ORIGINAL ARTICLE

Antidiabetic effect of Aqueous Extract of *Medicago Sativa* with Enhanced Histopathology of Pancreas in Alloxan Induced Diabetic Rats

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ABSTRACT

Aim: The present study investigated the effect of Medicago sativa extract and glibenclamide on biochemical parameters in the liver as well as on pancreas tissue in alloxan-induced diabetic rats.
Methods: Diabetes mellitus was induced in 28 out of 35 adult male albino rats, using an intra-peritoneal injection of 65 mg/kg body weight of alloxan. The diabetic rats were divided into four groups, two of which were administered orally by garlic extract (250 and 500 mg/kg) and a group composed of diabetic rats was given the standard drug, glibenclamide, orally at a dose of 2.5 mg/kg. The control rats (normal and diabetic) were fed normal saline, once daily for 21 d. The specimens were prepared for light microscopic examination. In parallel, the related biomedical parameters such as glucose and insulin levels had been estimated in pancreas, statistically analyzed and compared between the groups.
Results: The aqueous extract of *M. Sativa* significantly reversed (P<0.05) the manifestation of alloxan on the levels of serum glucose & insulin, C-peptide, malondialdehyde formation, Nitric oxide, glucose-6-phosphate dehydrogenase (G6PD), antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase). Histological pictures of pancreas showed pathological changes which was in concurrence with the biochemical results.
Conclusion: Extracts of *Alfalfa* improved hyperglycemia and other biochemical alterations noticed in alloxan-diabetic rats. These effects may be due to the presence of a high content of flavonoids which acts synergistically as antioxidants.
Keywords: Diabetes mellitus ; Pancreas; *M. Sativa*; Glibenclamide.

INTRODUCTION

Pancreas is a vital endocrine-exocrine organ that produces several hormones and enzymes. Its enzymes help in the digestion of carbohydrates, fats, and proteins whereas its hormones such as insulin regulate carbohydrate metabolism in the body and maintains passage of glucose across the cell membrane. Therefore, any change in the function of the organ may directly affect the physiological function of the body[1]. Diabetes mellitus (DM), an endocrine metabolic disorder of multiple etiologies manifested by consistent elevated levels of glucose in the blood resulting from defects in insulin secretion, insulin action, or both, and occurs in almost all populations of the world a variable prevalence[2,3]. Recently there has been a growing interest in alternative therapies, including the use of plant foods, to treat diabetic patients[4].

Alfalfa (*Medicago sativa*) or green gold is a high-yielding perennial legume that is cultivated worldwide with rich nutritional characteristics and bioactive compounds, that are used in traditional medicine due to being high in protein, calcium, β-carotene vitamins, including(B, C, E, and K), chlorophyll,c owmarine derivative, choline, essential amino acid, flavonols, lime, magnesium, phosphorous, protein, silicon, potassium, sterol, iron and Saponins and low in percentage of cellulose, lignin, and xylans,. It contains many enzymes, including amylase, invertase, and pectinase, and so it can be used as digestive aids[5-7].

Glyburide (glibenclamide) is one of the sulfonylurea compound chemically, 5-chloro-N- [2- [4-cyclohexyl carbamoyl sulfamoyl) phenyl] ethyl]-2-methoxy benzamide,to be good alternative for insulin. Oral hypoglycemic agents are an attractive option to insulin because of their lower cost and ease of administration, which increase patient compliance which is produced its effect via stimulating of endogenous insulin release from pancreas, enhancing peripheral tissue utilization of glucose, or by decreasing the absorption of glucose by intestine[8-10].

This study aimed at testing the ameliorative effect of two low doses of Moringa seeds powder (50 and 100 mg/kg body weight) on alloxan induced diabetic male rats.

MATERIALS AND METHODS

Induction of Diabetes: Alloxan is known as a useful drug to induce diabetes in experimental animals. After overnight fasting diabetes was induced in rats by intraperitoneal injection of alloxan monohydrate (Sigma, St. Louis, MO, USA) dissolved in 1ml distilled water at a dose of 65mg/kg BW.
Preparation of Medicago sativa Extract: Leaves of Medicago sativa after collection were allowed to shade dry and the dried leaves were ground to a fine powder, which was then used for extraction in a soxhlet apparatus for up to 10 hours using 95% ethanol at a boiling temperature of 60°C. The extract obtained from soxhlet extraction was

allowed to cool and then filtered to remove the residue. The filtrate was then concentrated at 65°C by rotavapour to get a fine powder that was refrigerated at 4°C until further use[11].

