The ameliorative effect of aqueous extract of bay leaves on kidney and liver in alloxan diabetic albino rats

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Abstract

Alloxan is diabetogenic agent has a distinct pathological effects interfering with the physiological functions of the pancreatic beta cells. Laurel (Laurus nobilis), bay leaves, is being used either as spices due to its flavoring capacity and aroma or in the traditional medicine due to its pharmaceutical activities. The present study aimed to evaluate the antidiabetic and anti-inflammatory activities of Laurus nobilis on liver and kidney of alloxan diabetic albino rats. Twenty four adult male albino rats (Rattus norvegicus) were equally divided into four groups. Group 1: served as control (kept without any treatment). Group 2: animals in this group were orally given the aqueous extract of bay leaves (200mg/kg body weight/day) for 4 weeks. Twelve rats were injected with a single i.p (150mg/kg body weight) of alloxan to induce diabetes. Then, the diabetic animals were randomly divided into three groups, six animals in each one. Group 3: diabetic animals in this group left without any other treatment till the end of the experiment. Group 4: the diabetic animals were given with the aqueous extract of bay leaves (200mg/kg body weight/day) orally for 4 weeks. The results showed that there is no difference in the histological, immunohistochencial, and biochemical results between the control group and animals administered the aqueous extract of bay leaves. When animals treated with alloxan, a highly significant decrease in their body weights was recorded. The renal cortex of alloxan diabetic animals showed more apparent pathological changes included glomerular atrophy or inflammation (glomerulitis), winding capsular space and degenerated tubular epithelial cells. Moreover, the liver of the diabetic animals revealed obvious pathological changes include degenerated hepatocytes with vacuolated cytoplasm and pyknotic nuclei in addition to marked leucocytic infiltration and congested blood vessels. Concerning the biochemical results a highly significant increase in the levels of blood glucose, urea and creatinine and the activities of liver enzymes (AST and ALT) was recorded. On the contrary, a highly significant decrease in catalase activity and increase malondialdehyde (MDA) level were recorded. Immunohistochemical results showed negative caspase-3 expression nearly in all the hepatocytes and renal tubular cells in animals in control and bay leaves groups. While PCAN expression appeared positive in few of hepatocytes and renal tubular cells in the same groups. On the other hand, diabetic animals (group 3) showed a marked decrease in PCNA expressions and an increase in caspase-3 expression. When diabetic animals were given the aqueous extract of bay leaves, an obvious degree of improvement was observed where most of hepatocytes and renal tubular epithelial appeared normal. In addition, all the previously determined biochemical parameters were restored to normal state. Concerning the immunohistochemical observations in these groups, both PCNA and caspase-3 expressions were appeared in few number of hepatocytes and renal tubular cells. These findings suggested that the aqueous extract of bay leaves has antidiabetic, antioxidant and hepatorenal protective potentials on diabetic rats.

Keywords. alloxan, bay leaves aqueous extract, kidney, liver, histopathological, immunohistochemical, biochemical
Introduction

*Diabetes mellitus* is a chronic metabolic disorder characterized by hyperglycemia due to over production of glucose [1] or an unlimited lack of insulin secretion or by both [2]. It is multifactorial disease characterized by hypercholesterolemia, hypertriglyceridemia and lipoprotein abnormalities [3], raised basal metabolic rate [4], defect in reactive oxygen species scavenging enzymes [5] and high oxidative stress induced damage to pancreatic beta cells [6]. Diabetes for long time resulted in many complications include, cardiovascular, nephropathy, neuropathy, retinopathy and foot ulcer [7].

Many studies were performed on the natural plants which have antidiabetic properties with almost no side effects, low cost and easy availability. These plants treated diabetes successfully due to their various phytochemicals such as polyphenol, catechins, saponins and flavonoids [8]. Among these plants, *Laurus nobilis* (bay) which has many pharmaceutical activities include antidiabetic effect [9], antioxidant and wound-healing effect [10], antimicrobial and antirheumatism effects [11] and hepatoprotective effect [12].

Many chemicals can induce diabetes in experimental animals such as alloxan and streptozotocin [13], dithizone [14] and glucocorticoids and corticosteroid [15]. Alloxan is an oxygenated pyrimidine derivative considered as toxic glucose analogue found as alloxan hydrate in aqueous solutions [13]. It is used to induce diabetes in different experimental animals as rats, rabbits, mice and doges with different grades of disease severity by varying the dose of alloxan used [16].

The purpose of this study is to evaluate the ability of the aqueous extract of bay leaves to prevent or/and alleviate the effect of diabetes on kidney and liver in adult male albino rats through histopathological, biochemical and immunohistochemical studies.

