Antidiabetic, antihyperlipidemic and histopathological analysis of *Panax quinquefolium* extract on alloxan induced diabetic rats

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**ABSTRACT**

**Objective**: To investigate antidiabetic, hypolipidemic histopathological analysis of *Panax quinquefolium* aqueous (AGE) extract in alloxan induced diabetic rat by administering intraperitoneal dose (100 mg/kg body weight / day). **Methods**: Optimal cutoff level of each of the four plasma glucose values of oral glucose tolerance test in alloxan diabetic rat was done. Other parameters as liver profile, renal profile and total lipid levels were determined in normal and alloxan induced diabetic rats after intraperitoneal administration of the extract for 30 days. Histopathological changes in diabetic rat organs (pancreas, liver and kidney were also observed after extract treatment. **Results**: Daily intraperitoneal administration AGE (100 mg/kg body weight) and glimebride (10 microg/g body wt) showed beneficial effects on blood glucose level (P<0.001) as well as improving kidney, liver functions and hyperlipedemia due to diabetes. The extract treatment also showed enhanced serum insulin level and body weight of diabetic rats as compared to diabetic control group. Furthermore, the extract has a favorable effect on the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes. **Conclusion**: *Panax quinquefolium* possesses antidiabetic property as well as improve body weight, liver profile, renal profile and total lipid levels. AGE has also favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes.

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**INTRODUCTION**

*Diabetes mellitus* (DM) is a very commonly occurring metabolic disorder characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. DM occurs due to absolute or relative deficiency of insulin or insulin resistance[9]. This metabolic disorder affects approximately 4% of the population worldwide and is expected to be increased by 5.4% in 2025. Because DM is associated with oxidative stress, it alters the cellular microenvironment in many different types of tissues causing myriad untoward effects, collectively referred to as ‘diabetic complications’. Two cellular processes affected by diabetes are inflammation and apoptosis[24].

Glimepiride is a medium-to-long acting sulfonylurea anti-diabetic drug. sometimes classified as the first third-generation sulfonylurea and sometimes classified as sec-
ond-generation. Glimepiride acts as an insulin secreta-
gogue. It lowers blood sugar by stimulating the release
of insulin by pancreatic beta cells and by inducing in-
creased activity of intracellular insulin receptors.[28]

Synthetic drugs usually come with considerable side
effects, such as hypoglycemia, drug-resistance, dropsy,
and weight gain. In recent years due to the adverse ef-
effects of synthetic hypoglycemic drugs, interests in alte-
nate therapeutic approach have become very popular.
Nowadays, herbal drugs are gaining popularity in the
treatment of diabetes and its complications due to their
efficacy, low incidence of side effects and low cost.

The medicinal plants provide a useful source of oral
hypoglycemic compounds for the development of new
pharmaceutical leads. Some of the plants which are being
used for the treatment of diabetes have received sci-
cientific or medicinal scrutiny and even the WHO expert
committee on diabetes recommends that this area war-
rant further attention.[27]

(Panax quinque folius L.) American ginseng is
slow-growing perennial plants with fleshy roots, belong-
ing to the Panax genus in the family Arali-aceae. Gin-
seng is one of the most widely used herbal medicines
and is reported that root extract of ginseng has a wide
range of therapeutic and pharmaceutical applications
for antioxidant, antidiabetic and vasorelaxation effects.[1].

MATERIALS AND METHODS

Glimepiride

The drug was purchased from (Delta Pharma for
Pharmaceuticals, Egypt). Oral dose: Oral administra-
tion of (10 microg/g body wt) daily in accordance
with[15].

Plant material

Mature whole Panax quinque folius plants were
collected and authenticated as Panax quinque folius
by Dr. H. Ezzat, Department of Pharmacognacy, Fac-
culty of pharmacy, Minia university.

Intraperitoneal dose: 100 mg/kg of body weight
per day in accordance with[10].

Extract preparation of Panax quinque folius
(American ginseng)

The plant roots were soaked in cold water for 2 h,
and then cut into small pieces less than 2mm in diam-
eter. These pieces were mixed with hot water about
95°C for 1 h, and then filtered. The filtrates were evap-
orated and lyophilized. The dried powders were sus-
pended in bath solution. The suspension was centri-
fuged for 10 min at 3000rpm and the supernatant was
used for the experiment.

