CLINICAL AND PATHOLOGICAL EFFECTS OF PARENTERALLY ADMINISTERED CHLOROFORM AND AQUEOUS EXTRACT OF CALOTROPIS PROCERA IN RABBITS


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ABSTRACT

Study was carried out to evaluate the pathological effects of parenterally administrated “Calotropis procera extracts” in rabbits. Fresh flowery buds of C. procera were collected from the District Lahore, Pakistan. Total of 35 rabbits of either sex were purchased from the animal house of University of Veterinary and Animal Sciences, Lahore. Rabbits were randomly distributed in to 3 major groups (A= C. procera chloroform extract, B= C. procera water extract, C= Normal saline). Groups A and B were further subdivided into three sub-groups (A1, A2, A3, B1 and B2 and B3) of 5 and C (n=5) were used as a control group. Biochemical findings showed a significant increase (P<0.05) in serum level of creatinine, urea and ALT in group A3 at day 30 as compared with group C. Gross lesions consisting of severe hemorrhages, lung emphysema in group A3 and lobular congestion, multifocal emphysema were observed in group B3. Histopathological lesions such as hyperplasia of Kupffer cells, hepatic cell degeneration and eruption of bronchiole epithelium were observed in group A3. It is concluded that higher dose (10 mg/kg BW) of chloroform extract of the C. procera had cardiotoxic and hepatorenal toxic effect and cause lungs emphysema in rabbits.

Key words: Calotropis procera, ALT, Urea, Creatinine, Rabbits

INTRODUCTION

Calotropis procera (C. procera); Ait F. Asclepiadaceae locally known as ‘Ak’ or ‘Arka’ common throughout the tropics of Asia and Africa, is a wild plant with multifactor characteristics (Bharti et al. 2010). It is an erect much branched 2–3 m high having purple spotted white flowers. Plant grows throughout the year in unfarmed soils, arid and dry zones (Singh et al. 2005). In an earlier study various medicinal properties such as a laxative, anthelmintic, purgative, anti-inflammatory and diuretic have been documented (Iqbal et al. 2005). Different parts of C. procera and its latex have shown analgesic; wound healing and anti-bacterial properties in traditional medicine (Laitiff et al. 2010; Lima-Filho et al. 2010; Attah et al. 2012). Several studies have also exploited the toxic effects of the plant like hepatotoxic and cardiotoxic effect (de Lima et al. 2011).

Plant is mostly being used in crude form for various medicinal purposes. But crude form uses have certain limitations due to possibility of inadequate absorption of the required substances and unknown substances present in crude extract may lead to adverse effects. So it was necessary to establish extraction of the plant through standard methods and evaluate its possible toxicity in laboratory animals before its use in dairy animals to combat the needs of new drug regime for various chemotherapeutic purposes. Present study was planned to get the extracts of the C. procera flowery part through sequential extraction using hexane, chloroform, ethanol and aqueous solvents. Chloroform and aqueous extracts of C. procera were injected in rabbits through parenteral routes and toxicity of both extract of plant was assessed on the basis of biochemical markers (ALT, Creatinine and urea) gross and microscopic lesions particularly of liver, heart, lungs and kidney.

MATERIALS AND METHODS

Source of plant samples: Fresh flowery buds of C. procera were collected from the District Lahore (Pakistan), identified and authenticated by a botanist by comparing with specimen (voucher number 3207) stored in the herbarium of Botany department, Punjab University Lahore, Pakistan. Flowers were dried in shade at room temperature (25°C). Dried flowers were grounded to a fine powder by wily mill (standard model 4) stored in glass bottle and kept at room temperature (25°C) for further usage.

Extraction by Soxhlet apparatus: Representative ground samples of C. procera were subjected to sequential (non-polar to polar) extraction with n-hexane, chloroform and ethanol using soxhlet apparatus. And aqueous extraction; crude aqueous extract (CAE) of the reference plant flower’s powder of 100 gm was mixed with 600 ml of distilled water in a flask of 1.5 liter volume capacity and boiled for 1.5 hr. Following cooling to 40°C, the ‘brew’ was filtered using Whatman No.1 filter paper.
Only filtrate obtained in CAE and extracted material from chloroform extraction of the flowery buds of the *C. procera* was concentrated in vacuum rotary evaporator. Finally obtained concentrated materials were stored at 4 °C for further use.

