000 rpm for 10 min at 4 °C. The clear supernatant sera were quickly removed and immediately stored at-80 °C till used for further analysis of biochemical parameters.

Biochemical estimations

Serum samples were analyzed to estimate the levels of glucose according to Tietz [13] and insulin according to Reeves [14]. Insulin resistance was estimated using homeostasis model assessment (HOMA-IR) from fasting serum glucose and insulin using the following equation [15]:

HOMA-IR = fasting serum glucose (mg/dl) × fasting serum insulin (µU/L)/405

Serum creatinine, urea and uric acid were estimated by colorimetric methods according to Tietz [13], while levels of aspartate transaminase (AST), alanine transaminase (ALT) and glutamyltransferase (γGT) in sera were determined colorimetrically following Schumann and Klauke [16]. Serum albumin and total proteins were measured according to the method of Burtis *et al.*[17]. Globulin was calculated by subtracting albumin from total proteins [13]. Serum total lipids, total cholesterol [18]; triglycerides[19] and HDL[20] were estimated colorimetrically using high quality kits according to manufacturer's protocol; while VLDL was calculated as triglyceride/5 and LDL was calculated applying the Friedwald's equation [21].

Friedewald's equation: LDL (mg/dl) = TC-HDL-[TG/5].

Risk factor 1 = TC/HDL

Risk factor 2 = LDL/HDL

Statistical analysis

The results were expressed as mean±SEM of 10 rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Duncan post Hoc test using the SPSS/17.0 software. Values were considered statistically significant at P<0.05.

RESULTS

Fig. 1. Depicts that diabetic rats showed marked decline (P<0.05) in their body weight (-4.55%) when compared to the control group. Supplementation of AJE to the diabetic rats for 30 days returned the body weight towards normalcy.

Table 1 shows that levels of insulin were significantly (P<0.05) decreased (-38.50%) and levels of glucose were significantly (P<0.05) increased (300.50 %) in sera of diabetic rats relative to the corresponding control rats. Oral administration of AJE to the diabetic rats for one month improved significantly (P<0.05) the levels insulin and glucose in sera relative to the control group.

In addition, HOMA_IR values were significantly higher (P<0.05) in diabetic rats (147.19%) when compared to the corresponding controls, while treatment of diabetic rats with AJE returned HOMA_IR values to normalcy.

Marked elevations (P<0.05) in the activities of AST (43.81%), ALT (49.24%) and γ GT (323.68%) were recorded in sera of the diabetic rats when compared to the values of the corresponding controls. On the other hand, treatment of diabetic rats with AJE significantly (P<0.05) abolished the disturbances occurred in the activities of these enzymes.

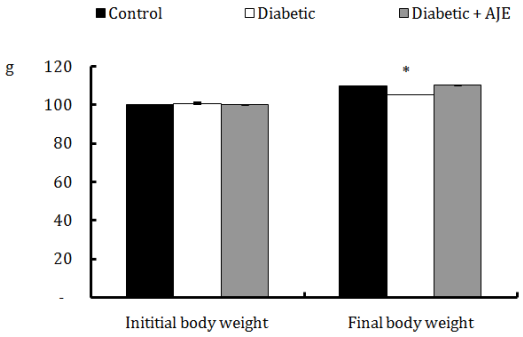

Fig. 1: Body weight changes in the control and experimental male albino rats
Values are expressed as mean±SEM for 10 rats in each group. *P<0.05, AJE: *Artemisia judaica* extract

Parameters of serum protein profile (total proteins, albumin, globulin and A/G ratio) in control and experimental groups are presented in table 3. Diabetic animals showed marked decline (P<0.05) in serum total proteins (-28.38%), albumin (-26.41%) and globulin (-31.01%) relative to the corresponding controls. Treatment of diabetic rats with AJE resulted in significant (P<0.05) modulation of the measured serum protein profile parameters. The values of A/G ratio showed non-significant changes between control and experimental groups.

Table 4 shows the indices of lipid profile in sera of control and experimental groups of rats. Diabetic animals showed marked elevation (P<0.05) in total lipids (201.69%), total cholesterol (64.11%), triglycerides (113.34%), LDL (104.93%), VLDL (113.37%) and ratios of TC/HDL (102.66%) and LDL/HDL (159.42) accompanied with marked decline in HDL (-20.47 %) relative to the corresponding controls. Treatment of diabetic rats with AJE improved the sera lipid profile indices.