Material and Methods

**Alloxan**

Alloxan (2,4,5,6-tetra oxy pyrimidine; 2,5,6-pyrimidinetetronate), as reddish powder, was purchased from Eldawlia Company, Tanta City, Egypt. About 2g of alloxan were dissolved in 20ml distilled water and were given intraperitoneally to rats by using diabetic syringe as a single dose (150mg/kg body weight) [17].

**Induction of diabetes**

At the beginning of the experiment the basal glucose level was determined after fasting all animals (twenty four rats) overnight to be sure that these animals not diabetic. Then twelve rats of these animals were intraperitoneally injected with a single dose of alloxan (150mg/kg body weight). Alloxan chemically established the diabetic condition 72 hours after the treatment. In order to assess this effect; a bit of rat’s tails were cut for a drop of the blood on a specified space of the glucometer. The animals with plasma glucose level above 180mg/dl were considered as diabetic [18].

**Plant material and extraction**

*Laurus nobilis* is traded as bay leaves or laurel. Bay leaves were purchased from local market in Dubai, Emarat. They were cleaned with water then air dried. The aqueous extract of bay leaves was prepared according to Elmastas et al. [19] where 20g of bay leaves were crushed and were soaked in 400ml of boiling water using magnetic stirrer for fifteen minutes. Then, this solution was filtered over a Whatman No. 1 paper. The filtrate was frozen and lyophilized in a lyophiler (Lab Conco, Freezone 1) at 5μm Hg pressure at -50°C. The extract was stored at 4°C until used and was given orally by gastric intubation to animals at a dose level of 200mg/kg body weight/day, for four weeks.

**Animals**

Twenty four adult male albino rats (*Rattus norvegicus*) weighing 120-140g were used. Animals were obtained from experimental rat house localized in Helwan, Egypt. They were housed in a standard rat cages and were acclimatized at controlled temperature (24 ± 2°C) 12 hour dark/light cycle. Animals were fed a standard commercial pellets diet and water was allowed *ad libitum*. All the experiments were done in compliance with the guide for care and the use of laboratory animals approved by Faculty of Science, Menoufia University, Egypt (Approval No. MUF5/F/HI/2/17).

**Experimental design**

Twenty four rats were equally divided into four groups, Group 1: served as control group (kept without any treatment). Group 2: animals in this group were orally given the aqueous extract of bay leaves (200mg/kg body weight/day) for 4 weeks. Then, the diabetic animals were divided randomly into three groups, six animals/group. Group 3: the diabetic animals of this group were left without any other treatment till the end of the experiment. Group 4: the diabetic animals were orally administered the aqueous extract of bay leaves (200mg/kg body weight/day) for 4 weeks.

**Histological preparation**

At the end of the experiment (4 weeks) all animals were fasten for 12 hours, but had access to water, then they were sacrificed by cervical decapitation and were dissected; the two kidneys and parts of the liver were removed, washed in saline and were fixed in 10% neutral formalin. The fixed tissues were dehydrated in ascending series of alcohol, cleared in two changes of xylene and were embedded in molten paraffin wax. Then 5 μm thickness sections were cut by rotary microtome, mounted on cleaned slides and stained with haematoxylin and eosin.

**Biochemical analysis**

At the end of the experiment, blood samples were withdrawn from the heart in plain tubes, left to clot and were centrifuged at 3000rpm for 10 minutes to collect sera then stored at −20°C. Sera were used to determine the levels of glucose according to Trinder [20]. Renal serum markers, blood urea and creatinine, were measured using commercially available kits. The activities of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as were determined according to Gella et al. [21]. Oxidative stress was detected by measuring malondialdehyde (MDA) level according to Buege.
and catalase (CAT) activity was determined according to Sinha [23].

**Immunohistochemical studies:**
Sections of kidneys and liver were observed immunohistochemically for visualizing proliferation cell nuclear antigen (PCNA) and cysteine-aspartic proteases (caspase-3) using suitable antibodies of anti-PCNA and anti-caspase stains [24].

**Statistical analysis**
The present data were expressed as mean ± standard deviation (Mean ± SD). Statistical analysis was performed by one way analysis of variance (ANOVA) test. Intergroup differences were considered to be highly significant at $P \leq 0.001$ and significant at $P \leq 0.05$.

**Results**

**Effect of the different treatments on body weight**
There was non-significant change between the initial and final body weight of control rats and animals treated with the aqueous extract of bay leaves. However, the diabetic animals (alloxan group) recorded a highly significant decrease ($P \leq 0.001$) in the final body weight when compared to the control group. When the diabetic animals treated with the aqueous extract of bay leaves, a highly significant increase ($P \leq 0.001$) in the final body weight was recorded comparing with alloxan group.

