

## Evaluation Of The Toxicological Effects Manifested After Long term Administration Of Aqueous *Calotropis procera* Plant Extract In Male And Female Rabbits

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### ABSTRACT

**Background:** Treatment with aqueous extract of *Calotropis Procera* was examined only for short periods. So, The present study was designed to examine the influences of long term administrations of aqueous extract of *Calotropis Procera* on male and female rabbits.

**Material and methods:** The extract was daily oral administered to thirty adult rabbits divided into two sets each set contain 3 groups (5/each) for a period of 8 weeks. The doses administered were 80 and 40 mg/kg body weight as high and low doses for both male and female test groups.

Percentage of body weight change, serum biochemistry (Alanine amino transferases (ALAT), aspartate amino transferases (ASAT) , Alkaline phosphatase, total protein, albumin, globulin, A/G ratio, urea and creatininre), hematological parameters (PCV, WBCs, RBCs, Platelets and Hb) and the blood indices (M.C.V., M.C.H. and M.C.H.C.) were examined.

**Results:** Results showed that there was no mortality or clinical signs observed among the treatment and the control groups. No significant changes in the percentage of body weight gain, hematological parameters, blood indices has been noticed in all treated groups

Highly significant decreases in serum total protein and albumin in male and female rabbits has been recorded at high doses. While the globulin and A/G ratio revealed no significant changes. Serum ASAT , urea and creatinine levels were highly significant increases ( $P < 0.01$ ) and the activity of ALAT and ALP was significant increases ( $P < 0.05$ ) in male and female rabbits at high doses only.

**Conclusions:** Some toxicological effects were observed after administration of aqueous leaves extract of *Calotropis Procera* at high doses in the long term for male and females rabbits

**Key word:** *Calotropis Procera*, biochemical parameters, Haematology; body weight gains, rabbits

### INTRODUCTION

*Calotropis procera* is a species of flowering desert plant in the dogbane family, Apocynaceae, that is native to North Africa, Tropical Africa, Western Asia, South Asia, and Indochina (Miller & Morris, 1987). The excavation at Helwan in Egypt showed that the plant was in use in Neolithic period in Egypt (Greiss, 1955). It is known as Ushar or Madar in Greeco-Arab medicine and known as Apple of

Sodom, a name derived from the Hebrew Tapuah Sdom also, the plant known as aak for its medicinal properties in Ayurveda (Oudhia & Dixit, 1994). The green globes are hollow but the flesh contains a toxic milky sap that is extremely bitter and turns into a gluey coating resistant to soap (Kumar *et al.*, 2004).

Preliminary phytochemical screening of fresh leaves of *Calotropis procera* in water

extract revealed the presence of phenols, saponins, tannins, glycosides and mixtures of cardenolides (Murti *et al.*, 2010; Pouokam *et al.*, 2011 and Soto *et al.*, 2011).

*Calotropis procera* is used in traditional system of medicine for the treatment of variety of disease conditions that include leprosy, ulcers, and tumors and piles, diarrhoean somatic, sinus fistula and skin diseases. The leaf part is used to treatment of jaundice (Murti *et al.*, 2010) while, milky white latex obtained from the plant exhibits potent anti-inflammatory activity in various animal models that is comparable to standard anti-inflammatory drugs ( Dewan *et al.*, 2000 and Sangraula *et al.*, 2002).

Toxicity of *C. procera* is reported after incidental ingestion of fresh leaves of Giant milkweed by sheep (ruminants) in the form of anorexia and diarrhea, (Mahmoud *et al.*, 1979). The Consumption of this plant leads to severe poisoning to livestock as well as man (Lewis and Elvin-Lewis, 1977). Administration of Giant milkweed leaves to sheep is responsible of tachycardia and transitory cardiac arrhythmia, it also has radiotoxic & hepatotoxic effects after 30 min of administration (District *et al.*, 1983).

The controversial reports about poisoning effects of this plant confused many scientist. So, we planed this work to evaluate and compare the toxic effects of *C. procera* aqueous extract leaves on both sex of rabbits. Also, to evaluate the dose which could be used as medicinal plant for long term without side effects.

