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

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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, cazuela, algodón extranjero in Spanish, Frensh 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:

Proportion of Weight Win = Weight at the end of the experiment - Weight at the beginning of the experiment / Weight at the beginning of the experiment × 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 $p \leq 0.05$ 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.

%Weight win	Groups
30.34±6.68	I
40.92±1.9	II

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.

	Group	0	2W	4W	6W	8W
W	I	18.33±0.74b	16.61±0.73a	18.2±0.89b	20.28± 1.43c	22±1.39cd
	II	17.86±0.97	18.23±1.06	19.29±1.26	22.23±1.84	23.71±1.53
H	I	91.44±3.24b	92.78±4.41b	95.56±3.0c	73.56±2.89a	71.22±2.99a
	II	92.4±5.79a	90±5.36a	88.6±4.11a	91.4±3.54a	89.8±4.49a
R	I	23.44±1.33a	22.44±1.71a	22.89±2.01b	29.44±2.14b	32.33±3.86bc
	II	23.8±1.66	25.4±1.7	25±1.79	24±2.35	24.4±1.54
T	I	39.61±0.11	38.39±0.17	38.29±0.2	39.6±0.09	39.41±0.08
	II	39.02±0.11	39.2±0.26	39.06±0.24	39.14±0.24	39.04±0.23

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.

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

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

Shown in table 4, results of serum levels of urea and creatinine changes. It is significantly elevated at ($p < 0.05$) in values of Urea through all treated periods. The highest level was in the 8th week. Creatinine levels elevated through periods of exposure but significantly increased only in 8th weeks.

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), reveled 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 an 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).

Table 4: Influence of administration of aqueous extract of *C. procera* leaves on serum urea level (mg/dl), as well as, serum creatinine (mg/dl) in goats used in study.

Test	Group	0	2W	4W	6W	8W
UREA	I	23.03±2.34a	26.03±1.34b	30.91±2.71bc	30.96±3.21bc	31.6±2.47bc
	II	22.79±1.82	23.15±1.39	23.24±1.87	22.89±2.24	21.98±2.21
CREA	I	1.92±0.38a	2.11±0.33a	2.06±0.19a	2.08±0.18a	2.41±0.54b
	II	1.96±0.22	2.01±0.32	1.76±0.37	1.73±0.16	1.91±0.32

CREA= creatinine, data expressed in means ± SE (standard error).

Phytochemical checking of *C. 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

Abbas, B., A.E. Tayeb and Y.R. Sulleiman (1992). *Calotropis procera*: feed potential for arid zones. *Veterinary Record.*, **131(6):** 132.

Al-Snafi, A.E. (2015). The Constituents and Pharmacological Properties of Calotropis procera-An Overview. *International Journal of Pharmacy Review Research.*, **l5:** 259-75.

Begum, N., B. Sharma and R.S. Pandey (2010). Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. *J. Biofertil. Biopestici.,***1(1):** 1-6.

Basak, S.K., A. Bhaumik, A. Mohanta and P. Singhal (2009). Ocular toxicity by latex of *Calotropis procera* (Sodom apple). *Indian J. Opthalmol*., **57(3):** 232-4.

Boulos, L. (2000). Flora of Egypt, Vol. 2 Geraniaceae-Boraginaceae. Al Hadara Publishing, Cairo, Egypt.

Chavda, R., K.R. Validia and R. Gokani (2010). Hepatoprotective and Antioxidant Activity of Root Bark of *Calotropis procera* R.Br (Asclepediacea). *Int. J. Pharmacol*., **6(6):** 937-43.

Clarke, E.G.C. and M.L. Clarke (1977). Veterinary Toxicology Cassel and Collier Macmillan Publishers London: 268.

Dada, Y.O., M.T. Lamidi, K.I. Eghianruwa and F. Adepoju (2002). Effects of Oral Administration of the Latex of *Calotropis procera* on weights, Hematology and Plasma Biochemistry in Rats. *Trop. Vet.,* **20:** 4.