Table 1: The changes in the body weight of rats in the different experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g) (Mean ± SD)</th>
<th>Final body weight (g) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125.80±3.12</td>
<td>134.80±2.98</td>
</tr>
<tr>
<td>Aqueous extract of bay leaves</td>
<td>123.20±4.39</td>
<td>133.60±4.34</td>
</tr>
<tr>
<td>Alloxan</td>
<td>121.20±4.81</td>
<td>79.20±17.62***</td>
</tr>
<tr>
<td>Alloxan+Aqueous extract of bay leaves</td>
<td>120.20±3.33</td>
<td>103.60±2.73*</td>
</tr>
</tbody>
</table>

$n=6$ animals in each group

(***): highly significant at $P \leq 0.001$ comparing with the control group.

(**): significant at $P \leq 0.05$ comparing with the control group.

(a): highly significant at $P \leq 0.001$ comparing with alloxan group.

**The Kidney**

**Histological observations**
Histological examination of kidneys obtained from the control rats revealed normal structure of the renal cortex. It consists of the renal corpuscles and heterogeneous population of renal tubular segments, the proximal and distal convoluted tubules. The renal corpuscle consists of Bowman's capsule (closing a narrow capsular space) with a double walled epithelial and the glomerulus which consists of a tuft of capillaries of efferent and afferent arterioles. The proximal and distal convoluted tubules are lined by simple cuboidal epithelial cells containing round nuclei. The lumen of the distal convoluted tubules is wider than that of the proximal ones (Fig. 1).

**Immunohistochemical observations:**

**The expression of proliferation cell nuclear antigen (PCNA) in the renal cortex**
Immunostaining reaction of PCNA was confined to the nuclei. In kidney of control rats, a positive PCNA expression appeared as brownish color in a few number of nuclei of the epithelial cells lining the urinary tubules (Fig. 8). In rats treated with the aqueous extract of bay leaves a considerable number of positive PCNA staining tubular cells appeared (Fig. 9). Renal cortex of alloxan diabetic animals showed an obvious decrease of PCNA expression appeared (as faint brownish color) in the nuclei of few tubular cells (Fig. 10). On the other hand, normal PCAN expression was observed in the tubular epithelial of the diabetic animals which given the aqueous extract of bay leaves (Fig. 11).

**The expression of caspase-3 in the renal cortex**
Examination of renal cortex of the control animals as well as animals treated with the aqueous extract of pay leaves showed weak caspase-3 expression appeared as brownish color in the cytoplasm of few of the tubular epithelial cells (Figs. 12 & 13). On the other hand, animals treated with alloxan showed a strong caspase-3 expression in the cytoplasm of large number of tubular cells (Fig.14). While animals treated with the aqueous extract of bay leaves showed an obvious decrease in the expression of caspase-3 (Fig. 15).
Fig. 1: A photomicrograph of the renal cortex obtained of a control animal showing Bowman's capsule (thin arrow) glomerulus (G), capsular space (thick arrow), proximal convoluted tubules (P) and distal convoluted tubules (D).

Fig. 2: A photomicrograph of the renal cortex of an animal treated with the aqueous extract of bay leaves showing normal glomerulus (G), capsular space (arrow), proximal convoluted tubules (P) and distal convoluted tubules (D).

Fig. 3: A photomicrograph of the renal cortex of an animal treated with alloxan showing glomeruli (G) infiltrated with inflammatory cells, widen capsular space (arrow), proximal (P) and distal (D) convoluted tubules.

Fig. 4: A photomicrograph of the renal cortex of an animal treated with alloxan showing atrophied glomeruli (G), proximal (P) and distal (D) convoluted tubules with widen lumen and tubular epithelial with vacuolated cytoplasm (thin arrow) and pyknotic nuclei (thick arrow).

Fig. 5: A photomicrograph of the renal cortex of an animal treated with alloxan showing congested renal blood vessel (RV) and degenerated renal tubules contain proteinaceous casts within their lumens (arrows).

Fig. 6: A photomicrograph of the renal cortex of an animal treated with alloxan showing intratubular hemorrhage (arrow) and congested renal vessel (RV).

Fig. 7: A photomicrograph of the renal cortex of a diabetic animal treated with the aqueous extract of bay leaves showing nearly normal glomeruli (G), capsular space (arrow), proximal (P) and distal (D) convoluted tubules.
Fig. 8: A photomicrograph of the renal cortex of a control animal showing positive PCNA expression as brownish color in few nuclei of tubular cells (arrows).

Fig. 9: A photomicrograph of the renal cortex of an animal treated with the aqueous extract of bay leaves showing positive PCNA expression in a few nuclei of tubular epithelial (arrows).

