INFLUENCE OF AQUEOUS EXTRACT OF CALOTROPIS PROCERA LEAVES IN SUBCHRONIC POISONING OF GOAT.

Ahmad Hanash Al-Zuhairi1, Jenan M. Khalaf 2 and Al-Khafaji Nazar Jabbar3

1, 3Department of Internal Medicine, College of Veterinary Medicine, University of Diyala. Diyala, Iraq.
2Department of Internal Medicine, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract

Calotropis procera has been reported to be a medicinal and toxic plant to humans and animals. Fifteen local breed Iraqi goats, of 7-11 months old and 18.1 ±.84 kg.b.wt. divided randomly into two groups. Those of group I were exposed to 1/15 of LD$_{50}$ (162.4) mg/kg.b.wt. of extract for 8 weeks, while those in G .II were left without exposure as a control group. The dependent parameters were, clinical signs exhibited by animals during the study , in addition to levels of some biochemical parameters (GOT, GPT, ALP, Urea and Creatinine) concentration. The main changes observed during monitoring the animals were diarrhea, arrhythmia, anorexia and depression. Biochemically there were a significant increases of depended parameters. From this, we can conclude that aqueous extract of leaves of C. procera has are influences on goat used in current study.

Key words: Calotropis procera, Goats, Toxicity, Biochemical parameters.

Introduction

The family of Asclepiadaceae comprises about 180 genera and 2200 species of shrubs, vines, or herbs. The genus Calotropis includes three species of shrubs abound in equatorial and semitropical from Africa, also Asia and America, as well as, India: Calotropis acia Buch-Ham, Calotropis gigantea (L.) WT Aiton and Calotropis procera (Rahman and Wilcock 1991, Upadhyay, 2014). Both the Calotropis procera and the Calotropis gigantia species are found through the world, closely similar to each other in chemical actions, while the Calotropis procera is widespread which has a purple color of the blossom while the Calotropis gigantia has whitish blossom (Shanker, 2005). C. procera It is famous by many names like, in English called Swallow wort and giant milkweed, debaj, oshar, usher in Arabic, madar in Hindi, bomba, cazuella, algodón extranjeró in Spanish, French cotton, in French, Bois Canon, ipekag in Turkish and Alarka in Sanskrit (Parrotta, 2001, Shahat and Shihata, 2012, Quazi et al., 2013, Al-Snafi, 2015, Imosemi, 2016, DEEDI, 2016).

Calotropis procera is a single or multiple stemmed shrubs, of 2-6-meter height stems (Little et al., 1974, Orwa et al., 2009). Smooth in texture, grayish-green in color. Ripe stalk have a deeply fissure, cork-like phloem, tinted sprightly brown (Boulos, 2000 Parsons and Cuthbertson, 2001). Germinate abundantly in semiarid and arid area of the world without needed irrigation, pesticides, fertilizer, or other agronomic processes (Erdman, 1983). Commonly C.procera used in the conventional medical system. The C. procera used for treatment of tumors, soreness, asthma, dyspepsia and bronchitis, leprosy, liver and spleen diseases, as well as ulcers (Parihar et al., 2011). Consumption of oshar at fresh stage leading to intoxication of livestock, likewise, humans (Lewis and Elvin-Lewis, 1977). Livestock are exposure utmost commonly during time of aridity, hunger periods, or when animals transport from venue to other for find of good grassland (Hall, 1977). Camels, sheep and goats, will eat the leaves of oshar during droughts, but consumption is little (Abbas et al., 1992). C. procera contributes to a paramount role in rife discomfort, indolence and loss appetite in grazing livestock and in this case the reason which is rarely diagnosed as caused by eating of a sub-clinical dosage of this tree (Clarke and Clarke, 1977). Consumption of Swallow wort leaves in sheep is resulting in tentative cardiac arrhythmia, toxic effect on liver, as well as, radiotoxic after half an hour of consumption (District et al., 1983). The preliminary phytochemical checking of leaf powder of C. procera demonstrate, that the leaves consist of cardenolides, as
well as, steroids, glycosides, sugars, tannins, terpenoids, phenols, flavonoids, saponins and alkaloids (Begum et al., 2010, Murti et al., 2010, Shrivastava et al., 2013). Leaves of this plant also contained a bitterish structure (mudarine) and abundant glycosides, uscharin, calactin, calotropin and calotoxin (Meena et al., 2010, Russell et al., 2011).

Many investigations have been announced that the swallow wort possess diverse medical functions, but also can cause toxic influence. The current study was intended to research the effects of the sub chronic exposure of goat to aqueous leaves extracts of *C. procera*.

### Materials and Methods

#### Collection and preparation of the plant material

Fresh leaves of *Calotropis procera* used in current study were collected from villages of Diyala province, Iraq. The plant was identified by the Ministry of Agriculture, Directorate of Seeds Testing and Certification (D.S.T.C) in Abu Graib, Baghdad. The fresh leaves of *C. procera* were washed, air-dried in shade, at room temperature, subsequently dried leaves milled and the powder kept in an airtight container until use.