**Source of Rabbits:** Rabbits (1200-1700 gm BW) were purchased from the animal house of UVAS, Lahore. Total of 35 rabbits were randomly selected of either sex. These rabbits were randomly distributed in 3 major groups (A, B and C). The groups A and B were further subdivided into three sub-groups (A1, A2, A3, B1, B2 and B3) of 5. The Treatment given to each group is illustrated in Table 1. Rabbits were kept in clean metallic wire cages with soft bedding of wheat straw. They were supplied with clean food and water ad libitum. They were provided with 12 hr light/dark light cycle.

**Preparation of Medicinal plants solutions for the assessment of toxicity in rabbits:** Solutions of extracted material in chloroform and water of *C. procera* were made with normal saline at concentration of 2 mg/ml. Prepared solutions were kept at 4 °C temperature for 24 hr. The solutions were filtered using 0.22μm syringe filter in a sterilized media bottles and were kept at 4 °C temperature for future uses.

**Blood sampling of rabbits:** Blood samples from each rabbit were collected at day 0 (pre-medication), day 9 and day 30 (post-medication). Fur over the jugular vein was clipped with scissor and skin was disinfected with methylated spirit. Four ml blood was drawn from the jugular vein using disposable syringe. Blood sample was immediately transferred in 10 ml sterilized vacutainers without containing any- anti-coagulant and kept closed with caps at room temperature (25 °C) for 4 hr. Serum samples were separated from the blood samples using centrifugation at 3,000 rpm for 10 minutes and stored in serum cups labeled with the specific number at -20 °C until used for the determination of biochemical markers as presented below.

**Estimation of biochemical parameters:** Collected serum samples were used for estimation of different biochemical parameters i.e. urea, creatinine and ALT. Serum urea level measurements were done by Urease-GLDH enzymatic UV test using commercially available kit (Urea UV, Merck Pvt. Limited, France). Serum creatinine concentration was estimated by Kinetic Method without deproteinization Jaffé reaction using commercially available kit “creatinine Test Kit” (Crescent diagnostics, Saudi Arabia). Measurements of serum alanine aminotransferase (ALT) were done using commercially available kit (Crescent diagnostics, Saudi Arabia).

**Post-mortem Lesions:** Post-mortem was performed by sacrificing two rabbits of each group at the end of experiment period. Gross lesions of organs were observed and noted.

**Histopathological examination:** Histopathological examination of tissue samples of kidney, heart, lungs and liver collected from the necropsied rabbits were fixed in 10 percent buffered formalin. A routine procedure of dehydration in ascending series of ethanol, clearing with xylene and embedding in paraffin was used. The histopathological examination was carried out on all samples using methods as described by Bancroft and Gamble (2007).

**Statistical analysis:** Comparison of various treatments for different parameters was carried out by one way Analysis of variance (ANOVA) followed by post hoc test (Duncan’s multiple range test) using SAS program (SAS SYSTEM, SAS Inst., Cary, North Caro-lina v 9.1)

## RESULTS

### Effect of *C. procera* on biochemical parameters

**Creatinine:** Creatinine values of rabbits in different groups recorded on various days of experiment are shown in Table 2. A significant increase (*P<0.05*) in serum concentration of creatinine (1.226±0.073) was in group A3 at day 30 of experiment than groups A1, A2, B1, B2, B3 and C. A non- significant difference (*P>0.05*) was observed in serum creatinine concentration in treatments groups A1, A2, B1, B2 and B3 than control group C at the end day of experiment.