Study design: A total of 35 Male albino Wistar rats of age 4-5 weeks with a body weightof180 to 200g were procured and acclimatized for7 days to animal house and were randomly divided into 5groups each consisting of 5 rats as follows:

Group 1: Non-diabetic control-received distilled water (3ml/kg.b.w).

Group 2 : Diabetic control- received 1 ml of distilled water

Group 3: Diabetic - received Medicago sativa extract (60mg/kg.b.w).

Group 4: Diabetic - received Medicago sativa extract (120mg/kg.b.w).

Group 5 : Diabetic - received Glibenclamide (10 mg/kg.b.w).

They were housed and received normal basal diet and tap water *ad libitum* in a constant environment (room temperature 28 ± 2°C, room humidity 60 ± 5%) with a 12/12 h light/ dark cycle.

Medicago sativa and glibenclamide doses were administered daily via oral gavage. Food and water intake were monitored daily whilst body weights were determined weekly.

Insulin levels were measured by Enzyme-linked Immunosorbent Assay (ELISA) technique. G6PDH activity was assayed by the method of Beutler. Catalase, **MDA** GPx, G6PD, NO levels were measured by spectrophotometric kit.

At the end of the experimental period the rats were sacrificed using ether anesthesia. pancreas were dissected ,fixed in 10% buffered formalin, and then dehydrated by successively passing through a gradient mixture of ethanol and water. The samples were rinsed in xylene and embedded in paraffin. 5 μm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation. The stained sections were examined and photographed under a light microscope[12,13].

Statistical analysis :One-way-ANOVA-using- SPSS-statistics. The p-value of more than 0.05 was considered as not significant.

RESULTS

Pancreatic insulin SOD, GPx, G6PD, NO and catalase levels were significant ($P < 0.05$) decreased while MDA level was significant increase in diabetic control rats as compared with normal control rats.

Treatment with aqueous extract of *M. Sativa* significantly enhanced the activity of SOD, GPx, G6PD, NO and catalase while MDA level decreased. Glibenclamide treatment produced significant increases in SOD, GPx, G6PD, NO and catalase while MDA decreased, compared to the diabetic control group.

Figure.1: Effect of *M. Sativa* on serum insulin levels in diabetic rabbits.

