

Experimental evaluation of analgesic and anti-inflammatory potential of leaves of *Antidesma menasu* on wistar albino rats

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ABSTRACT

Background: *Antidesma menasu* is a folk plant of Euphorbiaceae occurring in and around Udupi district in India. It is effectively used in folklore medicine for the management of arthritis, inflammatory bowel disorder and low backache. The present study aimed to evaluate the anti-inflammatory and analgesic activity of the plant in Wistar albino rats. Aqueous extract of leaves of *A. menasu* were evaluated for possible analgesic and anti-inflammatory activities in Wistar albino rats.

Materials and Methods: Eddy's hot plate test and radiant heat test were used for investigation of analgesic effect. Effect of extract on the acute inflammation was investigated on Carrageenan induced paw oedema, and chronic inflammation was investigated on cotton pellet induced granuloma tissue formation test. The test extract was subjected for preliminary phytochemical examination as per standard methodology.

Results: The test drug found to inhibit the carrageenan induced paw oedema significantly ($p < 0.05$) and there was moderate increase in the latency period in the analgesic test. The results suggest that the test group has high significant anti-inflammatory potential and there is moderate analgesic activity. The histopathological examination of adrenal cortex, spleen and thymus showed a normal cytoarchitecture comparable with that of normal control group. The preliminary phytochemical screening of the aqueous extract of the plant *A. menasu* revealed the presence of flavonoids, saponins, steroids and phenols.

Conclusion: The data obtained supports the traditional folklore therapeutic claim about its anti-inflammatory and analgesic activity. Further scientific investigation is required to establish its analgesic and anti-inflammatory property in other experimental models and clinical settings.

Key words: *Antidesma menasu*, arthritis, carrageenan, chronic inflammation, flavonoids.

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INTRODUCTION

Antidesma menasu (Euphorbiaceae) is found as herbs or shrubs along the tropical Himalayas and West Bengal, It is found commonly throughout the South Canara district of Karnataka in India in rainy season. It is a folk remedy for the management of low backache, arthritis, muscle pain, neuralgias by folklore practitioners of Udupi.^[1-3] These symptoms are mainly associated with inflammation at different parts of the body. There is search for better non-steroidal anti-inflammatory and analgesic

drugs with lesser side effects.^[4-6] The present drugs used for inflammation and pain like NSAIDs are associated with greater side effects, so there is a rising scope for traditional medicines. Emphasis on the use of plant material as a source of medicine for wide variety of human ailments have increased recently.^[7,8]

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Triterpenoids derived from *Antidesma menasu* showed diuretic activity.^[9] A survey of the literature revealed that the anti-inflammatory activity of *Antidesma menasu* has not been documented. Hence this study was undertaken to evaluate the anti-inflammatory and analgesic activity in albino rats.

MATERIALS AND METHODS

Plant collection and authentication

Leaves of *A. menasu* were collected from the medicinal plantation of our Institution during December 2011. It was authenticated by department of Pharmacognosy at SDM Research Centre Udupi. A voucher specimen (No. 199/12121701) had been deposited for further future reference.

Preparation of extract

The leaves of *A. menasu* were shade dried and 20 g of powder was soaked in 2 L of distilled water for 24 h, it was filtered, and then reduced to 80 ml by boiling on water bath. The aqueous extract (AAM) was prepared fresh every day before administration. For the study, two test drug doses were chosen by extrapolating human dose (6g/ kg) into rat dose on the basis of body surface area (500 and 1000 mg/kg).

Experimental animals

Albino rats of Wistar strains of either sex between 150 to 250 g were obtained from animal house attached to department of Pharmacology, SDM Research Centre, Udupi. The experimental protocol was approved from the institutional ethical committee (Ref.no. SDMC-RA/IAEC-2011-12DG 05ab). The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%.

Animal grouping: Adult albino Wistar rats of either sex weighing 180 g to 220 g, 24 rats were divided into four different groups, six in each group. Control group were administered with normal tap water at a dose of 10 ml /kg in 0.5% gum acacia, the standard group were administered with Ibuprofen 100 mg/kg as a suspension with 0.5% gum acacia, the test groups were administered with AAM at a dose of 500 mg/kg for test group I and 1g/kg for test group II.

Analgesic activity

Eddy's Hot plate method

The analgesic activity of AAM was assessed using hot plate method of Eddy and Leimbach (1953).^[10] The temperature was maintained at $55 \pm 0.2^{\circ}\text{C}$. Animals licked their limbs and jumped as an indication of pain. These rats were treated with suspensions as follows: control group received normal water in 0.5% gum acacia. The test groups received 500 mg/kg and 1g/kg of AAM. The standard group received Ibuprofen 100 mg/kg by the oral route.^[11] One hour after dosing group specific drugs, rats were placed on the hot plate and the time until either licking and jumping occurs was recorded by a stop watch. The latency period was recorded before and after 1h, 2h, 3h and 4h following oral administration of group specific drugs. The cut off time of 12 sec was employed for hot plate test.

