Biochemical effects of *Cleome droserifolia* on hepatic dysfunction in alloxan-induced *Diabetes mellitus* in male albino rats

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ABSTRACT

**Background:** *Cleome droserifolia* is considered to have protective effects against several diseases. The hepatic dysfunction associated with *Diabetes mellitus* (DM) has been reported and was found to be associated with oxidative damage. This study was conducted to evaluate the role of *Cleome droserifolia* to protect against alloxan -induced liver dysfunction in rats.

**Method:** Alloxan was administered i.p. in a single dose (150 mg/kg) to adult male rats. Alloxan-induced diabetic rats were orally administered Methanol water extract of *Cleome droserifolia* (MWCD) (0.31 g/kg body weight) daily in accordance to (Nicola et al., 1996) of rats daily for 30 days after alloxan injection. **Result:** Alloxan administration to rats resulted in significant elevation of serum transaminases (sALT and sAST), depletion of hepatic reduced glutathione (GSH), catalase (CAT) and glutathione peroxidase (GPx), elevation of lipid peroxides (LPO) expressed as malondialdehyde (MDA). Significant rises in liver tumor necrosis factor-alpha (TNF-α) and caspase-3 levels were noticed in alloxan-induced diabetic. Treatment of the alloxan-induced diabetic rats with MWCD significantly prevented the elevations of sALT and sAST, inhibited depletion of hepatic GSH, GPx, CAT and inhibited MDA accumulation. Furthermore, MWCD had normalized serum total proteins and hepatic CAT, TNF- α and caspase-3 levels of alloxan-induced diabetic rats In addition, MWCD prevented the alloxan -induced apoptosis and liver injury as indicated by the liver histopathological analysis. Results showed significant correlation in either alloxan MWCD group between TNF-α and each of serum ALT, AST and liver GPX, CAT, GSH, MDA and caspase-3 levels. **Conclusion:** our data indicate that MWCD protects against alloxan -induced liver injury in rats through antioxidant, anti-inflammatory and antiapoptotic mechanisms. However, further merit investigations are needed to verify these results and to assess the efficacy of MWCD therapy to counteract the liver dysfunction and oxidative stress status.  © 2015 Trade Science Inc. - INDIA
INTRODUCTION

Although, the spread of folk traditional medicine, recent pharmaceutical research is also focusing on marine organisms that have developed biologically unique molecules as sulphated poly saccharides for their biological activity\[^1\] As a consequence of the research efforts, it is clear that the marine environment represents an important source of unknown natural compounds whose medicinal potential must be evaluated. Recent studies in the field of diabetic research have revealed promising compounds, isolated from natural sources, with proven antidiabetic activity\[^17\].

_Cleome droserifolia_ (Forssk.) Del., is a plant of the Cleomaceae family. Its leaf decoction is widely used by the Bedouins of the southern Sinai for the treatment of diabetes, Skin diseases and open wounds\[^3\]. Alloxan, a \(\beta\)-cytotoxin, has demonstrated severe physiological and biochemical derangements of the diabetic state. The alloxan rats exhibited severe glucose intolerance and metabolic stress as well as hyperglycaemia due to a progressive oxidative insult interrelated with a decrease in endogenous insulin secretion and release\[^4\].

When alloxan monohydrate is injected into various laboratory animals, destruction of insulin-secreting \(\beta\) cells in the islets of Langerhans occurs, while other cells (\(\alpha, g, d\)) are resistant to alloxan. Disappearance of \(\beta\) cells within a few days is accompanied by typical and permanent hypoinsulinaemia and hyperglycaemia. Alloxan-treated animals were considered as excellent tools to study the pathogenesis of human diabetes, although in alloxan diabetes, in contrast to T1D in humans, there is no autoimmune component and no insulin resistance as in T2D. Thus alloxan diabetes can be regarded as a pure form of hypoinsulinaemia\[^24\].

Alloxan is toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the Glucose transporter -2 (GLUT2). In the presence of intracellular thiols, especially glutathione; alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid\[^1\]. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity\[^23\].

In this study, we aimed to evaluate the role of _Cleome droserifolia_ intake to alloxan-intoxicated rats via monitoring the liver histopathological changes and the go insight the changes of different biochemical parameters such as serum alanine transaminase (sALT), aspartate transaminase (sAST) and endogenous hepatic antioxidants e.g. reduced glutathione (GSH), and catalase (CAT) enzyme levels; lipid peroxides expressed as malondialdehyde (MDA). Moreover, the hepatic tissue damage marker; tumor necrosis factor (TNF-\(\alpha\)) and an apoptotic marker; caspase-3 were measured.