## MATERIALS AND METHODS

### *Collection of Plant and extract preparation*

Fresh leaves of *Calotropis procera* used in this experiment were harvested from Fadan Karshe in Kaduna State, Nigeria .The plant was

identified at the Federal College of Forestry, Plateau State, Jos, Nigeria. Two and half kg of the fresh leaves had been extracted with distilled water for 24 hours and then dried at 40°C in hot air oven in the Laboratory of the Biochemistry Department of NVRI, Vom to obtain dry crude extract of the *Calotropis procera*. The stock solution was prepared by dissolving 10 gm of the dried aqueous extract in 250 ml of distilled water (40 mg/ml). The stock solution was stored in the refrigerator at 4 °C.

### *Experimental animals and Groups*

Thirty white New-Zealand adult male and female rabbits 11± 2 week old and weighing 1.5 ± 0.3 kg in average were divided into 6 groups, 3 male and 3 female groups designated as (G1) and (G4) control, (G2) and (G5) daily treatment with high dose aqueous extract of *Calotropis procera* (80mg/kg body weight) for 8 weeks and (3) and (6) daily treatment with low dose of aqueous extract of *Calotropis procera* (40mg/kg body weight) for 8 weeks .

### *Collection of blood sample*

At the end of the experiment, all the surviving animals were sacrificed and blood was collected by cardiac puncture and venipuncture into tow bottles, the first one containing ethylene diamine tetraacetate (EDTA) for haematology analysis and the second bottle without EDTA. Sera were separated by centrifugation at 3000 rpm for 15 minutes and keep at - 40 °C till used for biochemical assays.

### *Percentage of body weight gain:*

All animals groups (control and treated ) were individually weighed in order to detect any changes in their body weights. The percentage of body weight gain was calculated as follows

$$\text{Percentage of body weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{M.C.H.C.} = \frac{\text{gm. Hgb./100 ml.} \times 100}{\text{Packed Cell Volume (PCV)}}$$

**Biochemical analysis**

Serum total protein was evaluated according to the method of **Doumas et al. (1975)** and serum albumin by method of **Doumas (1971)**, while Alanine amino transferases (ALAT) and aspartate amino transferases (ASAT) were determined as the method of **Reithman and Frankel (1957) and the Alkaline phosphatase (ALP)** by the method of **Babson (1965)**.

Serum creatinine levels were determined by **methods of Heinegard and Tiderstrom (1973)**, while serum urea was determined as the method of **Marsh et al. (1965)**.

**Hematological analysis**

The hematological parameters ; Packed Cell Volume (PCV), hemoglobin concentration (Hb), Red Blood Cells count (RBC), White Blood Cells count (WBC) and blood Platelets count were determined by methods described by **Baker et al. (1998)**.

The blood indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) for particular blood samples were also calculated as the following.

**Mean corpuscular volume (M.C.V.)**

$$\text{M.C.V.} = \frac{\text{Packed Cell Volume (PCV)} \times 10}{\text{RBC in millions/cu.mm.}}$$

**Mean Corpuscular Hemoglobin (M.C.H.)**

$$\text{M.C.H.} = \frac{\text{gm. Hgb./100 ml.} \times 10}{\text{RBC in millions/cu. mm.}}$$

**Mean Corpuscular Hemoglobin concentration (M.C.H. C.)**

**Statistical analysis of the data:**

The obtained data were expressed as a mean ± standard error (SE) and the analysis were revised by Quattro Pro statistical program for windows program version 2- Microsoft Windows version 7. The significance of difference between the means was calculated according to Student’s t-test. Statistical significance was accepted at  $P < 0.05$ .

**RESULTS**

The results for % of body weight gain are shown in table1 below. All the rabbits showed a consistent increase in weight. The result is not surprising as the rabbits did not show any sign of anorexia and were feeding very well (table. 1). The hematological test results for male and female test groups are shown in tables 2 and 3 .The results revealed no significant difference between the groups with all the values obtained lying within the normal range.