Chemicals

Alloxan was purchased from Loba chemie Pvt. Ltd.
Mumbai, India. Total cholesterol (TC), serum high-den-
sity lipoprotein (HDL), serum Creatinine (SC), serum
urea (SU), alanine transaminase (ALT), serum aspar-
tate transaminase (AST) and triglyceride (TG) standard
kits were obtained from Erba diagnostics Mannheim
Gambh, Germany. Blood glucose level was measured
using Elegance glucose meter (CT-X10) of Conver-
gent Technologies, Germany. All reagents used in study
were analytical grade.

Animals

40 White male albino rats weighting about 190±10
g were used as experimental animals in the present in-
vestigation. The animals were housed in standard
polypropylene cages and maintained under controlled
room temperature (22±2 æC) and humidity (55±5%)
with 12 h light and 12 h dark cycle and were fed a
standard diet of known composition, and water ad libi-
tum. The chow was purchased from El-Gomhoria Com-
pany, Cairo, Egypt. They were housed for two weeks
for accommodation. Our work was carried out in ac-
cordance with the guidelines of El Minia University for
animal use. These animals were used for induction of
Diabetes mellitus.

Induction of diabetes

Rats were made diabetic by a single intraperitoneal
injection of alloxan monohydrate (Loba Chemie,
Bombay; 150 mg/kg i.p.) in sterile saline. Twelve days
after Alloxan injection, rats with blood glucose level of
>200 mg/dL were separated and used for the study.
Blood glucose levels were measured using blood glu-
cose test strips with elegance glucometer (Frankenberg,
Germany) at weekly intervals till the end of study (i.e. 3
weeks). Blood glucose estimation was done on 0, 1, 2
and 3 hours after administration of extract orally at the
30th day of starting experiment.
TABLE 1: Effect of AGE on optimal cutoff level of each of the four plasma glucose values of oral glucose tolerance test in alloxan induced diabetic rats (A-D)(n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting glucose level</th>
<th>1. h glucose level</th>
<th>2. h glucose level</th>
<th>3. h glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (N)</td>
<td>69.12±2.3</td>
<td>112.3±9.8</td>
<td>89.3±7.2</td>
<td>74.9±6.8</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>142±12.3 ***a</td>
<td>465±41.8 ***a</td>
<td>435±30.25 ***a</td>
<td>412±38.5 ***a</td>
</tr>
<tr>
<td>Diabetic +Glimepride(G)</td>
<td>176±16.2 ^a</td>
<td>484±40.28 ^a</td>
<td>401±29.8 ^b</td>
<td>314±27.1 ^c</td>
</tr>
<tr>
<td>Diabetic+ AGE (AGE)</td>
<td>109±9.3 ^c</td>
<td>405±39.65 ^c</td>
<td>381±32.0 ^c</td>
<td>374±25.36 ^b</td>
</tr>
</tbody>
</table>

* Values significantly different compared to normal P***<0.001; ^ Values are expressed as means ± SE. Means not sharing common letter are significantly different (p<0.05) based on one –way ANOVA with Tukey’s post –hoc test.

TABLE 2: Effect of AGE on serum insulin level in alloxan induced diabetic rats (A-D)(n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum insulin levels (ng/ml) After 30 days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (N)</td>
<td>1.18±0.07</td>
</tr>
<tr>
<td>Diabetic control (DC)</td>
<td>0.48±0.07****</td>
</tr>
<tr>
<td>Diabetic + Glimepride (+G)</td>
<td>0.62±0.04 ^a</td>
</tr>
<tr>
<td>Diabetic+ AGE (AGE)</td>
<td>0.78±0.06 ^b</td>
</tr>
</tbody>
</table>

* Values significantly different compared to normal P***<0.001; ^ Values are expressed as means ± SE. Means not sharing common letter are significantly different (p<0.05) based on one –way ANOVA with Tukey’s post –hoc test.