#### Extraction of the plant material

The dried ground plant (50 g) was mixed with 2 L of distilled water and left for 72 h. at room temperature. The aqueous extract was filtered using Whatman filter paper and evaporated to a dark green gummy residue.

#### Acute toxicity of *C. procera*

The estimation of oral LD$_{50}$ in male albino rats was done according to Dixon (1980) by using the Up and Down method.

Animals: Fifteen healthy local breed goats, 7-11 months old and body weight of 18.1±.84 kg. were randomly divided into two groups, (I and II), group I daily treated with (162.4 mg/kg) body weight of aqueous extract of *Calotropis procera* for 8 weeks and group II serving as the control, non-treated.

The dependent parameter was clinical examinations, including pulse and respiratory rate, body temperature and body weight, in addition to monitoring for any abnormal changes in behavior, appetite, or other signs.

#### Percentage of weight win

Individually goat in control and exposure groups were weighed to determine any alteration in their weights. Proportion of weight win were measured as follows:

\[
\text{Proportion of Weight Win} = \frac{\text{Weight at the end of the experiment} - \text{Weight at the beginning of the experiment}}{\text{Weight at the beginning of the experiment}} \times 100
\]

Blood samples were collected from the jugular vein (5ml) in a test tubes without anticoagulants according to Pugh (2002) and centrifuged at 3000 rpm for 5 min. The sera obtained were stored at -4°C until use.

Biochemical analysis: Alanine aminotransferases (ALT) and aspartate aminotransferases (AST) were determined as described by commercial kit SGOT, SGPT from AGAPE DIAGNOSTICS SWITZERLAND. Alkaline phosphatase (ALP) by BIOLABO, France. Serum creatinine level was determined by creatinine kinetic method kit BIOLABO, France, while serum urea level was estimated by using a commercially available kit (UREA Berthelot from LINEAR CHEMICALS S.L).

#### Statistical analysis

The result collected were analyzed using analysis of variance (ANOVA, 2 way) according to Gomez and Gomez (1984), collected data are presented as the mean with standard error ± and p<0.05 was considered to be statistically significant difference.

### Results

#### Acute toxicity study of plant extracts

Determination of acute toxic median lethal dose (LD$_{50}$) was conducted in the male albino rat by up and down method, according to Dixon (1980), the value of LD$_{50}$ was 2435.25 mg/kg B.W.

Clinical signs observed: The main clinical signs appeared on animals through daily observation of the treated animals were, loss of appetite, diarrhea and some time passed soft feces and showed dullness, progressive weakness, intermittent diarrhea and depression, on heart auscultation observed irregulars heartbeat (arrhythmia).

All animal groups showed weight gain but these in group II were significant higher than those in group I (Table 1).

Table 3, showed the influence of the aqueous extract of *C. procera* leaves used in current study on biochemical parameters, before, within (2, 4, 6, 8 weeks) of administration for tests and control goats. GOT (AST) significantly increased in those of G I during the 6th and 8th weeks. The serum of GPT(ALT) significantly increased in G I during all treated weeks, the highest level was at 6th and 8th weeks. ALP level significantly increased in group I during the 8th weeks.

#### Table 1: Effect of administration of *C. procera* aqueous extract on weight win in goats.

<table>
<thead>
<tr>
<th>%Weight win</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.34±6.68</td>
<td>I</td>
</tr>
<tr>
<td>40.92±1.9</td>
<td>II</td>
</tr>
</tbody>
</table>

Data expressed statistically = as Mean and Standard error (SE).
Table 2: Effect of daily oral administration of C. procera aqueous extract on the body weight, heart and respiratory rates and body temperature of goats used in study.

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>0</th>
<th>2W</th>
<th>4W</th>
<th>6W</th>
<th>8W</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>I</td>
<td>18.33±0.74b</td>
<td>16.61±0.73a</td>
<td>18.2±0.89b</td>
<td>20.28±1.43c</td>
<td>22.08±1.39cd</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>17.86±0.97</td>
<td>18.23±1.06</td>
<td>19.29±1.26</td>
<td>22.23±1.84</td>
<td>23.71±1.53</td>
</tr>
<tr>
<td>H</td>
<td>I</td>
<td>91.44±3.24b</td>
<td>92.78±4.41b</td>
<td>95.56±3.0c</td>
<td>73.56±2.89a</td>
<td>71.22±2.99a</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>92.4±5.79b</td>
<td>90±5.36a</td>
<td>88.6±4.11a</td>
<td>91.4±3.54a</td>
<td>89.8±5.49a</td>
</tr>
<tr>
<td>R</td>
<td>I</td>
<td>23.44±1.33a</td>
<td>22.44±1.71a</td>
<td>22.89±2.01b</td>
<td>29.44±2.14b</td>
<td>32.33±3.86bc</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>23.8±1.66</td>
<td>25.4±1.7</td>
<td>25.7±1.79</td>
<td>24±2.35</td>
<td>24±1.54</td>
</tr>
<tr>
<td>T</td>
<td>I</td>
<td>39.61±0.11</td>
<td>38.39±0.17</td>
<td>38.29±0.2</td>
<td>39.6±0.09</td>
<td>39.41±0.08</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>39.02±0.11</td>
<td>39.2±0.26</td>
<td>39.06±0.24</td>
<td>39.1±0.24</td>
<td>39.04±0.23</td>
</tr>
</tbody>
</table>

W=Body weight, H=heart rate, R=respiratory rates ,T=body temperature, Data expressed statistically as Mean and Standard error (SE).