Tail flick test

Effect of test drug on the latency of tail flick response, which represents the pain threshold, was measured in rats employing the procedure of Gujaral and Khanna (1956).^[12] The tail flick response was measured with the help of an analgesiometer. Basal reaction time of animals to radiant heat was recorded by placing the tail 2-2.5 cm from distal end of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) was taken as the end point.

A cut-off period of 10 sec was kept to avoid damage to the tail. The drug was administered once daily for 5 consecutive days. On 5th day, one hour after drug administration, tail flick response was recorded at 30, 60, 120, 180 and 240 min.

Anti-inflammatory activity

Acute anti-inflammatory activity

Carrageenan induced hind paw oedema test was done in rats by method of Winter et al.^[13] The acute anti-inflammatory activity was evaluated by Carrageenan induced paw oedema method in Wistar albino rats. Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan solution into sub plantar surface of rat's hind paw. The group specific drugs were administered 1h before the carrageenan injection. The paw volume up to the tibio-tarsal articulation was measured using a Plethysmometer (PLM-01 PLUS Orchid Scientifics) at basal, 1h, 3h, and 6h after carrageenan injection. The anti-inflammatory activities were expressed as percentage decrease in paw oedema ($V - \text{paw volume}$), calculated using the formula:

$$\frac{(V_{\text{control}} - V_{\text{test}}) \times 100}{V_{\text{control}}}$$

Chronic anti-inflammatory activity

The effect of test drug on cotton pellet induced granuloma formation in rats was studied as per the method described by Garcia et al.^[14] The rats were anaesthetised under ketamine (80 mg/kg, i.p.). The dorsum was shaved and swabbed with 70% (v/v) alcohol. Mid-line incision of 1cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of small blunt forceps. One sterile cotton pellet weighing 100 mg (prepared by rolling of a cotton piece of 100 mg and sterilised by autoclaving for 30 min under 15 lbs pressure) was inserted per tunnel and closed the incision with interrupted sutures after expelling the air from the tunnel. Group specific drugs were administered for seven con-

secutive days starting from the day of implantation.

The rats were sacrificed on 8th day and dissected for collection of thymus, spleen, adrenal glands. Implanted cotton pellets were removed and cleaned of extraneous tissues and dried by placing them in a hot air oven overnight at 80 °C and then weighed. The difference between the initial weight and the final weight of the pellet after drying was taken as the weight of granuloma tissue. The result was expressed as mg of granulation tissue formed per 100 g body weight.

The weight of the adrenal gland, spleen and thymus were noted and preserved in 10% formalin and sent for histopathology. In addition, blood samples were collected to estimate biochemical parameters.

Statistical analysis

The experimental data were expressed as mean \pm SEM. Statistical analysis was carried out by one way analysis of variance followed by Dunnett test. A level for $p < 0.05$ was considered to be statistically significant.

RESULTS

Analgesic activity

In hot plate analgesic test, ibuprofen showed statistically significant increase in the reaction time. AAM at dose of 500 mg/kg and 1g/kg showed increase in the reaction time at 2nd and 3rd hour of drug administration; however, the observed changes were statistically not significant (Table 1).

In tail flick response test, ibuprofen showed statistical significant prolongation in the response time 1h after drug administration. AAM at 500 mg/kg dose, showed statistically significant prolongation in the response time during initial 30 min and 1h but response time measured at 2h, 3h and 4th h did not show significant changes (Table 2).

The results of the above tests indicate presence of mild to moderate central analgesic activity in the test drug- the mechanism of which remains to be determined.

Anti-inflammatory activity

In carrageenan induced paw edema test, ibuprofen had significantly suppressed biphasic responses of carrageenan induced inflammation. AAM at 500 mg/kg, showed statistically significant decrease in the paw edema during first and second phase of carrageenan induced

inflammation, which clearly shows presence of anti-inflammatory activity in the test drug at therapeutic dose (500 mg/kg) (Table 3).

In cotton pellet implanted granuloma formation test, ibuprofen showed marked and statistically significant decrease in the granuloma tissue weight in comparison to control group. AAM at 500 mg/kg, showed statistically significant decrease in granuloma tissue weight in comparison to control group (Table 4).

Table 1: Effect of aqueous extract of *Antidesma menasu* leaves on thermal stimuli induced pain in rats (Hot-plate test)

Group (dose)	Duration of latency of jumping response in (sec) at various time intervals				
	Basal	1h	2h	3h	4h
Control (10 ml/kg)	7.18 ± 0.73	8.27 ± 0.99	8.92 ± 1.04	8.72 ± 0.70	7.76 ± 0.79
Ibuprofen (100 mg/kg)	6.73 ± 1.10	10.82 ± 0.73*	10.78 ± 0.63	9.98 ± 0.52	9.9 ± 0.64
AAM (500 mg/kg)	4.90 ± 0.69	7.27 ± 0.90	8.84 ± 0.89	8.01 ± 0.74	7.09 ± 0.68
AAM (1 g/kg)	7.17 ± 0.81	8.69 ± 0.38	9.84 ± 1.12	10.17 ± 0.80	7.97 ± 0.51

Values are represented in Mean ± SEM, *p < 0.05 vs. control group.