**Urea:** Serum levels of urea in rabbits of different groups determined during the experiment are summarized in Table 2. The serum urea level of groups A3 and B3 on day 30 were significantly higher (*P<0.05*) in comparison to control group. A significant increase (*P<0.05*) in mean serum concentration of urea was observed in group A3 at day 30 of experiment than those of groups A1, A2, B1, B2 and C. Serum urea values of group B3 at the end of experiment were significantly higher (*P<0.05*) than groups A1, B1, B2 and C.

**Alanine Aminotransferase (ALT):** The serum ALT values recorded in rabbits of various groups during the experiments are given in Table 2. A significantly higher (*P<0.05*) value (68.600±2.592) of ALT in group A3 was recorded at the end of the experiment in comparison to group B1 and control group C.

**Gross pathological lesions:** At the end of experiment (day 30), two rabbits from each group were euthanized for post-mortem and histopathological studies. Gross pathological lesions were restricted to rabbits in group A3 consisting of severe hemorrhages, congestion and emphysema of lungs. In group B3 lesions were congested liver and hepatomegally. In all treatment groups, no gross lesions were observed in intestines, pancreas, spleen and...
gonads. Necropsy did not reveal any abnormal findings in gluteal muscles at injection site in any treatment groups. No post-mortem lesions were seen in control group C.

**Histopathological findings**

**Liver:** Histopathological changes of liver were restricted to rabbits (group A3), consisting of hyperplasia of Kupffer cells; severe congestion and hemorrhage and degeneration of hepatocytes. Liver of rabbits in group B3 showed mild congestion and focal haemorrhages. Vaculation of cytoplasm and acute cellular swelling were evident only in rabbits of group B3.

**Kidney:** Microscopic lesions in kidneys of rabbits (group A3) hemorrhages, moderate tubular cell swelling, epithelial necrosis, karyolysis, cystic dilated tubules and some of cells have vaculation of cytoplasm were seen. In group B3 lesions were observed as moderate congestion and hemorrhages. Normal kidney architecture was seen in rabbits of groups A1, A2, B1 and B2.

**Heart:** Histopathological changes in heart were found in rabbits (group A3) consisting of mild sub-endocardic hemorrhages and congestion. Normal cardiac architecture was seen in rabbits of group A1, A2, B1 and B2. Necropsies of rabbits (group B3) demonstrated moderate degeneration, congestion and multi-focal sub-endocardic hemorrhages.

**Lungs:** Rabbits of group A2 exhibited lesions as mild emphysema, congestion and hemorrhages. Necropsies of rabbits (group A3) revealed severe alveolar lung hemorrhages, focal congestion and eruption of bronchiale epithelium (Fig.2). Necropsies lesions such as mild hemorrhages, emphysema were seen in rabbits of group B3 also (Fig.3).

**Table 1:** Showing the treatments groups of *C. procera* extracts for toxicity evaluation in rabbits A=C. procera chloroform extract, B= *C. procera* water extract, C=DMSO

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Sub groups</th>
<th>Dose</th>
<th>Administration route</th>
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<tbody>
<tr>
<td>A</td>
<td>A1</td>
<td>1mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
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<tr>
<td></td>
<td>A2</td>
<td>5mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
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<tr>
<td></td>
<td>A3</td>
<td>10mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
</tr>
<tr>
<td>B</td>
<td>B1</td>
<td>1mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
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<td></td>
<td>B2</td>
<td>5mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
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<tr>
<td></td>
<td>B3</td>
<td>10mg/ml</td>
<td>I/M, on alternative days, 5 doses</td>
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<tr>
<td>C</td>
<td>-</td>
<td>5ml</td>
<td>I/M, on alternative days, 5 doses</td>
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**Table 2:** Mean measurements of Creatinine, Urea and ALT in rabbits treated with various concentrations of *C. procera* extracts in comparison to control during the experiment. A1= *C. procera* chloroform extract at 1mg/kg BW, A2= *C. procera* chloroform extract at 5mg/kg BW, A3= *C. procera* chloroform extract at 10 mg/kg BW, B1= *C. procera* water extract at 1mg/kg BW, B2= *C. procera* water extract at 5mg/kg BW, B3= *C. procera* water extract at 10 mg/kg BW, C= DMSO (0.98 %) at 5 ml/kg BW.