The data were tabulated in table (4) revealed highly significant decreases in serum total protein and albumin in males and females at high doses. While the globulin and A/G ratio revealed no significant difference between the groups.

The represented data (Table, 5and 6) indicated that marked elevation in the activity of ASAT, urea and creatinine, these elevations were statistically highly significant ( $P < 0.01$ ) in males and females at high doses. Furthermore, the levels of ALAT (IU/L) and ALP (IU/L) with the same previous dose showed statistically significant increases ( $P < 0.05$ ) in males and females.

**Table (1): Effect of daily oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract on the percentage of weight gain in male and female rabbits.**

Sex	Groups	%Weight gain
Male	Control(G1)	20.00± 1.52
	High dose (G2)	27.15± 3.44
	Low dose(G3)	22.16± 2.87
Female	Control(G4)	33.33± 4.22
	High dose(G5)	33.57± 3.11
	Low dose(G6)	22.93± 3.10

**Table (2): Values of the hematological parameters after oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract in male and female rabbits**

Sex	Groups	PCV (%)	Hb (gm/dl)	WBC (x10 <sup>3</sup> / mm <sup>3</sup> )	Platelets (x10 <sup>9</sup> /l)	RBC (x10 <sup>6</sup> / mm <sup>3</sup> )
Male	Control(G1)	36.40±03.65	12.76±1.05	09.52±00.85	198.00±16.08	6.23±0.23
	High dose (G2)	38.40±02.97	13.89± 0.23	10.70± 00.61	193.40±22.20	6.70± 0.44
	Low dose(G3)	37.20±03.03	13.98±0.28	09.90±01.16	230.00±34.08	6.56±0.25
Female	Control(G4)	36.25 ±04.79	12.55 ±02.72	09.43±00.38	205.50±14.80	5.96±1.35
	High dose(G5)	37.60±03.58	11.99±01.89	10.22± 01.35	227.00±16.09	6.00± 0.43
	Low dose(G6)	37.40±04.97	12.62±01.61	09.66± 02.40	202.40±27.94	6.26± 0.52

**Table (3): Values of some blood indices after oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract in male and female rabbits**

Sex	Groups	MCV (fl)	MCH (pg)	MCHC (g/dL)
Male	Control(G1)	58.42±4.62	19.38±2.56	35.06±1.74
	High dose (G2)	57.31±3.31	19.54±2.84	36.17±2.35
	Low dose(G3)	56.71±2.36	20.07±1.03	37.58±3.33
Female	Control(G4)	60.82±4.11	18.50±1.55	34.86±2.09
	High dose(G5)	60.67±5.89	16.32±2.99	32.71±3.77
	Low dose(G6)	59.74±3.00	19.27±1.12	33.74±2.87

The result expressed in means ± SE (standard error)

**Table (4): Effect of daily oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract on serum total protein, albumin, globulin and A/G ratio in male and female rabbits.**

Sex	Groups	Total protein	Albumin	Globulin	A/G ratio
Male	Control(G1)	5.55±0.226	3.41±0.174	2.14±0.197	1.59±0.123
	High dose (G2)	4.77**±0.095	2.70**±0.080	2.07±0.086	1.30±0.83
	Low dose(G3)	5.10±0.232	3.08±0.156	2.24±0.097	1.52±0.55
Female	Control(G4)	5.70±0.257	3.42±0.111	2.24±0.225	1.53±0.109
	High dose (G5)	4.82**±0.111	2.60**±0.075	2.22±0.156	1.17±0.097
	Low dose(G3)	5.20±0.127	3.08±0.117	2.12±0.066	1.45±0.069

**Table (5): Effect of daily oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract on serum ASAT (IU/L), ALAT (IU/L) and ALP (IU/L) in male and female rabbits**