Delmar, J.G and A. Bruce (1994). Concise Review for Primary-Care Physicians (Unexplained Dyspnea). *Mayo Clin Proc*., **69:** 657-63.

De Lima, J.M., F.J. De Freitas, R.N. Amorim, A.C. Camara, J.S. Batista and B. Soto-Blanco (2011). Clinical and pathological effects of *Calotropis procera* exposure in sheep and rats. *Toxicon*., **57(1):** 183-5.

Department of Employment, Economic Development and Innovation (*DEEDI*) (2016).Weeds of Australia - Biosecurity Queensland Edition Fact Sheet. website at www.biosecurity.qld.gov.au.

District, R., G. Wilson and G. Beere (1983). Toxicity experiments of rubber bush fed to cattle and sheep (*Calotropis procera*). *Technote.,* **30:** 1-3.

Dixon, W.J. (1980). Efficient analysis of experimental observation. *Ann. Rev. Pharmacol. Toxicol.*, **20:** 441-62.

Eissa, F.I and N.A. Zidan (2010). Haematological, biochemical and histopathological alterations induced by abamectin and *Bacillus thuringiensis* in male albino rats. *J. of Basic and Applied Sci*., **3(3):** 2497-505.

El- Shafey, A.A.M., M.M.E. Seliem, F. El-Mahrouky, W.M. Gabr and R.A. Kandil (2011). Some Physiological and Biochemical Effects of Oshar Extract and Abamectin Biocide on Male Albino Rats. *Journal of American Science.*, **7(12):** 254-61.

Erdman, M.D. (1983). Nutrient and Cardenolide Composition of Unextracted and Solvent-Extracted *Calotropis procera*. *J. Agric. Food Chem.,* **31(3):** 510-3.

Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Wiley and Sons, Inc. New York.

Hall, H.T.B. (1977). Diseases and Parasites of Livestock in the Tropics. Longman Group Ltd. : 273-5.

Imosemi, I.O. (2016). Evaluation of the Toxicity, Medicinal Use and Pharmacological Actions of *Calotropis procera*. *E.J.P.M.R.,* **3(9):** 28-36.

Jimoh, F.O. and A.A. Odutuga (2001). Changes in the activities of some diagnostic enzymes in some rat tissues following the consumption of thermally oxidized groundnut oil. Nig. *J. Biochem. Mol. Biol*., **16:** 173-6.

Lewis, W.H. and D.M.F. Elvin-Lewis (1977). Medical Botany: Plants that affect Man's Health. John Wiley, London.

Lin, Z. and Y. Will (2012). Evaluation of drugs with specific organ toxicities in organ specific cell lines. *Toxicol. Sci*., **126(1):**114-27.

Little, E.L. Jr., R.O. Woodbury and F.H. Wadsworth (1974). Trees of Puerto Rico and the Virgin Islands. Vol. 2. Agriculture Handbook 449. U.S. Department of Agriculture, Washingtion, DC.: 1024.

Mahmoud, O.M., S.E.I. Adam and G. Tartour (1979). The effects of *Calotropis procera* on small ruminants. I. Effects of feeding sheep with the plant. *J. Comp. Pathol*., **89:** 241-50.

Mbako, J.D., Z. Adamu, J.K. Afutu, A. Aliyu, S. David, M.B. Umar and C. Nduaka (2009). Effects of the aqueous extract of fresh leaves of *Calotropis procera* on haematological and biochemical parameters in female rabbits. *African Journal of Biotechnology*., **8(19):** 5071-5.

Meena, A.K., M.M. Yadav, U.S. Niranjan, B. Singh, A.K. Nagariya, K. Sharma, A. Gaurav, S. Sharma and M.M. Rao (2010). A review of *Calotropis procera* Linn and its ethnobotany, phytochemical and pharmacological profile. *Drug Invent Today.,* **2(2):** 185-90.

Murti, Y., B. Yogi and D. Pathak (2010). Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *International Journal of Ayurveda Research*., **1(1):** 14-7.