RESULTS

Antidiabetic activity

Single dose alloxan monohydrate (150 mg/kg) significantly (P<0.01) increases the blood glucose as shown in TABLE 1. After the daily intraperitoneal administration with AGE, for 30 days, significant decreased (P<0.01) in the blood glucose levels was observed in the diabetic rats (TABLE 1). The reduced insulin level in diabetic rats was also significantly improved by treatment of AGE. At the end of experiment (30th day) blood glucose level was (374±25.36) mg/dL of the groups treated with AGE (TABLE 2).

Effect on body weight of rats

In diabetic rats, continuous reduction in body weight was observed as shown in TABLE 2. AGE treatment significantly (P<0.05) improved the body weight of diabetic rats.

Effect on lipid profile

In diabetic rats, there was a significant increase of
TABLE 3: Effect of AGE on body weight, triglycerides (T.G) and total cholesterol (T.C) in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain</th>
<th>T.G</th>
<th>T.C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm</td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Normal (N)</td>
<td>37±3.21</td>
<td>74.2±6.8</td>
<td>87.2±8.4</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>-27±3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178.2±10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163.2±7.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Glimepride (G)</td>
<td>-21±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120.3±8.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.3±7.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+ AGE (AGE)</td>
<td>-23±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.3±7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108.2±8.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values significantly different compared to normal P<sup>***</sup>0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p<0.05) based on one way ANOVA with Tukey’s post–hoc test.

TABLE 4: Effect of AGE on HDL cholesterol and LDL cholesterol in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>HDL.C</th>
<th>LDL.C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Normal (N)</td>
<td>63.2±5.7</td>
<td>13.5±7.3</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>39.3±4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.2±4.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Glimepride (G)</td>
<td>45.2±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.1±3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+ AGE (AGE)</td>
<td>46.7±3.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.2±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values significantly different compared to normal P<sup>***</sup>0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p<0.05) based on one way ANOVA with Tukey’s post–hoc test.

**Effect on liver functions**

The effect of AGE on liver functions is represented in the TABLE 5. ALT, AST were significantly elevated in alloxan induced diabetes. The rats treated with AGE showed significant (P<0.01) reduction in the elevated levels of liver enzymes (transaminase) in a dose dependent manner as shown in TABLE 5.

**Histology of pancreas**

Histology of pancreas (Figure 1) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (1.a). In diabetic animals treated extensive damage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (1.b). On other hand, AGE treatment showing vacuolization of B cells of islets of langerhans (1.d).

**Histology of kidney**

Kidney function markers like urea and creatinine were elevated in the alloxan induced diabetic rats when compared with the normal rats. AGE reduced both the levels in dose dependent manner (TABLE 6).

**Effect on kidney functions**

Kidney function markers like urea and creatinine were elevated in the alloxan induced diabetic rats when compared with the normal rats. AGE reduced both the levels in dose dependent manner (TABLE 6).

**Histology of liver**

Photomicrographs of liver (Figure 2) showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein (2.a). In case of group II diabetic rats, the normal lobular structure was preserved. The central vein was prominent and prominently congested. Focal areas of hemorrhage were also seen. Fatty change was evident.

The portal tracts appeared normal (2.b). In group IV [diabetic rats + AGE mg/kg], showing kupffer cells activation (2.d).

**Histology of kidney**

Kidney function markers like urea and creatinine were elevated in the alloxan induced diabetic rats when compared with the normal rats. AGE reduced both the levels in dose dependent manner (TABLE 6).
TABLE 6: Effect of AGE on kidney parameters in normal and diabetic rats (n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (N)</td>
<td>25.41±2.14</td>
<td>0.65±0.07</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>66.14±2.14***a</td>
<td>1.31±0.01***a</td>
</tr>
<tr>
<td>Diabetic + Glimepride (G)</td>
<td>29.26±2.65 c</td>
<td>0.98±0.03 b</td>
</tr>
<tr>
<td>Diabetic + AGE (AGE)</td>
<td>41.21±2.33 b</td>
<td>1.09±0.04 b</td>
</tr>
</tbody>
</table>

* Values significantly different compared to normal P***<0.001; ** Values are expressed as means ± SE. Means not sharing common letter are significantly different (p<0.05) based on one-way ANOVA with Tukey’s post-hoc test.