Sex	Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Male	Control(G1)	91.42 ± 17.11	84.2 ± 18.23	46.48 ± 7.67
	High dose (G2)	139.33**±29.07	97.77*±9.15	64.39*± 11.89
	Low dose(G3)	100.39 ± 19.43	95.06 ± 16.09	48.37 ± 8.55
Female	Control(G4)	89.50 ± 18.01	83.83 ± 15.22	45.75± 8.35
	High dose(G5)	119.00**± 25.74	97.22*± 5.98	61.86*± 9.55
	Low dose(G6)	96.25 ± 14.32	88.33 ± 12.06	53.96± 5.45

**Table (6): Effect of daily oral administration of low and high dose (40 and 80mg/kg body weight) of *Calotropis procera* aqueous extract on serum urea (mg/dl) and creatinine(mg/dl) in male and female rabbits.**

Sex	Groups	Urea (mg/dl)	Creatinine (mg/dl)
Male	Control(G1)	34.44 ± 5.25	0.68 ± 0.12
	High dose (G2)	48.66**±6.30	1.17** ± 0.24
	Low dose(G3)	34.32 ± 3.73	0.89 ± 0.14
Female	Control(G4)	32.72 ± 4.33	0.59± 0.13
	High dose(G5)	50.48**± 7.49	1.30**± 0.26
	Low dose(G6)	37.87 ± 5.89	0.85± 0.19

The result expressed in means ± SE (standard error)

\* = Significant (P < 0.05)

\*\* = Highly significant (P < 0.01)

## DISCUSSIONS

Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care. Although many investigators has been reported that *C. procera* possess various medicinal functions, but it has some unknown toxics effects. This work designed to investigates the influence of the chronic aqueous leaves extract of *C. procera* on both sex of rabbits.

The results of the present study (Table, 1) revealed that, percentage of weight gain of all rabbits treated with aqueous leaves extract of *C. procera* showed no significant changes in these body weight. These results agreement with data observed by **Mbako et al. (2009)**, in rabbits and **Radunz et al. (1983)**, in cattle and sheep.

On other hand, *C. procera* plants causes anaemia and weight loss as a results of mild diarrhoea and loss of fluid or serous discharge could be attributed to erosion of mucous lining of the gastro-intestinal (**Mahmoud et al., 1979 and Dada et al., 2002**)

In all tested groups, the values of haematological parameters (PCV, Hb WBC, RBC and platelets) as well as blood indices (MCV, MCH and MCHC) showed no statistical significance and these values still remained within normal reference ranges, these data are in agreement with the reports of **El-Shafey et al. (2011) and Guy et al. (2011)**

In contradiction to these results, **Mahanmoud et al. (1979)** observed decreases in hemoglobin concentrations of the animals treated with *C. procera* extract, further suggests that prolonged administration of the

extract may cause anaemia due to the presence of some toxic reduced in this extract

The obtained results (Table, 6) showed, highly significant ( $P < 0.01$ ) increases in the levels of serum urea (mg/dl) and creatinine (mg/dl) in both male (G2) and female (G5) rabbits after administered high doses of aqueous extract of *Calotropis procera* only and the variations in the two previous parameters were not significant at low dose of *Calotropis procera* in both sex. These results go in agreement with the results of **Pouokam, et al. (2011)** who reported, marked increases in these parameters after administered aqueous extract of *Calotropis procera*.

These elevations may be due to the accumulation of toxic doses of the active principles of the plant extract, which causes damage of the kidney cells and failure of kidney functions (**Eissa and Zidan, 2010**). These accumulation may be cause degeneration of the tubular epithelial cells (**Parke, 1982**) and/or necrosis of the cells of the renal convoluted tubules (**Mahmoud et al., 1979**). This reduced renal blood flow associated with higher serum urea concentration may impair the secretary function of the kidney (**Whealton et al.,1994**)

The obtained results elucidated, highly significant increases ( $p < 0.01$ ) of ASAT and significant ( $p < 0.05$ ) increases in ALAT and ALP in both sex of rabbits ( G2 and G5) after administered high doses of aqueous extract of *Calotropis*. These increases may be due to damage/or dead liver cells under the toxic effects of high active principles of plant extract

and cause diffusion of these enzymes from the intracellular sites (Tilkian *et al.*, 1983 and Jimoh and Odutuga, 2001).

