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Evaluation of Pharmacological Activities of the Leaf Extracts of *Antidesma menasu* Miq.

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Abstract *— Antidesma menasu* is a folklore plant known for its anti-inflammatory and analgesic properties. Folklore practitioners use the leaves of this plant to cure swelling of body parts, lower back pains, arthritis in humans and swelling of shoulders and udders in animals. Development of a new drug requires pre-clinical studies of crude extracts on animal models. The aim of this study is to screen the plant extract for its pharmacological activities like anti-inflammatory and analgesic properties of aqueous and ethanol extracts. The leaf extracts obtained by soxhlet method. Standard protocols followed for the experiments. Analgesic and anti-inflammatory experiments performed with Wistar albino rats. The ethanol extract showed anti-inflammatory and analgesic effect but was not significant compared with standard drug. This finding further strengthens the medicinal properties of *A. menasu*.

Keywords *— Antidesma menasu, anti-inflammatory, analgesic, antimicrobial, acute oral toxicity*

I. INTRODUCTION

The interaction between chemicals and living systems is a frame work for understanding the actions of drug. Pharmacology is the revolutionary field to invent and test medicines. Many of the compounds in the clinic today have been identified through the work performed in a range of animal models. These models have provided us with an understanding of disease pathology and potential mechanistic pathways and have given us the means to prioritise new chemical entities before entry into the clinic [1].

Plant based derivatives take prime position in world medical scenario. In current practice, excessive use of synthetic drugs has diminished the efficacy of plant based therapies. These traditional medicines offer suitable alternatives in place of costly allopathic medicine which can be afford by a common man. Plants and animals have been utilised from ancient time parallels with development of new medicines. Despite of several ethical issues, animals are being used as a tool for design of physiological system of human for prognosis of human diseases. In animals, one can test newly synthesized drugs, vaccines or devices for safety and efficacy before being into human. Discoveries of novel drugs for human diseases need animal models to ascertain the properties of absorption, distribution, metabolism, elimination and toxicity (ADMET) as their systems are synergetic with human system [2]. Most of present day's drug discoveries were possible because of the use of animals in research. The greatest drug discoveries in the 19th and 20th centuries were possible due to the use of animals. There is a strong relationship between rapid progress in experiments on animals and progress in clinical medicine. Animals have been used and are still permitted for screening for drugs in bioassay and for preclinical testing including general and specific toxicity studies. This preclinical safety and efficacy data is needed for submission to drug regulatory authorities before the permission for further studies in humans are granted. Toxicity tests on animals provide critical information for assessing risk potential of a new drug [3].

From the literature it was found that Euphorbiaceae members act as an effective remedy for many diseases. They exhibit pharmacological activities such as anti-diarherial, anti-oxidant, anti-bacterial, anti-amoebic, anti-cancerous, anti-plasmodial, anti-inflammatory etc. They also act as effective drug against HIV, jaundice, infertility, neurosis, syphilis, small pox, Asthma and diabetes [4]. *Euphorbia hirta* is a potent medicinal plant exhibits different pharmacological properties such as anti-bacterial, anti-malarial, anti-inflammatory activity in mice, galactogenic activity in guinea pigs, anti-asthmatic activity, anti-diarrheal in mice, anti-oxidant, anti-fertility, anti-fungal activities were reported [5]. Pharmacological studies of five different species of *Tragia* (*T. involucrata, T. cannabina, T. spathulata, T. plukenetii, T. benthamii*) belongs to Euphorbiaceae were reported [6]. *Jatropha gossypiifolia* was reported to have anti-inflammatory, anti-microbial, anti-bacterial, anti-oxidant activity [7]. Anti-microbial, hepatoprotective, anti-inflammatory and anti-oxidant activity of *Euphorbia thymifolia* was reported by Sisodiya and Shrivastava [8]. Anti-inflammatory, anti-fungal, anti-viral, anti-spasmodic, cytotoxic, anti-

mutagenic, anti-bacterial and hepatoprotective activities of extracts of some plants belonging to genus *Euphorbia* are predicted [9]. The fruits of *Antidesma montanum* exhibits anti-oxidant activity and the leaves of this plant known to have anti-diabetic and anti-inflammatory properties [10].

Analgesic and anti-inflammatory potential of leaves *Antidesma acidum* on Wistar rats were proved [11]. The ethanol extract of the leaves of *Antidesma bunnies* at a dose of 200mg/kg has an effective anti-inflammatory activity against Carrageenan induced paw oedema [12]. Acute and chronic anti-inflammatory activity of the aqueous extract of *Antidesma menasu* was reported earlier [13]. Ethanol extract of leaves of *A. menasu* exhibited positive activity against *Staphylococcus aureus* while with other organisms the effect is negligible [14].