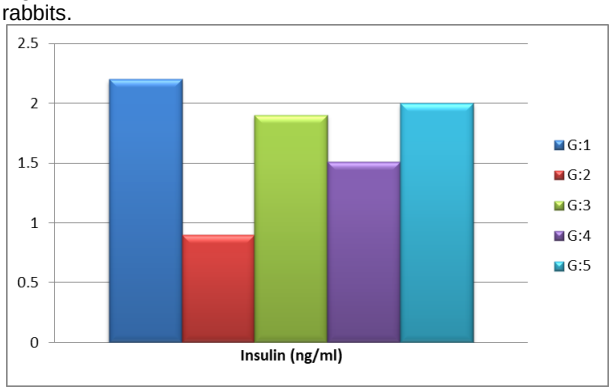

Figure.2: Effect of *M. Sativa* on serum GPx levels in diabetic rabbits

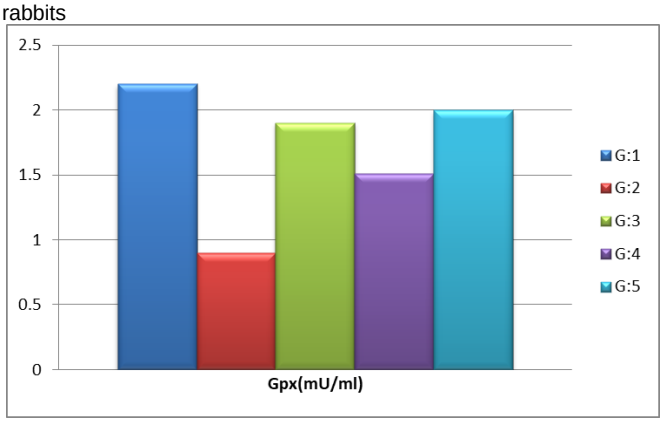

Figure.3: Effect of *M. Sativa* on serum catalase levels in diabetic rabbits

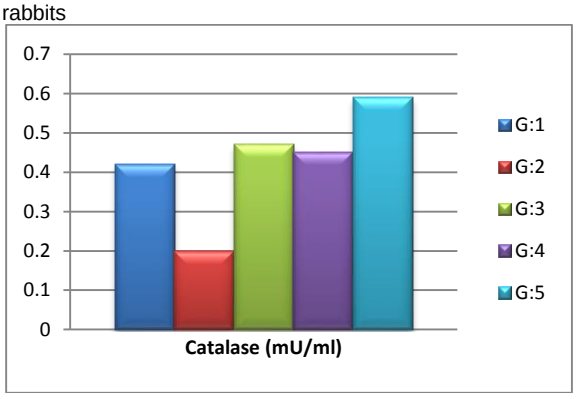

Figure.4: Effect of *M. Sativa* on serum NO levels in diabetic rabbits

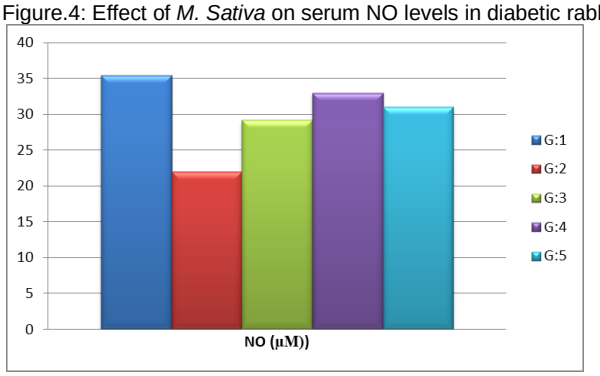

Figure.5: Effect of *M. Sativa* on serum G6PD levels in diabetic rabbits

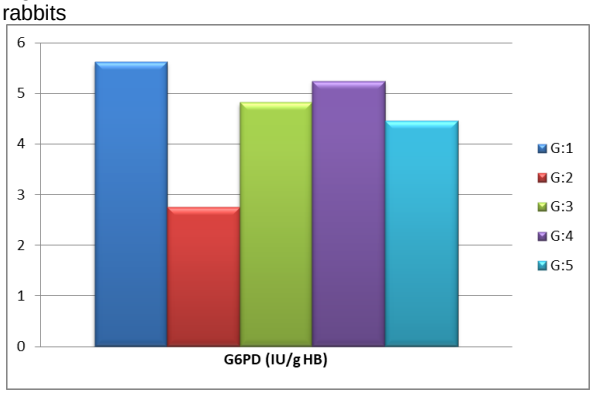

Figure.6: Effect of *M. Sativa* on serum MDA levels in diabetic rabbits

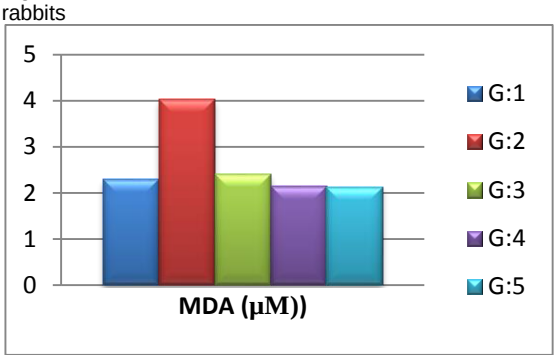

Table 3 - Effect of the Orally Administered *M. Sativa* Extract and Glibenclamide on Biochemical Parameters .

Parameters	Control	Group B	Group C	Group AD	Group E
Insulin (ng/ml)	2.2 ± 0.078	0.9 ± 0.059***	1.9 ± 0.08	1.51 ± 0.032**	2 ± 0.087*
GPx (mU/ml)	7 ± 0.24	1.9 ± 0.56	4.43 ± 0.75#	6 ± 0.46	5.82 ± 0.85
Catalase (mU/ml)	0.42 ± 0.09	0.2 ± 0.03*	0.47 ± 0.06#	0.45 ± 0.09#	0.59 ± 0.08#
NO (uM)	35.42 ± 1.17	22 ± 0.95**	29.2 ± 1.65#	33 ± 3.4#	31 ± 3.7#
G6PDIU/g HB	5.62±0.05	2.75± ±0.13	4.82 ±0.11	5.24± 0.17	4.46±0.09
MDA	2.29 ± 0.23	4.03 ± 0.23	2.40 ± 0.45**	2.15 ± 0.26**	2.13±0.20 a