Fig. 10: A photomicrograph of the renal cortex of an animal treated with alloxan showing negative PCNA expression in the nuclei of most tubular cells (arrows).

Fig. 11: A photomicrograph of the renal cortex of a diabetic animal treated with the aqueous extract of bay leaves showing positive PCNA expression (arrows) in few nuclei of the tubular epithelial.

Fig. 12: A photomicrograph of the renal cortex of a control animal showing positive caspase-3 expression as brownish color in cytoplasm of few tubular cells (arrows).

Fig. 13: A photomicrograph of the renal cortex of an animal treated with the aqueous extract of bay leaves showing positive caspase-3 expression in cytoplasm of few tubular cells (arrows).

Fig. 14: A photomicrograph of the renal cortex of a diabetic animal showing a positive caspase-3 expression in the cytoplasm of most tubular cells (arrows).

Fig. 15: A photomicrograph of renal cortex of a diabetic animal treated with the aqueous extract of bay leaves showing positive caspase-3 expression in the cytoplasm of few tubular cells (arrows).
The liver

Histological observations

Examination of the liver sections of control animals showed normal liver structure. The liver consists of numerous polygonal hepatic lobules which are made up of radiating plates, cords or strands of hepatocytes forming a network around central vein. The hepatocytes (cord-like arranged) characterized by acidophilic cytoplasm, round central basophilic nuclei with prominent nucleoli. The sinusoids are narrow blood space with irregular boundaries contained large irregularly shaped cells of the mono nuclear type named Kupffer cells which known to be active phagocytic cells (Fig. 16). The portal area, periphery of the hepatic lobules contained a branch of a portal vein, a hepatic artery and bile ductile which bound together by connective tissue (Fig. 17). Liver sections of animals treated with the aqueous extract of bay leaves for 4 weeks showed nearly normal structure of the hepatic tissue as in control group (Fig. 18).

Microscopic examination of liver section of the animals treated with alloxan showed impairment of the normal structural organization of the hepatic lobules and disrupt in the characteristic cord-like arrangement of the normal liver cells which led to disappearance of the sinusoids. Hepatocytes appeared severely degenerated where a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization and pyknotic nuclei. Moreover, congestion of the intrahepatic blood vessels and inflammatory leukocytic infiltrations were observed as a characteristic sequence of events of inflammation (Figs. 19 & 20). In addition, the portal areas characterized by dilated portal vein, bile ducts were hyperplastic and there was an obvious leucocytic infiltration (Fig. 21).

Examination of liver sections of the diabetic animals treated with the aqueous extract of bay leaves exhibited an obvious degree of improvement represented by almost completely absent of inflammatory leucocytic infiltration, no congested blood vessels and the hepatocytes restored their morphological and structural organization. The blood sinusoids appeared normal contained normal Kupffer cells (Fig. 22).

Immunohistochemical observations:
The expression of PCNA in liver issue

A positive PCNA expression was observed as brownish nuclear color in the hepatocytes of both control animals and that administered the aqueous extract of bay leaves for 4 weeks (Figs. 23 & 24). Treatment with alloxan revealed an obvious decrease in the expression of PCNA in most of the hepatocytes nuclei (Fig. 25). Normal PCNA expression appeared in the diabetic animals treated with the aqueous extract of bay leaves (Fig. 26).

The expression of caspase-3 in liver tissue

Examination of liver sections of the control animals as well as animals treated with the aqueous extract of bay leaves showed a negative expression of caspase-3 which appeared as brownish cytoplasmic reaction in most of the hepatocytes (Figs. 27 & 28). On the other hand, the apoptotic cells denoted by positive caspase-3 immunoreaction were more numerous in the hepatic cells of the diabetic animals (Fig. 29). The reaction appeared as brown dots granules filling the vicinity of cytoplasm in most of the hepatocytes. Negative caspase-3 immunoreaction was observed in several hepatocytes of diabetic animals that treated either with the aqueous extract of bay leaves except few hepatocytes still showed a positive caspase-3 reaction (Fig. 30).

Biochemical results

Changes in blood glucose levels

Animals treated with the aqueous extract of bay leaves daily for 4 weeks showed non-significant changes in the level of blood glucose when compared to the control ones. At the end of 4 weeks a highly significant increase ($P \leq 0.001$) was recorded in the level of blood glucose in diabetic animals when compared to the control group. When diabetic animals treated with the aqueous extract of the bay leaves, a significant decrease ($P \leq 0.05$) in the level of blood glucose was recorded comparing to the control group (table 2).

