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The Effect of Metformin and Ginseng on Alloxan-Induced Diabetic Rats: Hematological, Biochemical and Histopathological Studies

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ABSTRACT

The current study was performed to evaluate the anti-diabetic action of metformin and ginseng in alloxan-induced diabetes in rats. Forty male albino rats were used in this work, ten rats were left without any other treatment and kept as a control, the remaining thirty rats were injected with alloxan to induce diabetes and then divided into three equal groups, the first group was kept without any treatment during period of the experiment, the second one treated with metformin (300 mg/kg B.Wt.) and the third one treated with ginseng at dose level of 250 mg/kg B.Wt. for 30 consecutive days. The results showed a significant changes inthe hematological parameters, fasting blood glucose level, glycosylated hemoglobin (HbA1C) besides the activity of liver enzymes (aspartate aminotranseferase (AST), alanine aminotranseferase (ALT) and gamma glutamyltransferase (GGT)) and level of total protein in addition to lipid profile (Triglyceride (TG),total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) except high density lipoprotein (HDL)) in all groupscompared to control group. The liver and pancreas of all groups were collected and examined histopathologically. Metformin and ginseng were found to have ameliorative effect on biochemical and histopathological alterations caused by hyperglycemia. From our data it was concluded that ginseng can be used as an effective anti-hyperglycemic and antioxidant agent.

1. INTRODUCTION

Diabetes mellitus is the most common endocrine disorder resulting from defect in secretion or action of insulin or both (Sailaja et al., 2003). Persistent hyperglycemia of diabetes mellitus is associated with production of reactive oxygen species (ROS) due to glucose autoxidation and generation of advanced glycation end products which causing oxidative damage, particularly in liver, heart and kidney (Bonnefont-Rousselot et al., 2000). Historical records on traditional medicinal herbs revealed that *Panax ginseng* has been used for treatment of diabetes extensively and that ginsenosides, the major bioactive constituent in ginsengare thought to be the main component responsible for anti-diabetic action (Xie et al., 2005) through initiation of the secretion of glucagon-like peptide (GLP-1) from enterocytes, which stimulate insulin secretion, inhibiting glucagon secretion and promoting pancreatic β-cells proliferation (Liu et al., 2013). Metformin is an effective hypoglycemic drug that has been widely used for more than 50 years for treatment of type II diabetes mellitus(Choi et al., 2008; Hadi et al., 2013). Its hypoglycemic action is exerted through activation of activated protein kinase(APK) which induces muscle to take up glucose from blood and decrease glucose production by liver (Evans et al., 2005) and also has been reported to have some protective effect against complications of hyperglycemia (Wiernsperger, 2007). This study was carried out to evaluate the clinicopathologic and pathologic effect of metformin and ginseng in alloxan-induced diabetic rats.

2. MATERIAL and METHODS

2.1.Animals:

Forty apparently healthy albino male rats of 170 - 200 g body weight, three months old were obtained from Research Institute of Alexandria University. They were housed in cages under proper hygienic conditions. All of the animals were fed on a standard experimental diet which was formulated according to the requirement of laboratory animals (Reeves et al., 1993)and water *ad- libitum.*The animals were kept without any treatment for 10 days for adaptation before experiment.

2.2.Experimental Design and Treatment:

Thirty rats from forty were injected by intraperitoneal dose of alloxan (100mg/kg B.Wt.) (lobachemie, India) for induction of hyperglycemia.The glucose level was measured after three days from alloxan injection using an electronic glucose monitoring device (Omron®, Germany). The rats that have fasting glucose level more than 200mg/dl were kept as diabetic. The rats were divided into four equal groups as follow:

Group A (Control): normal control rats and kept without treatment for 30 days.

Group B (Diabetic untreated): alloxan-diabetic rats and kept without treatment for 30 days.

Group C (Metformin-treated group): alloxan-diabetic rats and received 300 mg/kg B.Wt. metformin***(Cidophage ® tablets)*** orally or 30 days according to Sartoretto et al. (2005).