Biochemical parameters of renal function elevated markedly (P<0.05) in sera of diabetic rats with percentage of change 82.42%, 95.44% and 248.08% higher than those of control rats for creatinine, urea and uric acid, respectively (table 5). Treatment of diabetic rats with AJE returned these parameters towards normalcy.

Table 1: Levels of serum insulin and glucose and values of HOMA_IR in control and experimental groups

Parameters	Groups		
	Control	Diabetic	Diabetic+AJE
Insulin (µU/l)	4.13±0.45	2.54±0.37*	3.80±0.68*
Glucose (mg/dL)	87.44±0.78	350.20±0.85*	94.04±0.62*
HOMA_IR	0.89±0.23	2.20±0.36*	0.88±0.08

Values are expressed as mean±SEM for 10 rats in each group. * P<0.05, AJE: *Artemisia judaica* extract, HOMA_IR: homeostasis model assessment of insulin resistance.

Table 2: Activities (U/l) of serum AST, ALT and ɤGT in sera of control and experimental groups

Parameters	Groups		
	Control	Diabetic	Diabetic+AJE
AST	37.02±0.59	53.24±1.08*	34.36±0.70*
ALT	22.46±0.56	33.52±0.60*	28.06±0.55*
ɤGT	2.28±0.12	9.66±0.08*	4.50±0.07*

Values are expressed as mean±SEM for 10 rats in each group. * P<0.05, AJE: *Artemisia judaica* extract, AST: aspartate transferase, ALT: alanine transferase, ɤGT: gamma-glutamyltransferase.

Table 3: Levels of serum proteins (g/dl) and A/G ratio in control and experimental groups

Parameters	Groups		
	Control	Diabetic	Diabetic+AJE
Total proteins	7.40±0.08	5.30±0.11*	8.20±0.09*
Albumin	4.24±0.07	3.12±0.06*	4.46±0.05
Globulin	3.16±0.05	2.18±0.07*	3.72±0.09*
A/G ratio	1.34±0.05	1.42±0.04	1.22±0.05*

Values are expressed as mean±SEM for 10 rats in each group. * P<0.05, AJE: *Artemisia judaica* extract.

Table 4: Levels of serum lipid profile indices (mg/dl) and values of TC/HDL and LDL/HDL ratios in control and experimental groups

Parameters	Groups		
	Control	Diabetic	Diabetic+AJE
Total lipids	474.00±8.12	1430.00±11.40*	463.60±5.00
Total cholesterol	141.08±0.37	231.52±0.53*	184.62±0.59*
Triglycerides	133.10±0.77	283.96±0.86*	147.72±0.45*
HDL	47.88±0.49	38.08±0.39*	43.70±0.54*
LDL	66.56±048	136.40±0.59*	111.48±0.61*
VLDL	26.62±0.16	56.80±0.17*	29.52±0.10*
TC/HDL	3.00±0.00	6.08±0.04*	4.22±0.06*
LDL/HDL	1.38±0.02	3.58±0.04*	2.54±0.05*

Values are expressed as mean±SEM for 10 rats in each group. * P<0.05, AJE: *Artemisia judaica* extract. HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, VLDL: very low density lipoprotein cholesterol.

Table 5: levels (mg/dl) of serum creatinine, urea and uric acid in control and experimental groups

parameters	Groups		
	Control	Diabetic	Diabetic+AJE
Creatinine	0.91±0.01	1.66±0.08*	0.92±0.01
Urea	32.46±0.55	63.44±0.59*	37.02±0.62*
Uric acid	2.08±0.06	7.24±0.09*	3.46±0.07*

Values are expressed as mean±SEM for 10 rats in each group. * P<0.05, AJE: *Artemisia judaica* extract.

DISCUSSION

Diabetes mellitus is one of the major diseases affecting many people on the globe. Traditional plant based remedies are still the first choice in the developing countries because of their cost effectiveness, easy availability and minimum or no side effects [22, 23]**.** More than 800 plant species have been identified throughout the world to have antidiabetic properties [24]**.** From these medicinal plants is *Artemisia judaica* which is commonly used in folk medicine in Egypt. The present investigation was carried out to examine the efficacy of AJE in reducing the metabolic abnormalities accompanied to alloxan-induced diabetes in male albino rats.