The objective of this study is to screen the ethanol and aqueous leaf extract of *Antidesma menasu* for anti-inflammatory and analgesic properties.

II. MATERIALS AND METHODS

Collection of Plant material

The leaves of *Antidesma menasu* were collected from Alike, Dakshina Kannada district, Karnataka. The plant was authenticated; herbarium samples were prepared and deposited.

Preparation of Extracts

Leaves were collected, shade dried, powdered using electric blender and preserved. Soxhlet extraction method [14] was carried out to get required amount of ethanol and aqueous extracts. 630g of sample was weighed and taken in thimble and placed it in a soxhlet extractor. 6.5 litres of ethanol was taken in the round bottom flask and boiled until 12 h. The extract as concentrated by distillation and solvent was removed by evaporation on a water bath. The extract was completely dried under vacuum. The percentage of dried extract recorded. Similarly aqueous extract also obtained. The extracts were stored at 4˚C for further experiments.

Experimental Animals

Experimental animals Wistar albino rats and mice of either sex were procured from Animal House, attached to the SDM Centre for Research in Ayurveda and Allied Sciences, Udyavara, Udupi. They were housed in clean polypropylene cages with stainless steel grills on top. Dried paddy husk was used as bedding material and was changed regularly. Rats were acclimatized for seven days before the initiation of experiment. They were exposed to 12 h light and dark cycle with relative humidity of 50-70% and temperature of 22± 3°C. Rats were fed with rat pellets and normal drinking water. Animals were marked with saturated Picric acid solution in water. These procedures were according to OECD test guidance [15].

Acute Oral Toxicity

Rats were grouped as head, neck, body and tail and their body weights were measured. Their basal behaviour before administration of drug was recorded. Constant range of doses i.e., 170mg/kg, 550mg/kg, 2000mg/kg and 2000mg/kg is given orally to the four rats according to their body weights. Then rats were kept under observation after the administration of drug. Signs and symptoms in their behaviour were observed and recorded after 1 h, 2 h, 3 h, 4 h, 24 h and 48 h of drug administration. The entire procedure was performed for both aqueous and ethanol extracts.

Acute Anti-Inflammatory Study

Acute anti-inflammatory activity of the crude drug was predicted by using Carrageenan induced paw oedema [13, 16]. Four groups of rats (Test 1, Test 2, control and standard) each group consisting 6 rats (head, neck, body, tail, forelimb and no mark) were selected and their body weights were measured. The amount of drug dosage for each rat was calculated by extrapolating the dosage in humans (6g/kg). Rats of Test 1 were administered a dose range of 200mg/kg and Test 2 were administered with a dose range of 400mg/kg of crude extracts orally. Rats belonging to control group are fed with normal water and standard group are administered with Brufin-400mg. The crude extract was mixed with CMC (Carboxy Methyl Cellulose) and oral administration was carried out for 7 consecutive days. On the seventh day after oral administration of the drug, 0.1 ml of 1% carrageenan was administered to the subplantar region of right paw of all the rats. The paw volume of each rat was measured (basal, after 30min, 1h, 3h, 6h and 24h) by using Plethysmometer. Percentage change in paw volume was calculated using the formula,

$$\% \text{ change in paw volume} = \frac{\text{Reading at 30min-Basal}}{\text{Basal}} \times 100$$

The entire test was performed for both aqueous and ethanol extracts.

Analgesic Activity

Acetic Acid Induced Writhing Test

Twelve mice were grouped into two groups (Group 1 male and Group 1 female) each containing six mice (head, neck, body, tail, forelimb, no mark) are selected and weighed. Another group of mice containing three mice is considered as Control. According to the body weight of the mouse, drug dosage given to be calculated. The drug is orally administered to all the 12 mice in each group for five consecutive days. On the fifth day, after drug administration, acetic acid induced writhing test is performed [17]. 0.1 ml of 0.6% acetic acid injected intraperitoneally to all male and female mice of group 1 one after another. Each animal was kept under keen observation for 20 min and number of writhes (abdominal constrictions) was recorded. Percentage inhibition was calculated by using the formula,

$$N_c\text{-}N_t/N_c \times 100$$

where, N_c is the number of writhes in control group and N_t is the number of writhes in test group.

Formalin Test

Formalin test was performed according to the standard protocol [18, 19] to predict the analgesic property of the crude drug. Two groups of mice (Group 2 male and group 2 female) each containing 6 mice (head, neck, body, tail, forelimb, no mark) are selected and body weight of each mouse was noted. Another group of mice containing three mice was considered as control. The drug is orally administered for five consecutive days and on fifth day, Formalin test was performed. Pain was induced by injecting 10% Formalin solution to the sub-plantar region of hind paw of mouse after 1 h of drug administration. Stop watch was kept on as soon as the injection was given. Mouse will start licking its hind paw and numbers of lickings were recorded for a time interval of 30 min. After counting the paw lickings, the change in the volume of injected paw as well as normal paw was measured using Plethysmometer.