Table 3: Influence of administration of aqueous extract of C. procera leaves on serum GOT (IU/L), GPT (IU/L), as well as, ALP level (IU/L) in goats used in the study.

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>0</th>
<th>2W</th>
<th>4W</th>
<th>6W</th>
<th>8W</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT</td>
<td>I</td>
<td>30.78±1.14a</td>
<td>31.69±1.52a</td>
<td>30.74±1.96a</td>
<td>32.53±1.99b</td>
<td>33.12±0.97b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>30.84±1.66</td>
<td>30.03±3.36</td>
<td>29.08±1.02</td>
<td>29.8±2.98</td>
<td>31.03±2.93</td>
</tr>
<tr>
<td>GPT</td>
<td>I</td>
<td>31.92±3.37a</td>
<td>34.38±3.07b</td>
<td>35.57±5.65b</td>
<td>37.46±4.1bc</td>
<td>37.61±5.44bc</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>31.7±2.62</td>
<td>32.66±1.81</td>
<td>33.88±4.42</td>
<td>34.06±3.32</td>
<td>32.06±2.57</td>
</tr>
<tr>
<td>ALP</td>
<td>I</td>
<td>165.86±8.02a</td>
<td>170.53±8.42a</td>
<td>170.88±10.33a</td>
<td>167.7±10.44a</td>
<td>180.6±9.5b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>165.14±7.41</td>
<td>165.4±6.17</td>
<td>167.35±9.2</td>
<td>169.33±8.17</td>
<td>167.35±7.4</td>
</tr>
</tbody>
</table>

Data expressed statistically as Mean and Standard error (SE).

Discussion

The results of the current study, revealed that weight win of all goats exposed to aqueous extract of this plant showed significant increases, while goats in a control group appeared highly significant increases. These result disagree with what revealed by Mbako et al., (2009), Shahat and Shihata, (2012), in rabbits, as well as, result recorded by Radunz et al., (1983), in livestock (sheep and cows). While Mahmoud et al., (1979), observed that weight was loosed in sheep, he attributed this to diarrhea and loss of fluid as a result off erosions of mucous lining of the gastrointestinal tract, Dada et al., (2002), revealed weight loss during orally administration of latex of Giant milkweed in rats. Cardenolides, which a cardiac-effective compounds that dampen the cellular membrane Na+/K+ ATPase, leading to electrolyte disorder, which result to influence on the heart electrical conductivity (Poindexter et al., 2007). So, the arrhythmia and lower heartbeats (dysfunction of heart) induced by aqueous extract of this plant in current study, can be refer to its consist of cardenolides. Significant elevation of respiratory rate at same time with decreased heart beats may be due to heart disturbances. Delmar and Bruce, (1994) revealed, that the Patients with cardiac disorder have a diminish greater heart beat than expectant. Lower cardiac product resulting to diminish maximal oxygen uptake and anaerobic metabolism at lower workloads. Respiration is usually more than prospective, however breathing reserve is within the normal range. Current study showed significant increases in values of GOT, GPT and ALP this agree with result observed by El-Shafeye, (2011), Shahat and Shihata, (2012). Rising in the level of GOT, GPT and ALP, is as a rule a signal to damage of liver (Chavda et al., 2010, Zhang et al., 2012). These rise may be caused by dead or damage of hepatocytes, under the toxic influence of highly effective materials of C. procera extract and at the end, this enzymes diffused from the intracellular position (Tilkian et al., 1983, Jimoh and Odutuga, 2001).

Significant increases of serum urea and creatinine after exposure to C. procera aqueous extract in current research perhaps caused by injury of the renal cells and/or dysfunction of the kidney under the venomous influence at composition of this plant (Eissa and Zidan, 2010). The Creatinine are removed from plasma via glomerular filtration, then excrete into the urine. Elevated in creatinine values is a signal of renal dysfunction (Smith and Hampton, 1990), this injury may be caused by cumulating of effective substance of the aqueous extract of C. procera in kidneys, cumulating of peril capable to Poisonous of the tubular epithelial cells (Parke, 1982).
Phytochemical checking of *Calotropis procera* leaves revealed the existence of cardiac glycosides, alkaloids, terpenoids, saponins, phenols, tannins and flavonoids, this Ingredients are recognized as a possess medical and pesticidal characteristic (Verma et al., 2013). De Lima et al., (2011) revealed that this plant is a hepatotoxic and cardio venomous. Other investigators have notarized prejudicial effect of this plant on renal function (Basak et al., 2009, Lin and Will, 2012).

**References**