Group D (Ginseng-treated group): alloxan-diabetic rats and received 250 mg/kg B.Wt. ginseng alcohol-based extract (***Nature's way company, USA)*** orallyfor 30 days according to Murtahy et al. (2014).

2.3.Hematological studies:

Rats of each group were euthanized after 30 days from the experiment beginning and blood samples were collected from retro-orbital venous plexus during euthenization using hematocrit tubes. About 0.5 ml of blood samples were collected in dipotassium salt of EDTA containing tubes as anticoagulant and used for hemogram evaluation according to Feldmanet al. (2000) and determination of glycosylated hemoglobin (Trivelli et al., 1971).

2.4.Clinicobiochemical studies:

About 5 ml of blood was placed in plain centrifuge tubes, left to clot and centrifuged at 3000 r.p.m for 15 minutes for separation of serum. The clear serum was carefully separated and immediately frozen at -20 until the time of biochemical analysis. The sera were used for spectrophotometric determination of glucose (Trinder, 1959),the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman and frankel, 1957) and gamma glutamyltransferase(GGT) (Szasz, 1969),in addition to total protein (TP) (Doumas et al., 1981), triglycerides(TG) (Trinder and Ann. 1969), total cholesterol (TC) (Allain et al., 1974), high density lipoprotein (HDL) (Gordon and Amer, 1977), very low density lipoprotein(VLDL)and low density lipoprotein (LDL) were calculated according to Friedewald, et al. (1972).

2.5.Histopathological studies:

After euthanization, small pieces of pancreasand liver were quickly collected and fixed in neutral buffered formalinsolution (10%) and processed by paraffin embedding technique. Five micron thick sections were stained with hematoxyline and eosin (H&E) according to Bancroft and Stevens, (1996).

2.6.Statistical analysis:

Statistical analyses were performed using the Statistical Analysis System software (SAS, 2011). Effects of treatments on hematological and biochemical parameters were assessed by the analysis of variance. Means were compared using one-way ANOVA test at a significance level of $P \leq 0.05$. Values are presented as means $\pm$standard errors.

3. RESULTS

3.1.Hematological Findings

As illustrated in Table (1), RBCs count showed a significant decrease in rats of group B only, while PCV%, Hb%, MCV, MCH, MCHC and platelets count did not record any significant changes in all of the treated groups compared to control group. Total leukocytic count and lymphocytic count showed a significant decrease in all treated groups (B, C and D) in comparison with control group, while granulocytic count, monocytic count and platelets count showed non-significant changes in all treated groups if compared with control group.Blood glycosylated hemoglobin level showed a significant increase in groups B and D, the highest value of blood glycosylated hemoglobin level was recorded in group B followed by groups D if compared with control group.

Table (1): The changes in hematological parameters and glycosylated hemoglobin levelin rats groups (A-D) four weeks post-treatment**(mean ± SE).**

Parameters	Control Gp. A	Diabetic Gp. B	Metformin Gp. C	Ginseng Gp. D
RBCs (10^6/µL)	7.45±0.28a	5.85±0.53b	6.78±0.31ab	6.65±0.30ab
PCV (%)	37.20±1.40a	35.76±1.33a	38.84±0.12a	36.67±0.82a
Hb (g %)	13.37±0.41a	11.90±0.81a	13.27±0.45a	12.68±0.53a
MCV (fl)	53.33±1.20a	52.25±1.70a	52.67±1.86a	52.25±1.11a
MCH(pg)	19.30±0.35a	19.28±0.59a	19.23±0.79a	18.75±0.27a
MCHC (%)	36.73±1.19a	36.59±0.44a	36.77±0.56a	37.28±0.35a
Platelets(10^3/µL)	535.00±13.05a	530.50±15.31a	565.00±11.59a	539.50±15.86a
WBCs (10^3/µL)	12.87±1.75a	7.57±0.42b	8.74±1.90b	7.88±0.38b
Granulocytes(10^3/µL)	2.63±0.43a	2.92±0.36a	2.95±0.40a	2.75±0.43a
Lymphocytes(10^3/µL)	8.99±1.68a	3.09±0.38b	4.19±0.86b	3.71±0.17b
Monocytes(10^3/µL)	1.26±0.09a	1.22±0.35a	1.59±0.71a	1.41±0.36a
HbA1c (%)	3.87±0.19c	8.13±0.13a	3.93±0.23c	6.55±0.48b

Means within the same row having different **small letters** are significantly different at $P \leq 0.05$.

