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Phyto-Pharmacological Study and Therapeutic Efficacy of *Calotropis procera* (Flower) Against Theileriosis in Cattle

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ABSTRACT

Calotropis species are common wasteland weeds, widely used as alternative therapeutic tool for the prevention or treatment of many diseases. This study was designed to evaluate the phytochemical analysis, acute toxicity studies and anti-theilerial acitivity of flowers of *Calotropis procera* in cattles. The results showed that the alkaloids, flavonoids, amino acids, saponin, tannins, steroids, glycosides and phenols were found in the twelve different solvent extracts of *Calotropis procera*. Acute toxicity studies revealed absence of toxicity symptoms upto 600 mg/kg body weight in mice. Comparative efficacy of Buparvaquone @ 2.5 mg/kg body weight and flower extract of *Calotropis procera* @ 0.3 mg/kg body weight was evaluated against the sub clinical theileriosis in cows. The haematological study revealed low Hb, PCV, TEC, TLC and increase in eosinophil percent in both the infected groups on day "0" (before treatment). Both the treatments improved altered levels of Hb, PCV, TEC, TLC and eosinophil percent within 21st day post treatment as compared to pre treatment values. The therapeutic study revealed that the percent efficacy of buparvaquone (100%) was higher as compared to *Calotropis procera* (83.67%). The study concluded that Buparvaquone and *Calotropis procera* found effective against sub clinical theileriosis in cows with improvement in haematological parameters.

Keywords: *Calotropis procera*, buparvaquone, theileriosis, phytochemical analysis, herbal medicine

Theileriosis is one of the most important haemoprotozoan disease cause devastating economic losses to the livestock industry and pose major constraints to the dairy industry throughout the world. *Theileria annulata* and *Theileria parva* are considered to be the most pathogenic species of *Theileriosis*. Tropical theileriosis is one of the most prevalent disease of cattle caused by *T. annulata* (Mirzaei, 2007) and is transmitted through *Ixodid* tick of genus *Hyalomma*. The effective drugs for the treatment of Theileriosis are available. Research work regarding the efficacy of these drugs has shown that buparvoquone, second generation hydroxynaphthaquinone found more effective. However, due to high cost, buparvoquone is not affordable for most of the farmers. Medicinal plants have remained the major sources of discovery of new drugs. In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin for the treatment of various diseases in man and animals. *Calotropis procera* family is small, erect and compact shrub, which is used in several traditional medicines to cure various diseases. This shrub has been known to possess analgesic, antitumor, antihelmintic, antioxidant, hepatoprotective, antidiarrhoeal, anticonvulsant, antimicrobial, oestrogenic and antimalarial activity. Durrani *et al*. (2009) conducted therapeutic trails of *Calotropis procera* and buparvaquone (Butalex) in experimental infection with *Theileria*

annulata in cross bred cattle and reported good efficacy of the plant.

Considering the easy availability and having medicinal properties, the flowers of *Calotropis procera* plant was selected to evaluate the phytochemical analysis (qualitative), acute toxicity study in laboratory animals and therapeutic efficacy against theileriosis in cattle.

MATERIALS AND METHODS

The flowers of *Calotropis procerawere collected,* shade dried and powdered after authentication by expert taxonomist, Department of Botony, Shri Shivaji Science College, Akola. The herbarium sheet of the authentic plant has been presented in the Department of Veterinary Clinical Medicine, PGIVAS, Akola.

Preparation of extract and determination of per cent extractability

The freshly prepared powder of flowers (25 g) of *Calotropis procera* was immersed in hydro-ethanolic solution (40% distilled water + 60 % ethanol) in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The contents of the flask were filtered through muslin cloth. The residue left in the flask was rinsed with little quantity of hydro-alcoholic solvent and filtered through the muslin cloth. The filtrate thus obtained was filtered through Whatman No. 1 filter paper. Final filtrate, so obtained was transferred to previously weighed large petri dish and was kept for evaporation of solvent at room temperature. After complete evaporation, the petri dish was once again weighed to know the amount of extract. The per cent extractability was determined. The extract was stored in airtight screw cap vials and kept in the desiccators until further used in this study.