Induction of diabetes by alloxan resulted in loss of body weight reflecting the catabolic effect of diabetes on protein metabolism by retarding protein synthesis and stimulating protein degradation [25]. In addtion, diabetic rats showed significant decrease (p<0.05) in levels of serum insulin accompanied with marked elevation (p<0.05) in levels of blood glucose when compared to the control rats. This may be due to damages caused by alloxan in beta cells which lead to decrease in endogenous insulin secretion. In the same context, Szkudelski [26] showed that Alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia. Treatment of diabetic rats with AJE returned the body weight, insulin and glucose levels towards normalcy. These results indicated that the AJE possesses significant hypoglycemic and anti-hypoinsulinemia effects that may be attributed to its content of flavonoids, essential oil, saponins, terpenes, and tannins. The hypoglycemic action of AJE may be through increase glucose uptake and glycogen synthesis, inhibiting for α-glucosidase and α-amylase, reduction of insulin resistance, reduction of oxidative stress and protecting against tissue damage, generation of beta cells in pancreas. These results are in agreement with a previous study suggested that *A. judaica* significantly reduced the blood glucose level in diabetic rats [27].

Furthermore, a new hormone called Betatrophin found to be secreted by liver and adipose tissues. This hormone prompts beta cells in the pancreas to multiply and produce more insulin [28]. AJE has a variety of chemical constituents such as essential oils (artemisyl-oil, apiperitone-oil, piperitone and trans-ethyl cinnamate), which have antioxidant, anti-inflammation and anti-hypo insulinemic activity [29]. Also, these essential oils might be stimulating normal beta cells for insulin production enhanced peripheral uptake of glucose where insulin increased significantly and caused regeneration of hepatocytes [30]. These hepatocytes produced more β-trophin enhancing insulin production by β-cells of pancreas and enhancing body weight.

HOMA-IR has considered as a robust tool for the surrogate assessment of insulin resistance [31, 32]. In the current study, HOMA_IR values were significantly higher (P<0.05) in diabetic rats when compared to the corresponding controls. This may be attributed to high glucose concentrations leading to the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity [33]. After AJE administration, the level of HOMA_IR returned to the normal values. These findings indicated that AJE exhibited antihyperglycemic properties, enhanced insulin release and peripheral uptake of glucose in alloxan-induced diabetic rats.

Activities of ALT, AST and γGT reflect the state of hepatocyte injury [34, 35]. In our study, there is an elevation in the activities of these enzymes in diabetic rats when compared with the control group indicating a state of hepatocyte injury. This may be attributed to hyperglycemia which promotes reactive oxygen species (ROS) accumulation, accelerates cellular damage and significantly contributes to the diabetic complications development and progression [36]. Furthermore, an overall significant reduction in serum total protein, albumin and globulin in diabetic animal's consequents with slight non-significant elevation in A/G ratio were

observed in the present study. These observations are in analogy to the results obtained [37] and Chandramohan *et al.* [38]. Hyperglucagonemia during insulin deficiency accelerates protein catabolism [39]. This may explain the reduction in serum total protein, albumin and globulin in diabetic animals as hypoinsulinemia increases the rate of protein degradation and may have a direct adverse effect on the synthesis and secretion of albumin and globulin.

Results of the present work showed that, treatment of alloxan-induced diabetic rats with AJE significantly reduced the activities of ALT, AST and γGT and serum protein profile parameters were returned back to the normal levels. These results indicated that AJE has a hepato protective effect and improving liver function. This may be due to the presence of flavonoids (apigenin, cirsimaritin, flavonoid glycosides) which have a hypoglycemic action in addition to a potent antioxidant action attenuating the oxidative stress induced by free radicals, so they can ameliorate the functions of the liver by protecting the hepatocytes and inhibiting the production proinflammatory mediators like TNF-α, IL-12, IL-2 cytokines which has been associated with inflammatory diseases [40].

Diabetic animals showed marked elevation ($P<0.05$) in the values of total lipids, total cholesterol, triglycerides, LDL,VLDL and ratios of TC/HDL and LDL/HDL accompanied with marked decline in HDL relative to the corresponding controls. Insulin has a potent inhibitory effect on lipolysis in adipocytes. Therefore, hypoinsulinemia is associated with excess lipolysis and increased influx of free fatty acids to the liver [41, 42]. This stimulates over production of triglycerides, LDL and VLDL by the hepatocytes [41]. According to Kinosian *et al.* [43], the changes in TC/HDL and LDL/HDL ratios are better predictors of coronary heart disease than the changes in LDL alone. In the present study, diabetic animals were subjected to the risk of coronary heart disease as evidenced by their high ratios of TC/HDL and LDL/HDL. These observations are consistent to earlier results obtained by many investigators [44-46]. Results of the current investigation showed that, AJE significantly ameliorated all the estimated sera lipid profile indices indicating that AJE has potential role of antioxidant activity and it can be used as anti-diabetic agent to decrease the risk of atherosclerosis. This may be attributed to the flavonoid compounds (apigenin, cirsimaritin) which were found in water extracts of *A. judaica*. These compounds act as antioxidants and reduce the levels of cholesterol and triglycerides significantly through their protective role against free radicals by scavenging activity [30, 47].