*Significantly different from non-diabetic control p < 0.05
**Significantly different from non-diabetic control p < 0.01
*** Significantly different from non-diabetic control p < 0.001
#Significantly different from diabetic control p < 0.05
##Significantly different from diabetic control p < 0.01

DISCUSSION

The current results reveals significant decrease in levels of serum insulin and marked elevation in levels of blood glucose in diabetic rats. This is attributed to the hypo secretion of insulin by the pancreatic β-cells, as alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia (14).The current observations are in analogy to earlier results obtained by (15,16).The concurrent treatment with alfalfa these parameters and nearly restored them to their normal levels. This curative effect is due to the active constituents present in alfalfa as trigonelline, which is the N-methyl derivative and main human metabolite of the vitamin nicotinic acid[17].

Hyperglycemia is the clinical hallmark of poorly controlled diabetes, which is known to cause protein glycation, also known as nonenzymatic glycosylation. It has been reported that various proteins, including hemoglobin, albumin, collagen, low-density lipoprotein, a crystalline fibronectin, undergo non-enzymatic glycation in diabetes[18].

Hence, estimation of glycosylated hemoglobin is a well-accepted biochemical parameter useful for the diagnosis and management of the disease. The increased glycated hemoglobin is associated with loss of β-cell function and has been implicated in the complications of diabetes mellitus[19,20].

The present study indicated significant increases in the activity of HbA1c in the STZ-induced diabetic rats and these levels were significantly reduced after treatment intervention with Medicago sativa which are concomitant with other findings[21-23].

Oxidative stress consider as interrelated contributing factors in pancreatic β-cell dysfunction and apoptosis[24,25]. Because of poor antioxidant capacity, beta cells are vulnerable to the oxidative stress induced by DM glucotoxicity (26).

Elevated level of MDA which considered one of the final products of lipid peroxidation (27). as well as reduced levels of key antioxidant enzymes, CAT and GPx levels in the pancreas of the diabetic control rats (Table 3), an indication of alloxan-induced oxidative stress. Glutathione is the mother of all antioxidants, the master detoxifier and maestro of the immune system. The low activity of GPx could be directly explained by the low content of GSH, since GSH is a substrate and cofactor of GPx. Enzyme inactivation could also contribute to low GPx activity[28-30]. A decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, thereby inactivating several membrane bound enzymes[31,32].

Treatment with alfalfa (60 and 120mg/kg) produced significant reductions in MDA as well as increased CAT and GPx levels demonstrating that the alfalfa leaves contain high concentrations of flavonoids and other phenols, which exert powerful antioxidant properties and capable of stimulating liver antioxidant enzymes as free radical scavengers and inhibitors of nitric oxide release and therefore may be capable of preventing tissue damage, it can protect the pancreas tissues from lipid peroxidation on diabetic rats (33,34).

Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) is the rate-limiting enzyme in the pentose-phosphate pathway, found in the mitochondria and cytosol. it converts

glucose-6-phosphate accompanied by nicotinamide adenine dinucleotide phosphate $NADP^+$ into 6-phosphogluconolactone and NADPH(Omotoso,2018). G6PD 6PD provides a source of reducing power against oxidative damage and is important for β-cell proliferation and prevention of β-cell death[33-35].

In alloxan-induced diabetic rats, the reduction of G-6-PDH activity in liver which obstruct glucose utilization through pentose phosphate pathway as this enzyme activity is controlled by insulin[33,36], and due to insufficient or limited production of NADPH which regenerates GSH, a physiologic antioxidant to scavenge glucose-generated free radicals[23].

The G6PDH activity was restored to normal level after treatment with Medicago sativa for 30 d. The restored G6PDH activity by Medicago sativa treatment confirmed the protective role of Medicago sativa against diabetes complications, since Medicago sativa administration showed a similar upturn with standard anti-diabetic drug glibenclamide.

In conclusion, Medicago sativa have potent anti-hyperglycemic and efficacies in alloxan-induced diabetic rats. These effects may be due their ability to improve the islets architecture, insulin secretory response and antioxidant activity.

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Ethical Approve: We declare that the study does not need ethical approval.

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