Phytochemical analysis

In phytochemical analysis (qualitative) flowers of *Calotropis procera* was extracted in twelve different solvent viz. acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether and xylene. These extracts were tested qualitatively for the presence of the active phytochemical constituents.

Acute toxicity studies

Acute toxicity was performed according to the OECD-423 guide lines. Twenty four swiss albino mice of either sex (20 – 25 g) were used in four groups. The animals were administered with 50, 300, 600 and 1000 mg/kg body weight of extract of *Calotropis procera* orally. The animals were observed for 24 hours, then for further 14 days for deaths and manifestation of any toxic effects. The toxic effects like agility, muscular tremors and convulsions were recorded.

Therapeutic study

The cattle presented to Teaching Veterinary Clinical Complex, Veterinary Polyclinic and Veterinary Dispensaries in and around Akola District, suspected with heamoprotozoan infection were screened for sub clinical Theileriosis by blood smear examination. The blood smear was stained with Giemsa stain and examined for the presence of blood protozoan parasite, followed by confirmation with PCR technique. Animals selected for screening based on the symptoms of rough hair coat, presence of ticks on the body, enlarged lymph node, lacrimation, reduced milk yield and appetite (Arunachalam *et al*., 2016).

Total 12 adult cows positive for sub clinical theileriosis were selected and divided randomly into 2 equal groups comprising of six animals in each group. First group (Group I) of cows positive for sub clinical Theileriosis was treated with *Calotropis procera* flower extract @ 0.3 mg/kg body weight given 8 doses orally on alternate days. Second group (Group II) of cows positive for sub clinical theileriosis was treated with Buparvaquone @ 2.5 mg/kg body weight intramuscularly as a single dose. All the treated cows were again tested for Theileriosis by polymerase chain reaction technique after treatment on 21st day to evaluate the efficacy of therapy.

Haematological parameters

Haematological parameters such as Haemoglobin (Hb), Packed cell volume (PCV), Total leucocyte count (TLC), Total erythrocyte count (TEC) Differential leucocyte

count (DLC) were estimated as per the method described by Benjamin (2001) on day "0" (before treatment) and on 7th and 21st day post treatment.

RESULTS AND DISCUSSION

Phytochemical (Qualitative) analysis of *Calotropis procera* was carried out to know the active constituents present in the above mentioned plants (Table 1). Alkaloids, flavonoids, amino acids, saponin, tannins, steroids, glycosides and phenols were found in the twelve different solvent extracts of *Calotropis procera*. The studies reported several compounds of confirmed biological activity such as alkaloids, flavonoids, amino acids, saponin, tannins steroids, glycosides and phenols from *Calotropis procera* which are in agreement with the present study (Hiren*et al.,* 2011; Sharma *et al.,* 2012; Patel *et al.,* 2014).

In acute toxicity studies, *hydro*-ethanolic extract of flower of *Calotropis procera* was administered at 50, 300, 600 and 1000 mg/kg body weight to set of six mice per group. The study showed absence of toxicity symptoms upto administration of 600 mg/kg body weight. There were no lethality but itching and restlessness was found @ 1000 mg/kg of the *Calotropis procera* extract.

The study revealed that *Calotropis procera* ethanolic flower extract was found non toxic and well tolerated @ 0.3 mg /kg body weight employed in this study as reported by Durrani *et al*. (2009). It was found that maximum tolerable dose 1000 mg/kg body weight in mice. The dose employed in this study for therapeutic evaluation was 0.3 mg/kg body weight which was 10 times lesser than maximum tolerable total dose and was very safe in cattle.