3.2. Clinicobiochemical Findings.

As shown in Table (2), comparatively with control group, fasting serum glucose level showed a significant increase in groups B and D,the highest value of serum glucose level was recorded in group B followed by groups D.Serum activities of AST and ALT showed a significant increase in groups B and D compared to control group but the highest increase in both of AST and ALT activities were recorded in group B followed by group D. Serum activity of GGT showed a significant increase in group B only in comparison with control group. Comparatively with control group, total protein level recorded a significant decrease in group B and D, whileserum levels of triglycerides and VLDL showed a significant decrease in group Donly. Serum level of total cholesterol and LDL levels recorded a significant increase in group B and D if compared with control group but their values were higher in group B than group D. Serum level of HDL did not record any significant change in all treated groups in comparison with control group.

Table (2): The changes in some serum biochemical parameters in rats groups (A-D) four weeks post-treatment**(mean ± SE)**.

Parameters	Control Gp. A	Diabetic Gp. B	Metformin Gp. C	Ginseng Gp. D
Glucose(mg/dl)	107.33±2.19c	270.00±15.34a	122.00±3.61c	164.75±8.33b
AST (U/L)	48.00±2.08c	78.25±2.06a	54.67±2.40bc	59.00±3.16b
ALT (U/L)	35.00±1.73c	64.00±1.96a	44.67±1.20bc	50.75±4.48b
GGT (U/L)	25.00±1.73b	44.75±2.32a	36.33±1.76ab	38.25±1.68ab
TP (g/dl)	8.93±0.27a	7.23±0.38b	9.17±0.52a	7.48±0.20b
TG (mg/dl)	244.00±2.52a	266.50±8.47a	246.00±9.61a	211.25±7.18b
TC (mg/dl)	94.33±2.03c	139.25±4.71a	97.33±3.84c	117.75±4.52b
VLDL (mg/dl)	48.67±0.33a	53.00±1.68a	49.00±1.53a	41.75±1.49b
LDL (mg/dl)	14.67±3.28b	62.25±3.20a	22.00±2.08b	52.50±3.07a
HDL (mg/dl)	31.00±1.53a	24.00±1.08a	26.33±2.73a	27.25±3.22a

Means within the same row having different **small letters** are significantly different at $P \leq 0.05$.

3.3.Histopathological Findings:

Histopathological examination of pancreas of alloxan-diabetic untreated rats (gp. B)showed severe vacuolation of β-cells of pancreatic islets (Fig: 1-a) and congestion of blood vessels with presence of shrunken islet (Fig: 1-b),in addition to infiltration of mononuclear inflammatory cells (Fig: 1-c).Metformin-treated rats (gp. C)showed mild vacuolation ofβ-cells of pancreatic islets with congestion of blood vessels (Fig: 1-d), but ginseng-treated rats pancreas (gp. D) showedmoderatevacuolation of β-cells of pancreatic islets (Fig: 1-d).

Liver of alloxan-diabetic untreated rats exhibited hypereosinophilia of some hepatocytes (Fig: 2-a), presence of large area of hepatic necrosis with infiltration of mononuclear inflammatory cells (Fig: 2-b), moderate infiltration of mononuclear inflammatorycells in the portal area (Fig: 2-c) and hyperplasia of biliary epithelium (Fig: 2-d). Metformin-treated group showed presence of limited area of hepatic necrosis (Fig: 2-e) with mild

infiltration of mononuclear inflammatory cells in the portal area (Fig: 2-f) but ginseng-treated group showed mild to moderate hydropic degeneration of hepatocytes with congestion of blood vessels(Fig: 2-g),besides area of hepatic necrosis with mononuclear inflammatory cells infiltration (Fig: 2-h).