Figure 1: Effect of AGE on pancreas of alloxan induced diabetic rats

Figure 2: Effect of AGE on liver of alloxan induced diabetic rats
Histology of kidney (Figure 3) in normal animals showed normal structure (3.a). In diabetic rats, mild thickening of the basement membrane of the arterioles of glomeruli along with mild change of density of mesangial mesangium were observed. No other significant changes were seen (3.b). After AGE 100 mg/kg treatment, these changes were improved towards normal condition (3.d).

**DISCUSSION**

Glucose is the key physiological regulator of insulin secretion; indeed, short-term exposure of β-cells to increasing glucose concentrations induces proliferation in a concentration-dependent manner. In addition to its effect on β-cell turnover, hyperglycemia also impairs β-cell secretory function. This glucotoxic effect is evident before apoptosis leads to a significant decrease in β-cell mass.

The present investigation indicated that a single dose of alloxan (150 mg/kg) intraperitoneally to adult male albino rats (210-220g) was suitable to induce histological changes of the islets of Langerhans characterized appearance, hypoinsulinemia and hyperglycemic state. The present dose as well as the observed histopathological and biochemical manifestations agree with the literature of

A gradual loss of β-cells due to apoptosis significantly hinders insulin production and inhibits cell viability. During apoptosis, cells shrink; chromatin condenses; DNA is cleaved into pieces at inter nucleosomal regions. A proactive way to increase β-cell viability is to decrease apoptosis level in order to retain the cell population and increase insulin production.

Oral administration of glimepiride causes significant decrease in levels of blood glucose in accordance with who revealed that glimepiride exerts its insulin-releasing effect mainly by inhibiting ATP-sensitive potassium channels. In the pancreatic β-cell this action induces depolarization of the cell membrane, allowing an influx of calcium in the cell. This in turn induces insulin release into the blood.

Pfützner et al revealed that the increase in the number of β-cells in the islets of Langerhan’s in glimepiride-treated diabetic rats in comparison to alloxan induced diabetic rats can be attributed to the fact that glimepiride affect the activation of the redox sensitive transcription factor NF (Kappa) β in alloxan induced diabetic rats. Although the mechanism of β-cell neoformation is not clear but there is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative /regenerative phase leading to nesideoblastosis (neogenesis of islets)

Antidiabetic effect of glimepiride was further examined by histological observations made on the pancreatic tissue of glimepride treated rats that show vacuolation and necrosis of some β cells of islets of Langer-
hans.

Intraperitoneal administration of AGE causes significant decrease in levels of blood glucose accordance with [26]. Also, intraperitoneal administration of AGE causes significant increase in level of serum insulin.

The antidiabetic effect of AGE may due to antioxidant activity of ginsenosides was involved in the anti-apoptosis activity as production of free radicals, such as NO and ROS was reduced by ginsenosides [14].

Antidiabetic effect of AGE was further evidenced by histological observations made on the pancreatic tissue of AGE treated rats that revealed few vacuolizations and slightly divided ß-cells in the islets of Langerhans.

The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL-cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/resistance. This results in excess fatty acid accumulation in the liver, which is converted to triglycerides. The impaired ability of insulin to inhibit free fatty-acid release leads to elevated hepatic VLDL-cholesterol production. The increased VLDL-cholesterol and triglyceride levels decrease the level of HDL-cholesterol and increase the concentration of small dense LDL-cholesterol particles by activation of lipoprotein lipase and lecithin acyl-cholesterol transferase [19].

In our study, elevated levels of serum TC, TG, LDL and VLDL-cholesterol and decreased HDL-cholesterol concentration in alloxan-induced diabetic rats are in accordance with [29].

On other hand, Induction of diabetes by alloxan resulted in loss of body weight in the diabetic control rats in accordance with that is directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis [3].