Changes in serum urea and creatinine levels

Data in table 3 showed the changes in the levels of serum urea and creatinine of animals in different groups. There was non-significant change in the concentration of these parameters between control animals and that treated with the aqueous extract of bay leaves. In alloxan diabetic rats, a highly significant increase ($P \leq 0.001$) in the levels of circulating urea and creatinine was recorded when compared to the control group. When diabetic rats treated with the aqueous extract of bay leave, a highly significant decrease ($P \leq 0.001$) was recorded in the levels of both urea and creatinine when compared to the alloxan diabetic group.

Changes in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities.

In animals daily treated with the aqueous extract of bay leaves for 4 weeks, an insignificant change in the activities of AST and ALT was recorded when compared to the control group. On the contrary, there was a highly significant increase ($P \leq 0.001$) in the activities of AST and ALT in alloxan diabetic animals when compared to the control group. When diabetic animals treated with the aqueous extract of bay leaves, a significant decrease ($P \leq 0.05$) in the activity of ALT and a highly significant decrease ($P \leq 0.001$) in the activity of AST were recorded comparing with the control group. (table 4).
Fig. 16: A photomicrograph obtained from liver section of a control animal showing normal hepatocytes, central vein (CV), hepatic lobule (HL), blood sinusoids (BS) and Kupffer cells (K).

Fig. 17: A photomicrograph obtained from liver section of a control animal showing portal area with portal vein (PV), bile duct (Bd) and hepatic artery (arrow).

Fig. 18: A photomicrograph obtained from liver section of an animal administered the aqueous extract of bay leaves showing normal hepatic lobules (HL), hepatocytes with nuclei (N), central vein (CV) and blood sinusoids space (Bs).

Fig. 19: A photomicrograph obtained from liver section of a diabetic animal showing congested blood vessel (BV), degenerated hepatocytes with cytoplasmic vacuolization (thin arrows) and pyknotic nuclei (thick arrows).

Fig. 20: A photomicrograph obtained from liver section of a diabetic animal showing leucocytic infiltration (arrow), degenerated hepatocytes with pyknotic nuclei (N) and cytoplasmic vacuolization (V).

Fig. 21: A photomicrograph obtained from liver section of a diabetic animal showing degenerated portal area with dilated portal vein (PV), numerous branches of bile ducts (Bd) and infiltrated leucocytes (arrow).

Fig. 22: A photomicrograph obtained from liver section of a diabetic animal treated with the aqueous extract of bay leaves showing normal liver architecture, central vein (CV) and considerable number of binucleated cells (arrows).
Fig. 23: A photomicrograph of liver section obtained from a control animal showing positive PCNA expression as brownish color in the nuclei of few hepatocytes (arrows).

Fig. 24: A photomicrograph of liver section of an animal treated with the aqueous extract of bay leaves showing positive PCNA expression in the nuclei of few hepatocytes (arrows).

Fig. 25: A photomicrograph of liver section obtained from a diabetic animal showing a negative PCNA expression in the nuclei of most hepatocytes.

Fig. 26: A photomicrograph of liver section obtained from a diabetic animal treated with the aqueous extract of bay leaves showing nearly positive PCNA expression in the nuclei of few hepatocytes (arrows).

Fig. 27: A photomicrograph of liver section obtained from a control animal showing expression of caspase-3 as brownish color in few hepatocytes (arrows).

Fig. 28: A photomicrograph of liver section of an animal treated with the aqueous extract of bay leaves showing expression of caspase-3 in few hepatocytes (arrows).

Fig. 29: A photomicrograph of liver section obtained from a diabetic animal showing positive caspase-3 expression (arrows) in large number of hepatocytes.

Fig. 30: A photomicrograph of liver section obtained from a diabetic animal treated with the aqueous extract of bay leaves showing positive caspase-3 expression (arrows) in few hepatocytes.
Table 2: Effect of different treatments on blood glucose levels.

<table>
<thead>
<tr>
<th>Weight Groups</th>
<th>Basal (initial) blood glucose (mg/dl) (Mean ± SD)</th>
<th>Final blood glucose (mg/dl) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.60±6.28</td>
<td>82.60±4.63</td>
</tr>
<tr>
<td>Bay leaves aqueous extract</td>
<td>83.40±2.15</td>
<td>80.20±4.47</td>
</tr>
<tr>
<td>Alloxan</td>
<td>81.00±55.99</td>
<td>370.00±55.99***</td>
</tr>
<tr>
<td>Alloxan+Bay leaves aqueous extract</td>
<td>85.40±15.43</td>
<td>145.00±9.74**</td>
</tr>
</tbody>
</table>

n= 6 animals in each group

(***): highly significant at $P \leq 0.001$ comparing with the control group.

(**): significant at $P \leq 0.05$ comparing with when the control group.