Statistical analysis

Data was represented as mean ± S.E.M. for analgesic and anti-inflammatory activity. Statistical analysis for pharmacological activity was carried out by one way ANOVA followed by Dunnett test.

III. RESULTS AND DISCUSSION

Table 1: Anti-inflammatory effect of *Antidesma menasu* ethanol extract on carrageenan induced paw oedema

Group	1 h Mean ±SEM	% paw volume	3 h Mean ± SEM	% paw volume
Control	39.01±9.12	-	84.60±12.08	-
Standard	29.57±6.21	24.198↓	42.96±10.55	49.2↓
Test-1	50.99±6.84	30.710↑	71.93±10.89	14.97↓
Test-2	46.70±9.58	19.712↑	87.22±13.90	3.096↓

Group	6 h Mean ± SEM	% paw volume	24 h Mean ± SEM	% paw volume
Control	78.81±8.44	-	19.04±3.54	-
Standard	32.85±7.48**	101.77↓	22.01±8.75	15.598↑
Test-1	79.33±8.40	0.659↑	18.46±4.48	3.046↓
Test-2	72.33±10.23	8.22↓	9.06±2.88	52.41↓

Data: Mean ± SEM, **p<0.01 compared to control

Table 2: Anti-inflammatory effect of *A. menasu* aqueous extract on carrageenan induced paw oedema

Group	1 h Mean ± SEM	% paw volume	3 h Mean ± SEM	% paw volume
Control	39.01±9.122	-	84.60±12.086	-
Standard	29.57±6.21	24.198↓	42.96±10.55	49.21↓
Test-1	66.98±6.93*	71.699↑	76±19.96	10.1↓
Test-2	65.35±7.99	67.52↑	80.61±7.14	4.71↓

Group	6 h Mean ± SEM	% paw volume	24 h Mean ± SEM	% paw volume
Control	78.81±8.440	-	19.04±3.547	-
Standard	32.85±7.48*	58.31↓	22.015±8.75	235.13↑
Test-1	68.34±17.43	13.278↓	63.81±9.14**	235.13↑
Test-2	58.30±6.29	26.024↓	54.88±7.73**	188.25↑

Data: Mean ± SEM, **p<0.01 compared to control

Table 3: Analgesic effect of *Antidesma menasu* ethanol extract on acetic acid induced writhing test

Group	Writhings in 20 min Mean ± SEM	% inhibition	Onset time Mean ± SEM	% inhibition
Control	27.66±8.65	-	6.14±1.37	-
Group 1 male	12.5±3.56	54.8↓	5.10±1.78	16.96↓
Group 1 female	11.5±3.06	58.42↓	2.39±0.51	61.01↓

Table 4: Analgesic effect of *Antidesma menasu* aqueous extract on acetic acid induced writhing test

Group	Writhings in 20 min Mean ± SEM	% inhibition	Onset time Mean ± SEM	% inhibition
Control	27.66±8.65	-	6.14±1.37	-
Group 1 male	32.2±10.64	16.41↑	3.128±0.22	49.08↓
Group 1 female	28.5±9.22	3.03↑	3.758±0.73	38.82↓

Data: Mean ± SEM, **p<0.01 in compared to control

Table 5: Analgesic effect of *Antidesma menasu* aqueous extract on formalin test

Group	5min Mean ± SEM	% paw volume	10min Mean ± SEM	% paw volume
Control	129.5±15.42	-	25.66±7.63	-
Standard	80.16±18.82	38.09↓	27.4±7.64	6.78↑
Group 2 male	117±15.78	9.65↓	17.6±4.47	31.41↓
Group 2 female	141±24.25	99.89↓	30.2±3.81	17.69↑

Group	20 min Mean ± SEM	% paw volume	Total Mean ± SEM	% paw volume
Control	35.5±8.79	-	250.16±31.37	-
Standard	13±4.95	63.38↓	165.33±37.75	33.9↓
Group 2 male	15.5±5.5	56.33↓	174.4±28.95	30.28↓
Group 2 female	34.25±15.46	3.52↓	219±41.48	12.32↓

Data: Mean ± SEM, **p<0.01 compared to control

Table 6: Analgesic effect of *Antidesma menasu* ethanol extract on formalin test

Group	5min Mean ± SEM	% paw volume	10min Mean ± SEM	% paw volume
Control	129.5±15.42	-	25.66±7.63	-

Standard	80.16±18.82	38.09↓	27.4±7.64	6.78↑
Group 2 male	110±14.01	15.05↓	33.8±7.01	31.72↑
Group 2 female	89.66±19.81	30.76↓	32.75±7.53	27.63↑