DM induces the growth of HSCs via MAP kinase pathways, which are activated by ROS produced by the NADPH oxidase system under the regulation of MAP kinase C. On other hand, hepatic oxidative stress induces proinflammatory cytokines, such as TNF-α, transforming growth factor-β (TGF-β), interleukin-1β, and interleukin-6, which are critical for HSC activation and perpetuation [25].

Intraperitoneal administration of AGE causes significant decrease in level of AST and ALT in accordance with [12] who attributed the antioxidant effect of ginsenosides in AGE. Hepatic protective effect AGE of was further evidenced by histological observations made on the hepatic tissue of AGE treated rats that revealed levels directly via increased lipoprotein lipase and decreased hepatic lipase activity. On other hand, the antilipidemic action of glimepiride may reside in their ability to stimulate insulin secretion and action.

Intraperitoneal administration of AGE causes significant decrease in the serum levels of triglycerides, total cholesterol and LDL-cholesterol in contrast to significant elevation in HDL-cholesterol and body weight in accordance with [2].

Yeo et al [30] revealed that AGE improves the lipid profile by regulation of the expression of genes associated with abnormal physiology associated DM, insulin, and adiponectin, which carry out critical functions in energy and lipid metabolism.

DM causes a disturbance in the uptake of glucose, as well as glucose metabolism. The liver plays an important role in the maintenance of blood glucose levels by regulating its metabolism.

The present investigation indicated that, a single dose of alloxan (150 mg/kg) intraperitoneally to adult male albino rats (210-220g) was suitable to induce histological changes in the liver of alloxan induced diabetic rats with characterized appearance, enlarged and swollen hepatocytes.

Alloxan causes significant increase in activity of sAST and sALT, in accordance with that is directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis [3].

In intraperitoneal administration of AGE causes significant decrease in level of AST and ALT in accordance with [12] who attributed the antioxidant effect of ginsenosides in AGE. Hepatic protective effect AGE of was further evidenced by histological observations made on the hepatic tissue of AGE treated rats that revealed
no histological changes.

Renal dysfunction is a slowly progressive process that is postulated to be accelerated by intervening diseases, such as diabetes, due in part to the addition of excessive stress and inflammation[16].

Alloxan led to a significant increase in serum urea and serum creatinine. A similar effect was recorded by[4], [23] revealed that enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea and creatinine. Furthermore, Alloxan increased the productions of reactive oxygen species, enhanced lipid peroxidation and protein carbonylation in association with decreased intracellular antioxidant defense in the kidney tissue.

Cao et al[5] suggests that development of diabetic renal dysfunction may due to activation of endoplasmic reticulum stress that can mediate progressive endothelial damage through growth and migration of vascular smooth muscle and inflammatory cells, alteration of extracellular matrix, apoptosis of endothelial cells, overexpression of inflammatory cytokines.

Oral administration of glimepiride causes significant decrease in level of serum urea and creatinine in accordance with[22].

In the present study the improvement in blood urea, serum creatinine and subsequent amelioration of histomorphological changes in kidneys of glimepiride treated rats can be attributed to the recovery of renal function which is explained by the regenerative capability of the renal tubules as good metabolic control is beneficial in slowing the progression of renal dysfunction in diabetes[17].

Glimepiride could ameliorate the glomerular and tubular lesions that characterize diabetic renal dysfunction and subsequently recover renal morphology and function. Renoprotective effect glimepiride of was further evidenced by histological observations made on the renal tissue of glimepiride treated rats that revealed normal structure of renal parenchyma.

Intraperitoneal administration of AGE causes significant decrease in level of serum urea and creatinine in accordance with[10].

Fu and Ji[71] indicates that ginseng components could prevent diabetic renal damage by attenuating the oxidative stress, inhibiting advanced glycation end product formation and of MAPK-Akt signaling cascade. Renoprotective effect of was further evidenced by histological observations made on the renal tissue of AGE treated rats that revealed no histological changes.

CONCLUSION

Although additional studies are needed, it could be suggested that AGE have Antidiabetic, Antihyperlipidemic mechanisms against Pancreas, liver and kidney injury induced by alloxan. The signaling mechanisms associated with protection against the liver damage and oxidative stress status induced by alloxan via intake AGE still need merit further investigations.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

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