Table 3: Effect of the different treatments on the levels of blood urea and creatinine.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Urea (mg/dl) (Mean ± SD)</th>
<th>Creatinine (mg/dl) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>38.24±1.02</td>
<td>1.050±0.07</td>
</tr>
<tr>
<td></td>
<td>Bay leaves aqueous extract</td>
<td>36.90±2.42</td>
<td>0.99±0.05</td>
</tr>
<tr>
<td></td>
<td>Alloxan</td>
<td>138.42±16.76***</td>
<td>3.79±0.32***</td>
</tr>
<tr>
<td></td>
<td>Alloxan+Bay leaves aqueous extract</td>
<td>38.33±5.22a</td>
<td>1.57±0.16a</td>
</tr>
</tbody>
</table>

n= 6 animals in each group

(***): highly significant at $P \leq 0.001$ comparing with the control group.

(a): highly significant at $P \leq 0.001$ comparing with alloxan diabetic group.

Table 4: Effect of the different treatments on the activities of liver enzymes (AST & ALT).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>ALT (mg/dl) (Mean ± SD)</th>
<th>AST (mg/dl) (Mean ± SD)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Control</td>
<td>20.24±1.31</td>
<td>31.12±3.10</td>
</tr>
<tr>
<td></td>
<td>Bay aqueous extract</td>
<td>22.28±2.67</td>
<td>31.65±1.13</td>
</tr>
<tr>
<td></td>
<td>Alloxan</td>
<td>58.46±5.37***</td>
<td>167.68±15.22***</td>
</tr>
<tr>
<td></td>
<td>Alloxan+Bay leaves aqueous extract</td>
<td>30.64±1.50a</td>
<td>53.16±6.17***a</td>
</tr>
</tbody>
</table>

n= 6 animals in each group

(***): highly significant at $P \leq 0.001$ comparing with the control group.

(***): significant at $P \leq 0.05$ comparing with when the control group.

(a): highly significant at $P \leq 0.001$ comparing with alloxan diabetic group.
Changes in malondialdehyde (MDA) level and catalase (CAT) activity

Table 5 showed insignificant difference in catalase activity and MDA level between control animals and that administered the aqueous extract of bay leaves. Alloxan diabetic animals recorded highly significant increase ($P \leq 0.001$) in MDA level and highly significant decrease ($P \leq 0.001$) in CAT activity comparing with the control group. When diabetic animals were treated with the aqueous extract of bay leaves, a highly significant increase ($P \leq 0.001$) in the activity of CAT and highly significant decrease ($P \leq 0.001$) in the level of MDA were recorded comparing with alloxan diabetic group.

Table 5: Effect of the different treatments on MDA level and CAT activity

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>MDA (n.mol/ml) (Mean ± SD)</th>
<th>Catalse (n.mol/ml) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.20±4.09</td>
<td>30.00±1.41</td>
</tr>
<tr>
<td>Bay leaves aqueous extract</td>
<td>96.20±5.65</td>
<td>28.60±4.13</td>
</tr>
<tr>
<td>Alloxan</td>
<td>140.00±11.92***</td>
<td>17.36±0.59***</td>
</tr>
<tr>
<td>Alloxan +Bay leaves aqueous extract</td>
<td>100.20±1.68**a</td>
<td>34.80±2.05**a</td>
</tr>
</tbody>
</table>

n= 6 animals in each group

(***): highly significant at $P \leq 0.001$ comparing with the control group.

(**): significant at $P \leq 0.05$ comparing with the control group.

(a): highly significant at $P \leq 0.001$ comparing with alloxan diabetic group

Discussion

The results of this study showed a highly significant decrease in the body weight of alloxan diabetic rats compared to control animals. This weight loss may result from catabolic disturbance. Granner [25] attributed the loss of body weight in diabetes to the loss in muscle and adipose tissues resulting from excessive breakdown of tissue proteins and fatty acids. Similarly, continuous reduction in body weight of the diabetic rats was observed by Nagy and Amin [26] who attributed this to catabolic effect on protein metabolism by retarding protein synthesis and stimulating protein degradation.

Examination of the renal cortices of alloxan diabetic rats in the present study revealed many pathological changes. The glomeruli appeared atrophied with dilated capsular spaces, degenerated renal tubules with wide lumen sometimes contained proteinaceous casts and the tubular epithelial cells showed pyknotic nuclei and cytoplasmic vacuolization. In addition, intratubular hemorrhage and congestion of renal blood vessels were seen. These results come in agreement with the fact that diabetes is combined with increased reactive oxygen species and oxidative stress. Similarly, Bassey et al. [27] and Yousry et al. [28] found the same pathological features in the kidney of diabetic rats and found that diabetes caused pronounced oxidative stress that affected renal functions and structure. Kumar et al. [29] suggested that the appearance of cells with increased acidophillia and pyknotic nuclei may be due to loss of cytoplasmic RNA and increased binding of denatured cytoplasmic proteins to eosin. The cytoplasmic vacuolization in kidney tubular cells may be attributed to lactate accumulation in the tubules of the kidney resulted in increased osmotic pressure with subsequent water influx [30]. Moreover, Aboonabi et al. [31] found that kidneys of diabetic rats showed shrinkage of glomeruli and tubular inflammation that might cause an abnormal production of cytokines and growth factors. Subsequently, they facilitate the synthesis of extracellular matrix proteins and their depositions in the glomerular level that finally lead to mesangial expansion, glomerular shrinkage and glomerular basement thickening.