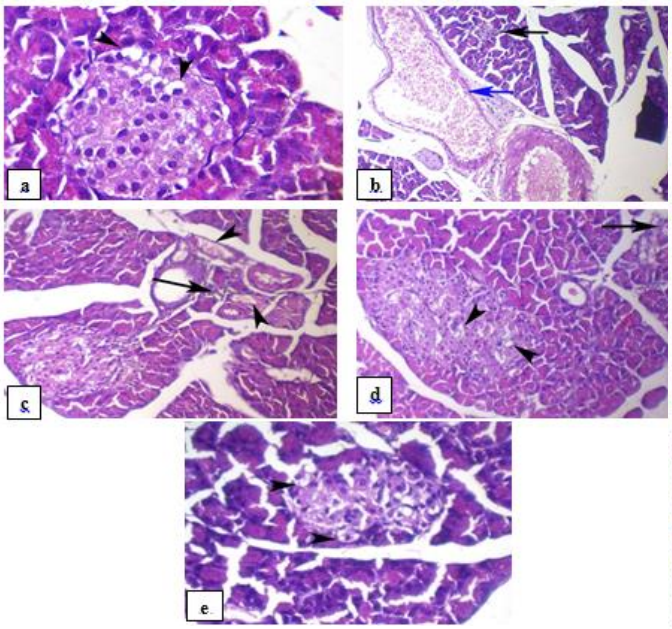

Fig (1): H&E stained sections of pancreas of rat of **(a)**– diabetic untreated group showing severe vacuolation of β cells of pancreatic islets (arrow heads). (×400)**(b)** - diabetic untreated group showing severe congestion of blood vessels (blue arrow) and shrunken islet (black arrow).(×200)**(c)**-diabetic untreated group showing congestion of blood vessels (arrow heads) and infiltrationof mononuclear inflammatory cells (arrow). (×200). **(d)**-metformin-treated group showing mild congestion of blood vessels (arrow) and mild vacuolation of β cells (arrow heads).(×200) **(e)**-ginseng -treated group showing moderate vacuolation of β cells of pancreatic islets (arrow heads).(×400)