Elevated levels of blood urea, creatinine and uric acid are likely evidence of impaired kidney functions. The obtained results from the current study showed high levels of renal markers in sera of diabetic rats relative to the corresponding control ones. Renal dysfunction indicated by elevation of renal markers in diabetic rats has been proved by many investigators [38, 44, 48]. Diabetes mellitus is characterized by hyper glycaemia that is strongly linked to nephropathy mediated via oxidative stress. This may be due to high activities of xanthine oxidase, lipid peroxidation as well as impairment of the urea cycle enzyme activities [49]. Treatment of diabetic rats with AJE reversed the renal markers towards the normal values indicating that *A. judaica* can ameliorate renal function abnormalities and provides protection against the oxidative renal damage through the antioxidant capacity of its constituents. These findings are in consistence with the results obtained [46] on treatment of diabetic rats with aqueous extract of *Olea europaea* plant. Also Chandramohan *et al.* [38]. Obtained similar results after treatment of diabetic rats with 3-hydroxymethyl xylitol for 45 days.

In conclusion, The present results suggest that AJE has a hypoglycemic acts as a beneficial agent against metabolic abnormalities induced by diabetes.

CONFLICT OF INTERESTS

Declared None

REFERNCES

1. Balkau B, Charles MA, Eschwege E. Epidemiological discourse on new criteria on diabetes. Mol Endocrinol 2000;2:229-34.
2. Rasineni K, Bellamkonda R, Singareddy SR, Desireddy S. Antihyperglycemic activity of Catharanthus roseus leaf powder in alloxan-induced diabetic rats. Pharmacogn Res 2010;2:195-201.
3. Hung TH, Peng G, Kota BP. Anti diabetic action of punica granatum flower extract: activation of PPAR-gamma and identification of an active component. Toxicol Appl Pharmacol 2005;207:160-9.
4. Thripathi BK, Sivastava AK. Diabetes mellitus: complication and therapeutic. Med Sci Monit 2006;12:RA130-147.
5. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;30:S42-S47.
6. Gupta R, Bajpai KG, Johri S, Saxena AM. An overview of indian novel traditional medicinal plants with antidiabetic potentials. Afr J Tradit Complementary Altern Med 2008;5:1-17.
7. Ganesh T, Saikat S, Thilagam E., Thamotharan G, Loganathan T, Raja C Pharmacognostic and anti-hyperglycemic evaluation of Lantana camara (L.) Var. Aculeate leaves in alloxan-induced hyperglycemic rats. Int J Res Pharm Sci 2010;1:247-52.
8. Shinwari ZK, Qaise M. Efforts on conservation and sustainable use of medicinal plants of pakistan. Pak J Bot 2011;43:5-10.
9. Kim SH, Hyun SH, Choung SY. Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol 2006;104:119-23.
10. WHO traditional medicine strategy 2002-2005. Geneva, WHO; 2002.
11. Tackholm V. Student Flora of Egypt. 2nd ed. Cairo University Press: Cooperative printing Co, Beirrut, Lebanon; 1974. p. 581.
12. Al-Mustafa AH, Al-Thunibat OY. Antioxidant activity Jordanian medicinal plants used traditionally for treatment of diabetes. Pak J Biol Sci 2008;11:351-8.
13. Tietz NW. Clinical Guide to Laboratory Tests. 3rded. WB Saunders company, Philadelphia; 1995. p. 518-22.
14. Reeves WG. Insulin antibody determination: theoretical and practical considerations. Diabetologia 1983;24:399-403.
15. Matthews DR, Hosker JP, Rudenski AS. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
16. Schumann G, Klauke R. New IFCC refer-ence procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper refer-ence limits obtained in hospitalized subjects. Clin Chem Acta 2003;327:69-79.
17. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. Philadelphia: WB Saunders; 2006.
18. Henry RJ, Cannon DC, Winkelman JW. Clinical chemistry principles and tetchiness. New York: Harper and Row; 1997. p. 1440.
19. Fossati P, Principe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28:2077-80.
20. Burstein RF, Scholnick VS. Biochemistry and methodology of lipids. J Lipid Res 1972;25:375-82.
21. Friedewald WT, Levey RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
22. Kameswara RB, Kesavulu MM, Guiri R, Apparao CH. Hepatic key enzyme in experimental diabetes. J Ethnopharmacol 1999;1:109-13.
23. Okigbo RN, Mmeka EC. An appraisal of phytomedicine in Africa. Sci Technol J 2006;6:83-94.
24. Eddouks M, Maghrani M. Phlorizin-like effect of Fraxinus excelsior in normal and diabetic rats. J Ethnopharmacol 2004;9:149-54.
25. Levine R. Insulin: the effects and mode of action of the hormone. Vitam Horm 1982;39:145–73.
26. Szkudelski T. The mechanism of alloxan and alloxan action in β cells of the rat pancreas. Physiol Res 2001;50:536-46.
27. Nofal SM. Antidiabetic effect of *Artemisia judaica* Extracts. Res J Med Med Sci 2009;4:42-8.
28. Yi P, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic β cell proliferation. Cell 2013;153:747–58.

29. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils. Food Chem Toxicol 2008;46:446-75.
30. Siddhuraju P, Becker K. Antioxidant popeties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringaoleifera lam.) Leaves. J Agric Food Chem 2003;51:2144-55.
31. Lann D, leroith D. Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am 2007;91:1063–77.
32. Antuna-Puente B, Disse E, Rabasa-Lhoret R. How can we measure insulin sensitivity/resistance? Diabetes Metab 2011;37:179-88.
33. Rossetti L, Giaccari A, Defronzo RA. Glucose toxicity. Diabetes Care 1990;13:610–30.
34. Pari L, Kumar AN. Hepatoprotective activity of Moringa oleifera on antitubercular drug induced liver damage in rats. J Med Food 2002;5:171-7.
35. Kim HC, Nam CM, Jee SH. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ 2004;328:983-7.
36. Diogo CV, Suski JM, Lebiedzinska M. Cardiac mitochondrial dysfunction during hyperglycemia-the role of oxidative stress and p66Shc signaling. Int J Biochem Cell Biol 2013;45:114-22.
37. Majekodunmi SO, Oyagbemi AA, Odeku OA. Ameliorative effects of the ethanolic seed extract of Mucuna pruriens in alloxan-induced biochemical alteration in male wistar rats. Pharmacologia 2014;5:177-83.
38. Chandramohan G, Al-Numair KS, Pugalendi KV. Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in alloxan-diabetic rats. Afr J Biochem Res 2009;3:198-204.
39. Nair KS, Halliday D, Matthews DE, Welle SL. Hyperglucagonemia during insulin deficiency accelerates protein catabolism. Am J Physiol 1987;253:208-13.
40. Koteswara RY, Fang SH, Tzeng YM. Anti-inflammatory activities of flavonoids isolated from Caesalpinia Pulcherrima. J Ethnopharmacol 2005;100:249-53.
41. Coppack SW, Jenson MD, Miles JM. *In vivo* regulation of lipolysis in human. J Lipid Res 1994;35:177-93.
42. Ohno T, Horio F, Tanaka S. Fatty liver and hyperlipidemia in IDDM (insulin-dependent diabetes mellitus) of alloxan treated shrews. Life Sci 2000;66:125-31.
43. Kinosian B, Glick H, Preiss L, Puder KL. Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. J Invest Med 1995;43:443-50.
44. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of aqueous extract and non polysaccharide fraction of Cynodon dactylon Pers. Indian J Exp Biol 2008;46:660-7.
45. Sivaraj A, Devi K, Palani S. Anti-hyperglycemic and Anti-hyperlipidemic effect of combined plant extract of *Cassia auriculata* and *Aegle marmelos* in streptozotocin (STZ) induced diabetic albino rats. Int J Pharmtech Res 2009;1:1010-6.
46. Helal EM, Yousef HN, khattab AM. Ameliorative effects of the olive leaf extract against alloxan-induced biochemical alterations in male wistar rats. Egypt J Biomed Sci 2013;34:675-90.
47. El-Wakkad AS, Ibrahim S, Mannaa F. Vitamin E supplementation and oxidative stress in a streptozotocin induced diabetic rats. Afr J Lab Med 2000;26:297-304.
48. Sabina EP, Baskaran UL, Martin SJ, Swaminathan M, Bhattacharya Y, Tandon S. Assessment of antidiabetic activity of the traditional indian ayurvedic formulation Brahmi gritham in streptozotocin-induced diabetic rats. Int J Pharm Pharm Sci 2014;6:347-51.
49. Anwar MM, Meki AR. Oxidative stress in streptozotocin induced diabetic rats: Effects of garli c oil and melatonin. Comp Biochem Physiol Part A 2003;135:347-539.