<table>
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<tr>
<th>Treatment Groups</th>
<th>Day 0</th>
<th>Day 9</th>
<th>Day 30</th>
<th>Day 0</th>
<th>Day 9</th>
<th>Day 30</th>
<th>Day 0</th>
<th>Day 9</th>
<th>Day 30</th>
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<td>0.640±e</td>
<td>0.660±e</td>
<td>0.738±e</td>
<td>29.000±e</td>
<td>34.000±e</td>
<td>35.600±e</td>
<td>53.000±e</td>
<td>54.000±e</td>
<td>58.800±e</td>
<td>0.092±c</td>
<td>0.106±c</td>
<td>0.076±b</td>
<td>1.870±a</td>
<td>3.591±b</td>
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<td>A2</td>
<td>0.520±e</td>
<td>0.760±e</td>
<td>0.780±e</td>
<td>32.200±e</td>
<td>36.200±e</td>
<td>37.600±e</td>
<td>53.000±e</td>
<td>55.200±e</td>
<td>60.400±e</td>
<td>0.063±a</td>
<td>0.107±c</td>
<td>0.066±b</td>
<td>3.152±a</td>
<td>2.437±b</td>
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<td>A3</td>
<td>0.620±e</td>
<td>1.180±c</td>
<td>1.226±c</td>
<td>34.000±e</td>
<td>41.200±e</td>
<td>48.200±e</td>
<td>51.600±e</td>
<td>65.000±e</td>
<td>68.600±e</td>
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<td>B1</td>
<td>0.620±e</td>
<td>0.670±e</td>
<td>0.760±e</td>
<td>30.200±e</td>
<td>33.400±e</td>
<td>34.600±e</td>
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<td>B2</td>
<td>0.604±e</td>
<td>0.780±e</td>
<td>0.804±e</td>
<td>31.600±e</td>
<td>36.400±e</td>
<td>35.600±e</td>
<td>52.000±e</td>
<td>58.400±e</td>
<td>60.400±e</td>
<td>0.058±a</td>
<td>0.038±c</td>
<td>0.048±b</td>
<td>2.379±a</td>
<td>1.208±b</td>
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<tr>
<td>B3</td>
<td>0.584±e</td>
<td>0.984±e</td>
<td>0.860±e</td>
<td>34.000±e</td>
<td>39.800±e</td>
<td>42.600±e</td>
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<tr>
<td>C</td>
<td>0.620±e</td>
<td>0.672±e</td>
<td>0.710±e</td>
<td>33.600±e</td>
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*Values are presented as mean ±SE. Mean having different superscripts (a, b and c) in same column are significantly different (P<0.05).*

DISCUSSION

Herbal medicinal drugs are mostly considered to be harmless but several authors (de Lima et al., 2011) reported the plant as hepatotoxic and cardiotoxic and some others researchers have documented the renal toxicity and hepatic toxicity of the plant (Corns, 2003; Basak et al. 2009; Lin and Will, 2012).