The group I treated with *Calotropis procera* flower extract @ 0.3 mg/kg body weight orally total eight doses administered on alternate day. Results revealed that out of 6 positive animals for theileriosis, 5 animals recovered completely on 21st day post treatment based on PCR assay indicated 83.67% effectiveness of treatment. The group II treated with Buparvoquone @ 2.5 mg/kg body weight intramuscularly as a single dose showed 100% efficacy on 21st day post treatment as evident by complete recovery of all six animals and based on post treatment PCR assay as well. The results of the present study indicated that the efficacy of buparvaquone was comparatively high as compared to *C. procera* after 21st day post treatment. Similar therapeutic study was also conducted by Durani *et al.* (2009) they evaluated efficacy of *C. procera* @ 0.3 mg/kg body weight orally (eight doses on alternate days) and Buparvoquone @ 2.5 mg/kg body weight intramuscularly as a single dose in experimentally induced infection of *Theileria annulata* in cross bred cattle. However, the results reported higher efficacy of *C. procera* (92.5%)

Table 1: Phytochemical analysis (qualitative) of Flower of *Calotropis procera*

Solvent used	Active principle										
	Alkaloides	***Flavon oides***	***Anthra quinone***	***Amino acid***	***Protein***	***Sap onin***	***Tanins***	***Sterol***	***Reducing sugar***	***Glyo sides***	***Phenolics***
Acetic acid	–	++	–	++	–	+	–	+	–	–	+++
Acetone	–	–	–	++	–	–	–	–	–	–	–
Benzene	+	–	–	–	–	++	++	–	–	+++	–
Chloroform	–	–	–	–	–	++	++	+	–	+++	–
Ethyl acetate	–	–	–	–	–	–	+++	+	–	–	–
Ethanol	++	–	–	+	–	++	+	++	–	–	–
Hexane	–	–	–	–	–	–	++	+	–	–	–
Hydroethanol	++	+++	–	+++	–	+	+	+	–	–	++
Methanol	–	–	–	+++	–	–	+	–	–	–	+
Petroleum ether	+++	–	–	–	–	+	+++	++	–	–	–
Xylene	++	–	–	–	–	++	+++	+	–	–	–
Water	+++	++	–	–	–	–	–	–	–	–	+

– Nil; + Mild; ++ Moderate; +++ Abundance

Table 2: Mean haematological values of *Calotropis procera* treated group and Buparvaquone treated group in Theileriosis affected cows