In contradiction to these results, Ali (2006) recorded decreases in ALAT and ASAT after 15 days of treatment with *Calotropis* extract (oshar). Also, Pouokam *et al.* (2011) observed that, no significant change was noticed in the activity of ASAT and ALP and significant decreases ( $p < 0.05$ ) were found in the activity of ALAT in smaller rabbits after administered high doses of aqueous extract of *Calotropis*

The obtained data (Table 4) showed that serum total protein and albumin were decreased after administered of high doses of aqueous extract of *Calotropis* in both sex of

rabbits. This decreases may be a result of the damaged effect of plant extract on liver. The liver is a major organ of protein synthesis and any diseases in the liver can cause damage of hepatocytes with changes in protein and free amino acid metabolism and decreases their synthesis in the liver and increase wasting via catabolism (Yousef *et al.*, 2006; Wallace, 2007 and El-Shafey *et al.*, 2011).

On the other hand, Mbako (2009) observed that, Serum albumin and total proteins of rabbits showed slight variations after administration aqueous extract of fresh leaves of *Calotropis procera* also reported in rats (Dada *et al.*, 2002). These variations were not statistical significance, this may be because the liver was not greatly damaged.

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## تقييم التأثيرات السامة بعد المعاملة لفترة طويلة بالمستخلص المائي لأوراق نبات *Calotropis procera* على ذكور وإناث الأرناب

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تهدف هذه الدراسة إلى تقييم ومقارنة التأثيرات السامة لتعاطي المستخلص المائي لأوراق نبات *Calotropis procera* لمدة طويلة على كلا من ذكور وإناث الأرناب وأيضا للوقوف على مدى صلاحية النبات للاستعمال في الطب الشعبي.

استخدم في هذا البحث عدد 30 أرناب (15 ذكور و15 إناث) قسمت كلا منهما إلى ثلاث مجاميع كل مجموعة تحتوى على خمس أفراد , المجموعة الأولى كمجموعة ضابطة والثانية تم إعطائها جرعة يومية كبيرة من المستخلص المائي لأوراق نبات *Calotropis procera* (80 ملجم /كجم من وزن الجسم) لمدة 8 أسابيع والمجموعة الثالثة تم إعطائها جرعة يومية صغيرة من المستخلص المائي لأوراق نبات *Calotropis procera* (40 ملجم /كجم من وزن الجسم) لمدة 8 أسابيع .

أوضحت النتائج عدم وجود أى تغيرات ذي دلالة إحصائية في وزن الجسم أو عدد كريات الدم الحمراء و البيضاء والصفائح الدموية وكمية الهيموجلوبين وPCV وكذلك أيضا في مؤشرات الدم (M.C.V., M.C.H. and M.C.H.C.) في جميع المجاميع (الذكور والإناث)

كما ظهرت النتائج أيضا وجود نقص ذو دلالة معنوية في مصلى البروتين الكلى و الالبومين فقط في المجموعات (الذكور والإناث) التي أعطيت جرعات كبيرة من المستخلص النباتي أما التي أعطيت جرعات صغيرة من نفس المستخلص فلم يحدث لها أى تغيرات معنوية

وقد دلت النتائج إلى حدوث زيادة معنوية في إنزيمات الكبد ووظائف الكلى في المجموعات (الذكور والإناث) التي أعطيت جرعات كبيرة من المستخلص النباتي أما التي أعطيت جرعات صغيرة من نفس المستخلص فلم يحدث لها أى تغيرات معنوية

مع أن بعض الأبحاث تجيز استعماله في الطب الشعبي ولكن لوحظ من النتائج السابقة أن هذا النبات له بعض التأثيرات السامة عند استعماله بجرعات كبيرة لمدة طويلة أما الجرعات الصغيرة فتعتبر مأمونة، للوقوف على الجرعة المناسبة للاستعمال، فهذا بحاجة إلى دراسات تكميلية مستقبلية خاصة لدراسة الجرعات التراكمية على المدى البعيد.