Toxicity of the plant extract was evaluated in performing liver function test. Liver is largest gland and detoxifying organ of animals. Toxic agents are detoxified by the liver and excreted from the body. However if toxic dose is beyond the tolerance level of liver, liver cannot function smoothly. AST and ALT are found in cytoplasm and get releases in serum; elevation in the concentration of AST, ALP and ALT is usually an indicator of hepatic injury (Chavda et al., 2010; Zhang et al., 2012). This study revealed that chloroform extract of C. procera when administered parenterally in animals at dose of 3-5 mg/kg body weight, liver function was not impaired by measuring ALT enzyme. But when it was injected parenterally in rabbits (group A3) at dose of 10 mg/kg body weight, ALT enzyme was significantly increase \( (P<0.05) \) which in turn shows the damage of hepatocytes as previously reported by Remien et al. (2012). In contrast to present findings of ALT, some researchers (Pouokam et al. 2011) reported significantly decrease in ALT serum in rabbits following orally administration of fresh leaves of the plant. In contrast to present findings some authors (Ramachandra et al. 2007) also reported the hepatoprotective effect of C. procera flowers. They administered 70% ethanolic extract of flowers of the plant at dose rate of 200–400 mg/kg against paracetamol-induced hepatitis in rats. After treatment they observed the dose depend response on biochemical markers. In present findings elevation of biochemical marker (ALT) of liver confirmed the toxicity of the C. procera chloroform extract at dose 10mg/kg body weight. It shows that parenterally administration of the plant extract (chloroform extract) at dose of 10 mg/kg body weight have some toxic effect. Difference to previous studies may be due to more potent toxic substance in chloroform extract. In addition to others factors, route of plant extract may attribute to the toxicity in animals. As some of the previous studies (el et al. 1998; Pahwa and Chatterjee, 1988) have also reported the toxic effect of whole latex of the plant following its parenteral and oral administration. They reported that poly-isoprene fraction present in whole latex of C. procera account for its toxicity.

Kidney is excretory organ of the waste substances like creatinine, urea and uric acid (Pouokam et al. 2011). In this work filtering capacity of kidney study was evaluated by measuring creatinine and urea in blood level. It was found that when extracts of plants extracts were injected parenterally at dose of 3 to 5 mg/kg body weight. There was no increased in creatinine and urea. However abnormally high serum levels of creatinine and urea were determined in rabbits treated with water and chloroform extract of C. procera at dose of 10 mg/kg body weight as compared to control groups. These findings suggested that extracts of the plants when injected parenterally at dose of 10 mg/kg body weight led to impair the kidney function. Impaired kidney function...
is mostly consistent with elevated serum creatinine and urea (Kumer et al. 1988).

Creatinine is eliminated from the plasma through glomerular filtration and excreted as a waste product into urine. Elevation in creatinine concentration indicated the impaired renal function (Smith and Hampton, 1990), possibly plant extracts ingredients may be accumulated into the kidney and may led to damage in kidney (Pouokam et al. 2011), accumulation of toxic substance may cause injuries to tubular epithelial cells (Parke, 1982). Creatinine is more specific indicator of kidney injuries (Pouokam et al. 2011), our findings confirmed the harmful effect of the plants extracts at dose rate of 10 mg/kg weight on the kidney, but contrast with another conclusion from Pouokam et al. (2011) describing no harmful effects on organs (kidney) during oral administration of fresh leaves of C. procera in rabbits.

The results obtained in necropsy examination of rabbits over 30 day experiment showed the toxicity lesions at the highest dose rate 10 mg/kg of both extract of C. procera. Biochemical and gross lesions findings were confirmed as microscopic lesions were seen in liver, kidney and heart of rabbits treated with either extract at dose of 10 mg/kg BW. Present histopathological study indicated that C. procera extracts at the highest dose rate have toxic effect in organs of treated rabbits. In line to present study hepatotoxicity and cardiotoxicity has been reported in a previous study (de Lima et al. 2011). Pahwa and Chatterjee,(1988) studied the toxicity of the plant and reported the lesions, very similar to our findings consisting of cytoplasmic granulation, Kupffer cell hyperplasia, hepatocytolysis and hemorrhage in the kidney. Histopathological and biochemical findings suggested that plant extracts at highest dose have toxic effect on liver, kidney, heart and lungs. Toxicity of plant has been reported in several earlier studies (El Badwi et al. 1998; Mossa et al. 1991). But in contrast to our findings several studies (Kumar and Padhy, 2011; Singhal and Kumar, 2009) reported hepatoprotective and renal- protective effect of the plant. Difference from present findings due to substances present in extract may produce toxic effect at high dose when administered parenterally as shown in present study. A previous study (el al. 1998) also explained the toxic effects of C. procera latex when they administered the extracts through intraperitoneal route as compared to oral route in goats.

REFERENCES