Blood Parameters	Intervals / Treatment Groups	0 Days	7 Days	21 Days	Pooled Mean
Hb	T1	8.03 ± 0.78	8.33 ± 1.03	8.38 ± 0.92	**8.25** ± 0.50
	T2	7.30 ± 0.84	8.02 ± 0.82	8.20 ± 1.19	**7.84** ± 0.53
	Pooled Mean	**7.67** ± 0.56	**8.18** ± **0.63**	**8.29** ± **0.72**	
PCV	T1	28.73 ± 2.01	32.01 ± 2.32	32.29 ± 3.96	**31.01** ± 1.62
	T2	27.92 ± 2.51	30.92 ± 2.21	33.79 ± 2.73	**30.88** ± 1.47
	Pooled Mean	**28.33** ± 1.54	**31.46** ± **1.54**	**33.04** ± **2.30**	
TLC	T1	8.26 ± 0.74	10.25 ± 0.51	10.84 ± 0.52	**9.78** ± 0.42
	T2	8.44 ± 0.87	9.80 ± 0.76	10.33 ± 0.69	**9.52** ± 0.46
	Pooled Mean	**8.35** ± 0.54^{A}	**10.03** ± **0.44** ^{B}	**10.58** ± **0.42** ^{B}	
TEC	T1	7.14 ± 0.49	7.61 ± 0.62	7.80 ± 1.14	**7.52** ± 0.44
	T2	6.54 ± 0.76	7.09 ± 0.94	7.78 ± 1.15	**7.14** ± 0.53
	Pooled Mean	**6.84** ± 0.44	**7.35** ± **0.54**	**7.79** ± **0.77**	
Lymphocyte	T1	63.00 ^{NS}± 3.57	59.33 ^{NS}± 3.47	53.83 ^{NS}± 5.71	**58.72** ± 2.54
	T2	63.83 ^{NS}± 4.98	58.67 ^{NS}± 3.60	56.00 ^{NS}± 3.51	**59.50** ± 2.35
	Pooled Mean	**63.42** ± 2.92	**59.00** ± **2.38**	**54.92** ± **3.21**	
Monocyte	T1	1.33 ± 0.21	1.33 ± 0.21	1.17 ± 0.40	**1.28** ± **0.16**
	T2	1.50 ± 0.34	1.17 ± 0.17	1.00 ± 0.00	**1.22** ± 0.13
	Pooled Mean	**1.42** ± 0.19	**1.25** ± **0.13**	**1.08** ± **0.19**	
Neutrophil	T1	34.00 ± 4.37	37.00 ± 3.56	35.33 ± 7.57	**35.44** ± 2.97
	T2	31.83 ± 4.94	38.67 ± 3.54	41.67 ± 3.60	**37.39** ± 2.42
	Pooled Mean	**32.92** ± 3.16	**37.83** ± **2.40**	**38.50** ± **4.11**	
Eosinophil	T1	3.17 ± 0.60	3.00 ± 0.36	0.83 ± 0.17	**2.33** ± 0.34
	T2	2.83 ± 0.60	1.50 ± 0.56	1.50 ± 0.50	**1.94** ± 0.34
	Pooled Mean	**3.00** ± 0.41 ^{B}	**2.25** ± **0.39** ^{B}	**1.17** ± **0.27** ^{A}	
Basophil	T1	0.17 ± 0.17	1.17 ± 0.17	0.00 ± 0.00	**0.44** ± 0.14
	T2	0.17 ± 0.17	1.33 ± 0.33	0.50 ± 0.22	**0.67** ± 0.18
	Pooled Mean	**0.17** ± 0.11 ^{A}	**1.25** ± **0.18** ^{B}	**0.25** ± **0.13** ^{A}	

Similar superscript indicates non significant variations

as compared with buparvoquone (75%) on 21st day post treatment. In the present investigation the efficacy showed by hydro-ethanolic extract of flower *of C. procera* was promising in treatment of subclinical theileriosis in cattle and can be considered to be a alternative option for treatment of sub-clinical theileriosis in cattle. However, affected animals showed complete clinical recovery after treatment.

Several pharmacological investigations including *in vitro* and *in vivo* studies using different parts of *C. procera* showed antimicrobial, anti-inflammatory, antioxidant, analgesic, antipyretic, anthelmintic, anti-malerial, antipyretic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective, antidiarrheal, anticonvulsant, enhancement of wound healing, antifertility and smooth muscle relaxant effect (Dolan *et al*., 1999; Sharma *et al.,* 2011; Al-Snafi, 2015).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the body. These phytochemicals are active constituents that exhibit some biological activities. In the present study

extract of flower of *Calotropis procera* was used which contained many biological active chemical including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids, saponins, calactin, calotoxin, calotropagenin, calotropin. Flowers also contained enzymes 3-proteinase and calotropain (protease). Other chemical constituents of *C. procera* flowers are lupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a-calotropeol, 3 epimoretenol, alactuceryl acetate and a-lactuceryl isovalerate as reported by Sharma *et al.* (2011) and Al-Snafi (2015).

In the present investigation the result achieved by the *C. procera* against subclinical theileriosis in cattle might be due to active principle present in this herbal medicinal plant. The exact mechanism by which the herbal preparation brought about recovery is not known. However, it could help in eliminating the theilerial parasite by their anti-oxidant, anti-malerial, anti-protozoal and immunomodulatory properties through their active principles as reported by several workers. Antithelerial activity of flowers of *Calotropis procera* was also reported by Durrani *et al.* (2009).