MATERIALS AND METHODS

Animal license

Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Egypt.

Animals and experimental design

130–190 g were used as experimental animals in this study. total of 30 adult male Swiss albino rats, weighing The animals were kept in wire-floored cages under standard laboratory conditions of 12 h/12 h light/dark, 25±2 °C with free access to food and water. The rats were randomly divided into three groups of ten animals, each as follows:

- **Group 1**: NC rats: normal control untreated rats received orally an equivalent volume of normal saline based on body weight.
- **Group 2**: (DC) Alloxan-induced diabetic rats: rats were treated with single dose of alloxan i.p. (150 mg kg-1) dissolved in normal saline.
- **Group 3**: (MWCD) _Cleome droserifolia_ rats: rats were orally administered MWCD (0.31 g/kg body weight day-1) for 30 days.

The selection of MWCD doses used in this study, was based on the work conducted by other investigators\[^22\]. After the last treatment, rats were fasted for 8 h. Animals were subjected to light ether anesthesia and sacrificed by cervical dislocation. The blood sample were collected and centrifuged to obtain serum in or-
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Estimation of serum hepatic enzymes (sAST and sALT)

To assess the liver function, the serum activity (U/l) of sAST and sALT were analyzed. The sAST was determined spectrophotometrically at 340 nm in presence of α-ketoglutarate, aspartate, NADH and malate dehydrogenase. The sALT was assayed in presence of α-ketoglutarate, pyruvate, NADH and lactate dehydrogenase at 340 nm.[34]

Determination of MDA in liver homogenate

The lipid peroxidation level in rat liver homogenate was measured as MDA which is the end product of lipid peroxidation that reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 532 nm.[19]. Phosphoric acid 1% (3 ml) and TBA 0.6% (1 ml) was added to 0.5 ml of liver homogenate in a centrifuge tube and the mixture was heated for 45 min in boiling water bath. After cooling, 4ml of n-butanol was added to the mixture and vortexed for 1 min followed by centrifugation at 20,000 rpm for 20 min. Organic layer was transferred to fresh tube and its absorbance was measured at 532 nm.[9].

Determination of GPx and CAT activities in liver homogenate

Determination of GPx activity in rat liver homogenate is based on the oxidation of GSH by GPx, using t-butyl hydroperoxide as a substrate, coupled to the disappearance of NADPH by glutathione reductase.[12]. The results expressed as mU/mg protein. The CAT activity was measured using H2O2 as substrate that can be decomposed by CAT enzyme. A mixture of 50mM phosphate buffer (pH 7.0), 20mMH2O2 and 0.1 ml liver homogenate in a final volume of 3ml was incubated at room temperature for 2 min. The change in absorbance at 240 nm in 2 min was calculated. One unit of CAT is defined as the amount needed to decompose 1 nmol H2O2 of per minute and the specific activity is expressed as μ moles H2O2 decomposed/min/mg protein.[7].

Estimation of GSH in liver homogenate

To estimate the GSH liver homogenate content, liver homogenate (0.5 ml) was mixed with 0.5 ml of 10% trichloroacetic acid. The contents were mixed well for complete precipitation of proteins and centrifuged at 2000 rpm for 5 min. An aliquot of clear supernatant (0.1 ml) was mixed with 1.7 ml of 0.1M potassium phosphate buffer (pH 8). A 0.1 ml of DTNB was added. After 5 min, the absorbance was measured at 412 nm against blank.[8]. The GSH value was expressed as mg/gm tissue.

Determination of TNF-α in liver homogenate

The determination of TNF-α in rat liver homogenate involved solid phase sandwich ELIZA using two kinds of high specific antibodies. Tetra methyl benzidine was used as chromagen. The strength of color measured at 450 nm is proportional to the quantities of rat TNF-α that expressed as pg/gm liver.

Estimation of caspase-3 level in liver homogenate

The caspase-3 colorimetric assay in liver rat homogenate (U/mg protein) based on the spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA (acetyl-Asp-Glu-Val-Asp p-nitroanilide). The pNA can be quantified using spectrophotometer at 405 nm.