Group	20min Mean ± SEM	% paw volume	Total Mean ± SEM	% paw volume
Control	35.5±8.79	-	250.16±31.37	-
Standard	10±5.56	71.83↓	165.33±37.75	33.9↓
Group 2 male	39±14.0	9.85↑	224.2±43.52	10.37↓
Group 2 female	18.75±6.66	47.18↓	161.5±30.74	35.44↓

Data: Mean ± SEM, **p<0.01 compared o control

Acute Oral Toxicity

No deaths were found in any of the concentrations after 24 and 48 h of drug administration. Hence the lethal dose of the drug will be more than 2000mg/kg. The gross behavioural study of each rat was found to be normal in all the above doses of drug and there was no adverse effect of the drug was predicted in above concentrations. This observation is comparable with earlier reports [11]. [13], [20], [21].

Anti-Inflammatory Activity

The experimental data from Carrageenan induced paw oedema of ethanol extract (Table 1) reveals, the standard drug (Brufin- 400mg) showed significant response for the anti-inflammatory activity, but the ethanol extract of the crude drug both in 200mg/kg and 400mg/kg has not shown any significant activity towards the tests performed. Aqueous extract at 200mg/kg and 400mg/kg has shown statistically significant ($p<0.01$) decrease in paw volume after 1h and 24 h of induction of paw oedema. In one of the earlier report by Sithara et al. [13] acute and chronic anti-inflammatory activity of the aqueous extract of *Antidesma menasu* at 500mg/kg showed significant decrease in the paw oedema. The chronic anti-inflammatory study performed (Cotton pellet induced granuloma formation) revealed a significant decrease in granuloma tissue weight at 500mg/kg in comparison with the control group.

In one of the study on methanol and chloroform stem and root extracts of *Inula cuspidata,* all the extracts exhibited dose dependent reduction in paw edema volume at different doses of 100 and 200 mg/kg. The methanol extract at both doses 100 and 200 mg/kg inhibit nitric oxide synthase, responsible for production of nitric oxide which also acts as one of the mediator in inflammation [20]. In another study [21] on ethanolic extract of stem bark of *Anogeissus latifolia* dose dependent inhibition of paw oedema (200 mg/kg) showed time dependent inhibition ($p < 0.05$ and $p < 0.001$ at 3 h and 5 h) of the mean raise in paw volume compared with control rats. The high dose of ethanol extract (400mg/kg) showed significant inhibition of the mean increase in paw volume (oedema) in time dependent manner as indicated by increased per cent inhibition of paw oedema compared to control rats. There are other earlier studies supports the present results which were reported in members of Euphorbiaceae [7], [8], [10], [11], [12].

Chronic Analgesic Activity

The experimental data from Acetic acid induced writhing test (Table 3 and Table 4) reveals that, both aqueous and ethanol extracts have shown decrease in percentage of writhes in test groups with the concentrations 200mg/kg and 400mg/kg when compared to control group. However, the data is not significant.

Experimental data from Formalin test (Table 5 and Table 6) reveals that, both aqueous and ethanol extracts of *Antidesma menasu* have shown decrease in percentage paw volume in both 200mg/kg and 400mg/kg but it is statistically non-significant.

The analgesic activity of methanol and chloroform extracts of stems and roots of *Inula cuspidata* in both the methods centrally acting hot plate model and peripherally acting acetic acid induced writhing model. Methanol and chloroform extracts protected mice against both hot and chemical induced noxious stimuli [20]. Effect on acetic acid-induced writhing ethanol extract of *Anogeissus latifolia* exhibited a significant change on writhing test. Post hoc test showed that extract caused significant inhibition of number of writhing at the dose of at 200 and 400 mg/kg. The maximum inhibition exhibited at 400 mg/kg (66.83%). The effects were comparable to that of standard drug aspirin that also showed significant inhibition (71.34%) of writhing [21]. *Antidesma acidum* has significant analgesic properties in aqueous extract [11].

Present study proves the anti-inflammatory and analgesic activity of both aqueous and ethanol extracts of *Antidesma menasu*. The results have shown that the leaf extracts exhibit moderate analgesic as well as anti-inflammatory activity. The aqueous extract of *Antidesma menasu* has got very significant anti-inflammatory activity when compared to the ethanol extract. The research plant did not show any adverse effect on gross behaviour of animals and deaths were not found till 2000mg/kg.

This study supports the opinion of folklore practitioners about the anti-inflammatory and analgesic activities of *Antidesma menasu* as well as isolating the active compounds from the extract and their pharmacological activity in different animal models are necessary to evaluate the plant for clinical use. These findings validated the claim for the traditional use of this plant in the treatment of pain and inflammatory ailments.

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