The haematological study (Table 2) revealed low Hb, PCV, TLC, TEC and increase in eosinophil percent in both the infected groups on day '0' (before treatment). Analysis of variance showed non-significant variation in haemoglobin concentration between different periods. However, pretreatment haemoglobin concentration in both the groups (Group 1 and Group 2) was low and apparently increased on 7th and 21st day post treatment over the pre treatment haemoglobin concentration of corresponding groups. The variation in PCV % between different periods revealed non-significant variation. However, PCV was apparently improved on 7th and 21st day over the pre treatment values of corresponding groups.

The analysis of variance revealed significant variation in the TLC between different periods ('0', 7th and 21st day), indicated significant increase in TLC on 7th and 21st day post treatment values (P>0.01), in both the groups. TEC did not differ significantly between different periods. However, TEC was apparently increased on 7th and 21st day over the pre-treatment values of corresponding groups. The analysis of variances revealed significant variation in eosinophil percent. Thus, indicated significant reduction in eosinophil percent on 21st day post treatment over the pretreatment percent in both the groups, indicated improvement in eosinophil percent in both the treatment groups. Neutrophil, lymphocyte and monocyte per cent did not differ significantly between different intervals.

Both the treatment showed improvement in TLC and eosinophil percent and apparently improvement in Hb, PCV, TEC, lymphocytes, neutrophils and monocytes over the pre-treatment levels indicated effectiveness of both the treatment in Theileriosis in cattle.

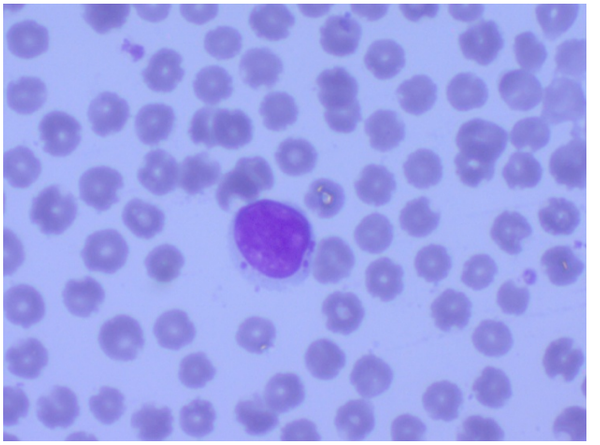

Fig. 1: Showing presence of *Theileria annulata* in lymphocytes

Such findings have already been reported by Col and Uslu, (2006) and Hasanpour *et al*. (2008). The decrease in erythrocyte count, packed cell volume and hemoglobin concentration resulted anemia in infected group. It has been reported that anemia occurs in lateral stages of theileriosis following parasitaemia (Mbassa *et al.,* 1994). Immune-mediated mechanism like erythrophagocytosis might be responsible for the destruction of erythrocytes infected with *Theileria* schizoints. Removal of piroplasm infected erythrocytes by macrophages in the reticuloendothelial system has been suggested as a cause of anemia. The decreased erythrocyte counts could be attributed to increased levels of activated complement products. Moreover, oxidized erythrocytes are destroyed by erythrophagocytosis, oxygen free radicals may also be responsible for anemia (Mbassa *et al.,* 1994).

On the basis of the results obtained in the present study, it is concluded that both the treatments found effective in eliminating thelerial infection along with improvement

in haematological parameters. The group treated with single dose of Buparvaquone @ 2.5 mg/kg body weight intramuscularly showed higher efficacy as compared to flower extract of *Calotropis procera*. It is further concluded that hydro-ethanolic flower extract of *C. procera* @ 0.3mg/kg body weight given total 8 doses orally on alternate days could be novel and affordable therapeutic regimen for sub-clinical theileriosis in cattle. However, future research on quantification of phytochemicals and efficacy of *C. procera* with different doses and period of therapy need to be evaluated in order to develop effective herbal medicine against theileriosis in cattle.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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