Liver histological examination

The liver tissues were removed, plotted with normal saline between filter paper and fixed in 10% neutral buffered formalin and subsequently embedded in paraffin and sliced into slices of 5 m thickness followed by staining with hematoxylin and eosin and examined under light microscope (Olympus BX-200, Tokyo, Japan).

Statistical analysis

Statistical analysis was carried out using GraphPad Instat software (version 3, ISS-Rome, Italy). Unless differently specified, groups of data were compared...
with un-paired t-test and one-way analysis of variance (ANOVA) followed by Tukey-kramer (TK) multiple comparisons post-test. Values of P<0.05 were regarded as significant. The data, as clearly indicated are reported in tables and figures as mean ± standard error (S.E).

RESULTS

Alloxan intake to normal rats showed significant elevations of sALT and sAST levels compared to the normal control rats, p < 0.001. The oral administration of MWCD to diabetic rats at a dose of 400 mg/kg body weight of rats for 30 days blocked the Alloxan-induced elevations of sALT and sAST as noticed by significant decrease compared to diabetic rats, p < 0.001. (TABLE 1).

TABLE 1 : Effect of MWCD on sALT and sAST in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>sALT U/l</th>
<th>sAST U/l</th>
</tr>
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<tbody>
<tr>
<td>Normal (N)</td>
<td>19.51±0.84</td>
<td>23.17±0.75</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>62.39±0.87***</td>
<td>84.29±0.94***</td>
</tr>
<tr>
<td>Diabetic+ MWCD (DC + MWCD)</td>
<td>44.27±0.38b</td>
<td>47.18±0.54b</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

HWHC exhibited significant increase in liver CAT level compared to the control rats, p < 0.001 (TABLE 3). Alloxan significantly decreased hepatic diabeteic rats produced significant increase in liver CAT level compared to diaibetic rats, p < 0.001 (TABLE 3).

On the other hand, alloxan significantly decreased the liver GPx level compared to control rats, p < 0.001 (TABLE 3). MWCD intake to alloxan-induced diabetic rats produced significant increase in GPx level compared to alloxan-intoxicated rats, p < 0.001 (TABLE 3).

Caspase-3-activity was significantly increased upon administration of alloxan to normal rats as compared to diabetic rats (Figure 4B). This increase was significantly declined upon HWHC intake to diabetic compared to alloxan–induced diabetic rats, p < 0.001 (TABLE 4).

Additionally, alloxan significantly increased the hepatic level of TNF-α compared to control rats (TABLE 4). Administration HWHC to alloxan -treated rats decreased the hepatic TNF-α level significantly compared to the diabetic rats, p < 0.001 (TABLE 4).

To confirm the protective effect of MWCD on alloxan-induced liver tissue damage, we performed histological examinations. The normal control rat liver showed normal architecture of hepatic lobules and hepatocytes. The hepatocytes form columns of cells adherent to each other by one or more surfaces. The sinusoids were variable in diameter and lined with discontinuous sheet of endothelial cells with flat nuclei (Figure 1).

However, Alloxan-treated rat liver elicited decrease in the number of hepatocytes and widely dilated central veins. The cytoplasm showed area of hemorrhage and inflammatory cell infiltration around the blood sinusoids which appeared widely dilated (Figure 2).

Alloxan –treated rats administered MWCD revealed degeneration of some hepatocytes and normal architecture of the others. The blood sinusoids are still

TABLE 2 : Effect of MWCD on level of hepatic MDA and hepatic GSH in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic TBARS mg/gm tissue</th>
<th>Hepatic GSH mg/gm tissue</th>
</tr>
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<tbody>
<tr>
<td>Normal (N)</td>
<td>46.39±5.2</td>
<td>16.23±1.23</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>1248±133***</td>
<td>10.23±0.69***</td>
</tr>
<tr>
<td>Diabetic+ MWCD (DC + MWCD)</td>
<td>590.39±33.4b</td>
<td>19.21±2.02b</td>
</tr>
</tbody>
</table>

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Architecture (Figure 3).

DISCUSSION

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[25].

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.

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 protein 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[33].