Examination of liver sections of alloxan diabetic rats revealed many pathological features including loss of the normal architecture of hepatic lobules, hepatocytes with pyknotic nuclei and cytoplasmic vacuolization, congested blood vessels and leukocytic infiltrations were observed. Similarly, Nagy and Amin [26] found the same histopathological changes in the liver of alloxan diabetic rats. Diabetes raised the oxidative stress by increasing the production of free radicals and diminishing cell antioxidant capabilities that may result in tissue damage in diabetic patients [32].

Proliferating cell nuclear antigen (PCNA) is a stable cell cycle-related nuclear protein and represents a reliable marker for the determination of proliferative activity [33]. It is expressed in late G1 and expressed maximally during S-phase of the cell cycle and its rate of synthesis correlated with the proliferative rate of cells [34]. In the present study, a marked decrease of PCNA expression was appeared in most of the
hepatocytes and the renal tubular cells of the diabetic animals which may attribute to the ability of alloxan to cause DNA fragmentation. These results come in agreement with Dkhil et al. [35] who found markedly reduction in PCNA-expression in testicular germ cells of diabetic rats which is an indication of disruption in proliferation in diabetes. Moreover, Otton et al., [36] concluded that high glycemia and the lack of insulin participates in the reduced proliferation capacity in diabetes. Sakurai et al. [37] found that the alloxan able to generate the reactive oxygen species then caused beta cells apoptosis and the insulin-1 cell death which may be linked to alloxan-induced DNA fragmentation. Alloxan caused DNA strand broken and damaged DNA activates nuclear poly (ADP ribose) synthetase, which deplete the cellular pool of NAD+, resulting in b-cells damage [38].

The caspase-3 protein is a member of the cysteine-aspartic acid protease (caspase) family. Caspase-3 is a marker of the early phase of apoptosis [39]. An obvious increase in caspase-3 expression in most of the hepatocytes and the renal tubular cells of the diabetic animals was observed. Similarly, Haligur et al. [40] observed increased apoptotic activity and the expression of caspase-3 in the hepatocytes and kidney tubular cells in diabetic rats. The same author attributed that to different mechanisms which may play a role in diabetes as hypoxia, apoptosis, and calcium influx in degenerative changes in different cells. Brezniceanu et al. [41] confirmed that apoptosis has been demonstrated to mediate cell death in a variety of renal diseases, including diabetic nephropathy. Indeed apoptosis was detected in renal proximal tubular cells of different species including experimental animals and patient with diabetes suggested that tubular apoptosis may precede tubular atrophy in diabetes [42].

In the current study, a highly significant increase in the level of plasma glucose was recorded in diabetic animals. This result may come in parallel with the fact that diabetes causes a disturbance in the uptake and the metabolism of glucose. Similarly, El-desouki et al. [43] observed the same results and concluded that the increased level of plasma glucose could promote destruction in β-cells of pancreas. Moreover, Al-Hilfy [44] attributed the elevation of blood glucose level after alloxan to its ability to produce oxygen free radicals that destroyed pancreatic β-cells and caused severe hypoinsulinaemia and hyperglycemia (type 1 diabetes). Moreover, Celec et al. [45] found that prolonged hyperglycemia in diabetes led to oxidation of protein and inflammatory changes in renal tissue.

A highly significant increase in the levels of serum urea and creatinine was observed in alloxan diabetic animals were considered as significant markers of renal functions. These changes may be due to the oxidative stress raised by alloxan. Similarly, Elseweidy et al. [46] found the same results in alloxan diabetic rats explained that by increased oxygen species and reduced antioxidative ability in diabetes that resulted into renal tubular injury and led to gradual loss of the renal functions. Moreover, Hfaiedh et al. [47] attributed these changes to disturbance in glucose metabolism and generation of reactive oxygen species accompanying chronic hyperglycemia in diabetes triggered oxidative stress, enhances lipid peroxidation, DNA damage and protein degradation and exhausts the antioxidative defense systems. Cao et al., [48] suggests that development of diabetic renal dysfunction may due to activation of endoplasmic reticulum stress that can mediate progressive endothelial damage, apoptosis of endothelial cells and over expression of inflammatory cytokines.