Orwa, C., A. Mutua, R. Kindt, R. Jamnadass and S. Antony (2009). Agroforestry database: a tree reference and selection guide version 4.0. World Agroforestry Center, Kenya.

Parihar, G., A. Sharma, S. Ghule, P. Sharma, P. Deshmukh and D.N. Srivastava (2011). Anti-inflammatory effect of *Calotropis procera* root bark extract. *Asian Journal of Pharmacy and Life Science*., **1(1):** 29.44.

Parke, D.V. (1982). The Handbook of Environmental Chemistry: 1 Springer Verlag, *Berlin.*, 141-78.

Parrotta, J.A. (2001). Healing plants of Peninsular India. CAB International, Wallingford, UK and New York.

Parsons, W.T and Cuthbertson, E.G. (2001). *Noxious Weeds of Australia*. 2nd ed. CSIROPublishing: Melbourne :712.

Poindexter, B., W. Feng, A. Dasgupta and R. Bick (2007). Oleandrin produces changes in intracellular calcium levels in isolated cardiomyocytes, a real-time fluorescence imaging study comparing adult to neonatal cardiomyocytes. *J. Toxicol. Environ. Health.*, **70:** 568-74.

Pugh, D.G. (2002). A text Book of Sheep and Goat Medicine. 1st edition. W.B. Saunders, USA., 0-7216-9052-7211.

Radunz, B.L., G. Wilson and G. Beere (1983). Toxicity Experiments of Rubberbush Fed to Cattle and Sheep *(Calotropis procera)*. *Technote*., **30:** 1-3.

Rahman, M.A and C.C. Wilcock (1991). A taxonomic revision of Calotropis (Asclepiadaceae). *Nordic Journal of Botany.*, **11:** 301-8.

Russell, D.J., M.H. Al-Sayah and F.M. Munir (2011). Volatile compounds produced by *Calotropis procera* (Family: Asclepiadaceae) leaves that aid in the repulsion of grazers. *Journal of Biodiversity and Ecological Sciences*., **1(3):** 191-6.

Shahat, M.A. and A.M.A. Shihata (2012). Evaluation of the toxicological effects manifested after long term administration of aqueous *Calotropis procera* plant extract in male and female rabbits. *Egypt. J. Hospital Med*., **47:** 291-300.

Shanker, A. (2005). Hand book of poisoning, bgalani publishing house, 2nd edition: 736.

Shoaib, Q., M. Kumkum and A. Sandeep (2013). *Calotropis procera*: An overview of its phytochemistry and pharmacology. *Indian Journal of Drugs*., **1(2):** 63-9.

Shrivastava, A., S. Singh and S. Singh (2013). Phytochemical investigation of different plant parts of *Calotropis procera. International Journal of Scientific and Research Publications.,* **3(3):** 1-4.

Smith, C.L. and E.M. Hampton (1990). Using estimated creatinine clearance for individualizing drug therapy: a reassessment. *D.I.C.P*., **24:** 1185-90.

Tilkian, S.M, M. Conover and A.G. Tilkian (1983). Clinical implications of laboratory tests. The C.V. Mosby Company, Louis, Toronto, London.

Upadhyay, R.K. (2014). Ethnomedicinal, pharmaceutical and pesticidal uses of *Calotropis procera* (Aiton) (Family: Asclepiadaceae). *International Journal of Green Pharmacy.,* 135-146.

Verma, R., G.P. Satsangi and J.N. Shrivastava (2013). Analysis of phytochemical constituents of the ethanolic and chloroform extracts of *Calotropis procera* using gas chromatography-mass spectroscopy (GC-MS) technique. *J. Med. Plants Res*., **7(40):** 2986-91.

Zhang, Y., Y. Huang, X. Deng, Y. Xu, Z. Gao and H. Li (2012). Iron overload-induced rat liver injury: Involvement of protein tyrosine nitration and the effect of baicalin. *Eur. J. Pharmacol.*, **(1-3):** 95-101.