The enlarged hepatocytes showed intense cytoplasmic staining with Periodic Acid-Schiff stain, and negative staining with Periodic Acid-Schiff Diastase. This is suggestive of glycogen accumulation and consistent with glycogenic hepatopathy. The present dose as well as

Table 3: Effect of treatments on activity of hepatic CAT, hepatic GSH-Px and hepatic SOD in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic CAT</th>
<th>Hepatic GSH-Px</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ moles H2O2 decomposed/min/mg protein</td>
<td>μg GSH consumed/min/mg protein</td>
</tr>
<tr>
<td>Normal (N)</td>
<td>234.25±9.58</td>
<td>681.23±26.36</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>174.36±11.89***</td>
<td>453.69±33.26***</td>
</tr>
<tr>
<td>Diabetic+ MWCD (DC + MWCD)</td>
<td>220.89±14.69b</td>
<td>608.21±30.21b</td>
</tr>
</tbody>
</table>

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Table 4: Effect of MWCD on activity of hepatic caspase-3 and level of hepatic TNF-α in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic caspase-3</th>
<th>Hepatic TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(U/mg protein)</td>
<td>(pg/gm liver)</td>
</tr>
<tr>
<td>Normal (N)</td>
<td>0.6±0.06</td>
<td>86±7.5</td>
</tr>
<tr>
<td>Diabetic control (DC)</td>
<td>1.8±0.05***&lt;a&gt;</td>
<td>498±40.25***&lt;a&gt;</td>
</tr>
<tr>
<td>Diabetic+ MWCD (DC + MWCD)</td>
<td>1.02±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235±21.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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Widely dilated and appeared congested and showed inflammatory cell infiltration. The diabetic rats administered MWCD showed marked regeneration of the hepatocytes with preservation of the normal hepatic architecture (Figure 3).
the observed histopathological and biochemical manifestations agree with the literature of[34].

Alloxan causes significant increase in activity of sAST, sALT, hepatic caspase-3 and levels of hepatic MDA and hepatic TNF-α. On other hand, Alloxan causes significant decrease in hepatic GSH content and activity of hepatic CAT, and GPx in accordance with[13].

In alloxan induced diabetic rats, the changes in the levels of sAST, sALT, are 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[4].

Francés et al.[10] revealed that DM promoted a significant increase in hydroxyl radical production which correlated with lipid peroxidation (LPO) levels. Besides, hyperglycemia significantly increased mitochondrial BAX protein expression, cytosolic cytochrome c levels, and caspase-3 activity leading to an increase in apoptotic index

Hamden et al.[14] revealed that liver is bombarded by ROS that can directly cause inflammation within the liver cells, which then release further pro-inflammatory cytokines, leading to more hepatocyte injury and accelerated apoptosis that affect the integrity and architecture of liver cells[21].

As consequence of DM, the hepatocellular accumulation of triglycerides, initially leads to an altered metabolism of glucose and free fatty acids in the liver. Increased expression of death receptors in response to this altered hepatic metabolism enhances the hepatocytes’ susceptibility for pro-apoptotic stimuli, thus eliciting excessive hepatocyte apoptosis and inflammation[35].

Ingaramo et al.[21] explained that DM enhances TNF-α in the liver, which may be a fundamental key leading to apoptotic cell death, through activation of caspase-3, NFkB led to an induction of iNOS and consequent increase in NO production.

Oral administration of MWCD causes significant decrease in level of hepatic TNF-α and activity of hepatic caspase-3 in accordance with[6] who explained that flavonoids, major active constituent in MWCD possess common effects, namely, induction of apoptosis involving the release of cytochrome c from mitochondria, activation of caspases and down-regulation or up-regulation of Bcl-2 family members, but also induction of cell cycle arrest and inhibition of survival/proliferation signals.

Hepatic protective effect MWCD of was further evidenced by histological observations made on the hepatic tissue of MWCD treated rats that showing kupffer cells activation.

In conclusion, although additional studies are needed, it could be suggested that Cleome droserifolia could partly protect hepatocytes through antioxidative, anti-inflammatory and antiapoptotic mechanisms against liver injury induced by alloxan. The signaling mechanisms associated with protection against the liver damage and oxidative stress status induced by alloxan via intake of MWCD still need merit further investigations.

In conclusion, although additional studies are needed, it could be suggested that Cleome droserifolia could partly protect hepatocytes through antioxidative, anti-inflammatory and antiapoptotic mechanisms against liver injury induced by alloxan. The signaling mechanisms associated with protection against the liver damage and oxidative stress status induced by alloxan via intake of Cleome droserifolia still need merit further investigations.

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