In the present study, highly significant increase in the activities of AST and ALT was observed in the diabetic animals. The change in the activities of these enzymes is directly related to liver injury and may cause changes in the metabolism in which they are involved. Similarly, Yuniarti and Lukiswanto [49] revealed that alloxan caused a significant increase in the activity of AST and ALT. Batran et al. [50] found that increasing in the activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of diabetic rats are also responsible for the increased gluconeogenesis and ketogenesis. Moreover, Stanely et al. [51] suggested that the elevation in the activities of transaminases may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan.

In the present study, diabetic animals recorded highly a significant decrease in the catalase activity and highly significant increase in the malondialdehdy (MDA) level. Similarly, Li et al. [32] observed a reduction in the activities of endogenous antioxidant enzymes such as catalase and this reduction can cause tissue degradations. Hayashi et al. [52] found that 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. It has been shown that the antioxidants status of tissues is an important factor in the development of diabetic complications [53]. Moreover, Salimi et al. [54] found the same results after alloxan induced diabetes in rats. Taki et al. [55] proved that the pathogenesis of diabetes nephropathy involve oxidative stress, an increase malondialdehyde level, advanced glycation end product that led to irreversible tissue damage.

Concerning the mechanism of alloxan Das et al. [56] reported that alloxan reacts with two-SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result of alloxan reduction, dialuric acid is formed which re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species and superoxide radicals.

The obtained results showed that treating diabetic animals with the aqueous extract of bay leaves improved the histopathological and biochemical changes induced in the kidney and liver by alloxan. These tissues appeared more or less similar to control. This improvement may be attributed to the antioxidant and anti-inflammatory activities of the aqueous extract of bay leaves. Similarly, Ravindran et al. [12] found that the laurel extract has a remarkable protective effect against liver damage and attributed this to its antioxidant activity which reduced the oxidative damage by blocking the production of free radicals and inhibits lipid peroxidation. In addition, Nayak et al. [57] reported that the aqueous bay leaf extract contained the alkaloids and monoterpenoids in addition to the flavonoids which reduced lipid peroxidation not only
by preventing or slowing the onset of cell necrosis but also by improving vascularity. The protective properties of the bay leaves extract probably due to combined action of the extract phytoconstituents as flavonoid and nonflavonoid origin such as, terpenes and terpenoids possessing antioxidative and antimicrobial activities [11&58].

Diabetic animals administered the aqueous extract of bay leaves showed normal expressions of PCNA and caspase-3 in the hepatocytes and renal tubular cells. Similarly, Abudahab et al. [59] found that bay leaves extract components mediated inhibition of proliferation and increased caspase-3 activity. The same authors attributed the strong antiproliferative activity of both leaves and fruits extracts to the flavonoids in addition to the pure volatile compounds include, 1,8-cineole, pinene, limonene, linalool, borneol, b-caryophyllene and caryophyllene oxide.

A highly significant decrease in the blood glucose level in the diabetic animals treated with the aqueous extract of bay leaves. This result comes in agree with Khan et al. [9] who reported that bay leaves reduce serum glucose level in people with diabetes. Aljamal [60] indicated that the antidiabetic activity of bay leaves attributed to the presence of polyphenols compound which affected the insulin sensitivity, glucose uptake and antioxidant status.

In the present study, a significant decrease in the activities of AST and ALT was observed in diabetic animals treated with the aqueous extract of bay leaves. Similarly, Gasparyan et al. [61] observed normalization tendency of AST and ALT activities after administration of bay leaves extract that indicated the hepatoprotective property.

The activity of CAT and the level of MDA were returned to normal status after treated the diabetic animals with the aqueous extract of bay leaves. Ravindran et al. [12] observed the same results and concluded that the bay leaves extract presumably offered protection against lipid peroxidation by quenching and detoxifying the free radicals. The antioxidant activities of lyophilized aqueous extract of bay leaves include, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities were evaluated by Patrakar et al. [62]. Moreover, Lakshmi et al. [63] reported that bay leaves displayed scavenging activity against hydroxyl radicals by inhibition of Fe$^{2+}$-ascorbate induced lipid peroxidation in diabetic rats.

**Conclusion**

This study concluded that alloxan induced diabetes in rats and caused adverse cytotoxic side effects on liver and kidney as indicators of diabetic complications. When the diabetic animals treated with aqueous extract of bay leaves showed similar degrees of improvement and reversed hepatonephropathy effects of diabetes. This study recommended bay leaves as ameliorating medicinal plant in management of diabetes mellitus and its complications due to its anti-inflammatory, antidiabetic and antioxidant properties.

**References**