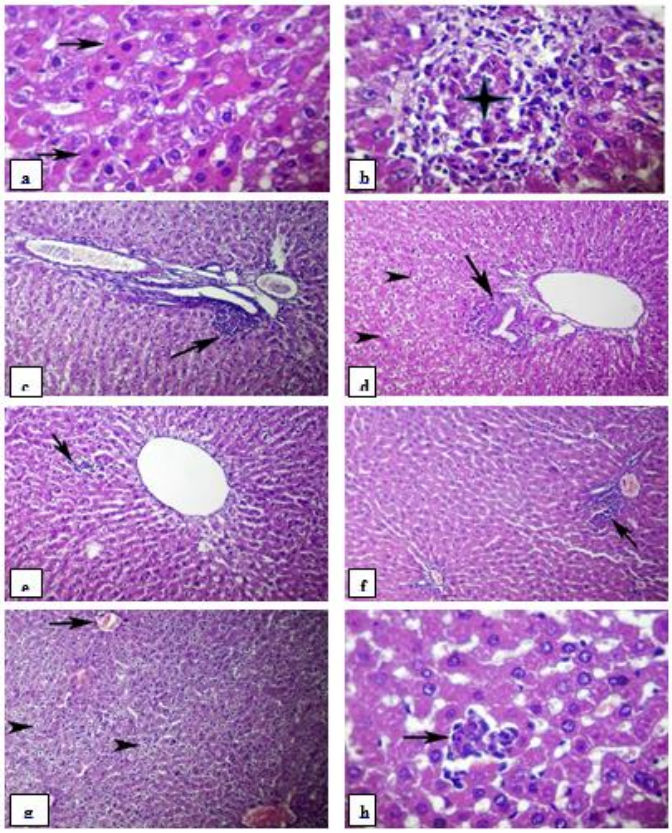

Fig (2): H&E stained sections of liver of rat of **(a)**– diabetic group showing hypereosinophilic hepatocytes. (×400)**(b)** - diabetic group showing focal hepatic necrosis with mononuclear inflammatory cells infiltration (asterisk). (×400)**(c)** – diabetic group showing moderate mononuclear cell infiltration in portal area (arrow). (×200)**(d)**-diabetic untreated group showing hyperplasia of lining epithelium of bile ducts (arrow) and hydropic degeneration of hepatocytes (arrow heads).(×200) **(e)**-metformin-treated group showing focal hepatic necrosis (arrow). (×200)**(f)**-metformin-treated group showing mild infiltration of mononuclear cell in portal area (arrow)(×200)**. (g)** ginseng -treated group, showing mild to moderate hydropic degeneration of hepatocytes and congestion of blood vessels (arrows).(×200)**(h)**-ginseng-treated group showing focal area of hepatic necrosis withmononuclear inflammatory cells infiltration.(×400)

4. DISCUSSION

Persistent hyperglycemia is resulting in an oxidative stress condition due to free radicals productionmainlyreactive oxygen species (ROS) which are considered the main initiators of organs damage caused by diabetes (Coskun et al., 2005).The decrease in RBCs count in diabetic untreated group compared to control group may be connected to the increase in non-enzymatic glycosylation of RBCs membrane protein due to hyperglycemia causing oxidation of these proteins with an increase in production of lipid peroxide which destroy RBCs membrane (Oyedemi et al., 2011). On the other hand, RBCs countwasinsignificantly increased in ginseng and metformin-treated groups compared to untreated diabetic group toward the normal level of control group,such result proved that both of ginseng and metformin have a potent hypoglycemic effect so, they can partially decrease non-enzymatic glycosylation of RBCs membrane protein(Aseervatham et al., 2010; liu et al., 2013). Concerning leukocytic picture, leucopenia with lymphopenia was recorded in all of the treated groups compared to control group. Lymphopenia recorded in these groups may be caused by diabetes which causes shorteningin the circulation time of lymphocytes (Kozlov et al., 1995).

Concerning serum glucose level, the diabetic untreated rats recorded a significant increase in serum glucosedue to destructive cytotoxic effect of alloxan on β-cells of pancreascausing hypoinsulinemia (Akah et al., 2009), but ginseng and metformin have an effective regulatory mechanism on glucose metabolism causing hypoglycemia, so ginseng and metformin-treated groups showed a significant decrease in fasting serum glucose level (Evans et al., 2005; liu et al., 2013).The untreated diabetic rats recorded a significant increase in level of blood glycosylated hemoglobin compared to control group, this may be due to persistent hyperglycemic conditionas glycosylated hemoglobin (HbA1c) is produced by non-enzymatic condensation of glucose molecules with free amino acids on the globin component of hemoglobin so, the increased level of glucose leading to elevation in HbA1c level (Beissuenger et al., 1993). In accordance with the result of Saleh,(2012) and Xiao et al. (2013), the hypoglycemic effect of ginseng and metformin led to a significant decrease in level of blood HbA1c of ginseng and metformin-treated groups compared to untreated diabetic group.

The significant hypoproteinemia in untreated diabetic rats and ginseng-treated diabetic rats (gps. B&D) compared to control group may be attributed to liver damage which proved histopathologically in this study causing a decrease in plasma proteins production (Latimer et al., 2003).While serum level of total protein weresignificantly increased in metformin-treated rats group compared to diabetic untreated rats. Such result agreewith the result of Ikewuchi et al. (2011)and this may be attributed to the hypoglycemic effect of metformin which ameliorating the oxidative stress of hyperglycemia causing hepatic affections.

In the same manner, the increased level of ROS (Oxidative stress) can produce hepatic damage, and as AST and ALT are present in large amount in hepatocytes, they leak from hepatocytes upon its destruction and as thepressure of damaged hepatocytes on bile canaliculi causing bile stasis and a resultant increase in serum activity of GGT (cholestatic enzyme), so the activities of AST, ALT and GGT were increased in diabetic untreated rats compared to control healthy animals group.Our results act in a harmony with the results of Hadi et al. (2013) and Ndidi et al. (2014). The activities of serum liver enzymes (AST, ALT and GGT) were decreased significantly in both of ginseng and metformin treated rats groups compared to untreated diabetic rats due to hypoglycemic effect of ginseng and metformin which decreasegeneration of ROSand lipid peroxidation, in addition to the ROS scavenger effect of ginseng which totally have a net result of marked relieving effect on hepatocytes damage by ROS. The same results were obtained by Moram, (2001) and Luka et al. (2013) who proved a significant decrease in serum level of AST, ALTand GGT upon treatment of diabetic rats with ginseng and metformin.Hypercholesterolemia which occurred in diabetic untreatedratsmay be caused by the increased cholesterol synthesis and the decrease in synthesis of bile salts from cholesterol due to decreased hepatic phenol-2 monooxygenase enzyme activity which responsible for formation of bile salts from cholesterol (El-Khamisy and Rezk, 2013). The increased level of LDL in diabetic untreated group may be explained by the decrease in number of peripheral LDL receptors or the reduction in LDL binding to its receptors (Osman and Kandil, 1999) or due to relative increase in LDL synthesis from the increased VLDL (Basak et al., 2013).Treatment of diabetic rats with ginseng caused a significant decrease in TG, TC, VLDL and LDL in accordance with Saleh, (2012) and Liu et al.(2013). Ginseng hypolipidemic effect may be a result of its ability to increase secretion of GLP (Glucagon like protein) from enterocytes which increase secretion of insulin which in turn activate LPL enzyme enhancing VLDL clearance from blood stream, in addition, ginseng can inhibit cholesterol biosynthesis so it has an effective hypocholesterolemic effect (Moram 2001; liu et al., 2013). Metformin glycemic control leading to a decreased rate of lipolysis of fat depots, decreasing free fatty acids libration and consequently decreasing hepatic production of VLDL (El Messaoudi et al., 2013), the decreased VLDL in turn decrease the level of LDL as LDL is produced from action of LPL on VLDL (Latimer et al., 2003).

Histopathological lesions occurred in pancreatic tissues of diabetic untreated rats group induced by alloxan injection which cause selective β-cells necrosis (Viana et al., 2004). Furthermore, persistent hyperglycemia caused by alloxan injection causing oxidative stress which resulting in further damage of pancretic β-cells which have intrinsically low antioxidant enzymes defense in a phenomenon called glucose toxicity (Robertson et al., 2004). Treatment with metformin resulted in a remarkable preservation of pancretic β-cells from further damage by oxidative stress as a result of its hypoglycemic effect in agreement with Salman et al. (2013). Also, ginseng-treated group showed moderate ameliorative effect on hyperglycemic β-cells damage and the possible explanation may be that ginseng has been shown to exhibit an anti-

apoptotic function on pancreatic β-cells via regulation of nitric oxide and reactive oxygen species production (ROS scavenging) (Kim and Kim, 2008).

The cause of hepatic lesionsof diabetic untreated rats may be depletion of enzymatic and non-enzymatic anti-oxidants of liver tissues due to over production of ROSresulting in excessive lipid peroxidation as well as fragmentation of mitochondria, Geolgi apparatus and rough endoplasmic reticulum leading to hepatocytes destruction (Abdultawab and Ayuob, 2013). In addition to its hypoglycemic and anti-hyperlipidemic effect, ginseng hepatoprotective effect may be conducted to the anti-oxidant properties of ginsenosides and its ameliorative effect against ROS damage in liver (Voces et al., 1999), so ginseng-treated groupexhibited a noticeable amelioration of hepatic lesions caused by diabetes mellitus. Metformin hypoglycemic effect has been proved to diminish oxidative stress and increase liver content of anti-oxidant enzymes preventing the oxidative damage of ROS on liver (Zhang and Tan, 2000) so, metformin showed an ameliorative effect on hepatic lesions of diabetes mellitus. From the obtained data, it could be concluded that treatment with metformin and to lesser extent ginseng has a potent effect against biochemical and histopathological alterations caused by diabetes in rats.

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