AAM - aqueous extract of *Antidesma menasu* leaves

Table 2: Effect of aqueous extract of plant *Antidesma menasu* on latency period of radiant heat test

Group (dose)	Duration of latency of tail flick response in (sec) at various time intervals					
	Basal	30 minutes	1h	2h	3h	4h
Control (10 ml/kg)	4.11 ± 0.10	3.75 ± 0.24	3.78 ± 0.24	4.43 ± 0.30	4.33 ± 0.40	4.58 ± 0.40
Ibuprofen (100 mg/kg)	3.75 ± 0.10	4.15 ± 0.18	4.56 ± 0.18*	4.10 ± 0.44	3.98 ± 0.10	4.45 ± 0.10
AAM (500 mg/kg)	3.60 ± 0.17	5.10 ± 0.20 [†]	4.95 ± 0.44*	4.90 ± 0.30	4.81 ± 0.30	4.70 ± 0.18
AAM (1 g/kg)	3.28 ± 0.20	4.10 ± 0.22	4.63 ± 0.13	4.63 ± 0.30	4.73 ± 0.10	4.40 ± 0.18

Values are represented in Mean ± SEM, *p < 0.05 vs. control group, [†]p < 0.001 vs. control group.

AAM - aqueous extract of *Antidesma menasu* leaves

Table 3: Effect of aqueous extract of *Antidesma menasu* in Carrageenan induced paw oedema test

Group (dose)	Percentage inhibition of Carrageenan induced paw oedema (ml)						
	Basal	1h	% Change	3h	% Change	6h	% Change
Control (10 ml/kg)	0.89 ± 0.06	0.33 ± 0.43	-	0.54 ± 0.10	-	0.51 ± 0.10	-
Ibuprofen (100 mg/kg)	0.88 ± 0.01	0.14 ± 0.04*	57.5 ↓	0.23 ± 0.04*	57.4 ↓	0.13 ± 0.00 [†]	74.50 ↓
AAM (500 mg/kg)	0.83 ± 0.04	0.19 ± 0.08*	42.4 ↓	0.49 ± 0.07	9.25 ↓	0.35 ± 0.10 [†]	31.37 ↓
AAM (1 g/kg)	0.88 ± 0.01	0.39 ± 0.57	18.1 ↑	0.68 ± 0.85*	26.8 ↑	0.48 ± 0.13 [†]	5.88 ↓

Values are represented in Mean ± SEM, *p < 0.05 vs. control group, [†]p < 0.01 vs. control group.

AAM - aqueous extract of *Antidesma menasu* leaves

Table 4: Effect of *Antidesma menasu* in cotton pellet induced granuloma tissue formation in rats

Group (dose)	Granuloma tissue	%change
Control (10 ml/kg)	767.66 ± 107.52	-
Ibuprofen (100 mg/kg)	105.84 ± 34.73 [†]	86.21↓
AAM (500 mg/kg)	341.16 ± 94.34 *	55.55↓
AAM (1 g/kg)	464.5 ± 110.54	39.49↓

Values are represented in Mean ± SEM.

*p < 0.05 vs. control group, [†]p < 0.01 vs. control group.

AAM - aqueous extract of *Antidesma menasu* leaves.

Biochemical parameters

There were no significant changes in SGOT, SGPT and ALP activity, but a statistically significant decrease was seen in the serum total protein content in AAM 1 g/kg groups on 8 days of test drug treatment. It indicates there might be moderate increase in the catabolism of serum proteins (Table 5).

Histopathological examination

The adrenal gland sections from control, standard, and AAM at 500 mg/kg and 1g/kg group were found to have same cytoarchitecture profile with well-developed cortex and medulla. This showed that the standard and test drugs have no influence on the adrenal gland cytoarchitecture (Figure 1).

The sections of spleen from control group exhibited normal cytoarchitecture, moderate increase in white pulp proportion was

observed in some sections but overall profile was normal. Moderate increase in white pulp was observed in standard group. In sections from both AAM at 500 mg/kg and 1000 mg/kg groups showed moderate to marked increase in the proportion of white pulp (Figure 2).

Thymus sections from the control group exhibited normal profile. In sections from standard group, a slight decrease in cellularity was observed. The sections from AAM at 500 mg/kg and 1000 mg/kg exhibited normal profile (Figure 3).

DISCUSSION

Acute inflammation conveniently described as vascular and cellular events, alteration in the microvasculature is the earliest response to tissue injury. These alterations include hemodynamic changes such as transient vasoconstriction, persistent progressive vasodilatation, followed by local hydrostatic pressure, stasis, leukocyte migration, and vascular changes in which accumulation of oedema fluid. In cellular events, phagocytosis, that is engulfment solid particulate materials by cells, causes the inflammation.^[15]

Since the secondary phase of oedema is significantly suppressed it can be suggested that the observed anti-inflammatory of *A. menasu* may be due to suppression of formation and release of the inflammatory mediators.

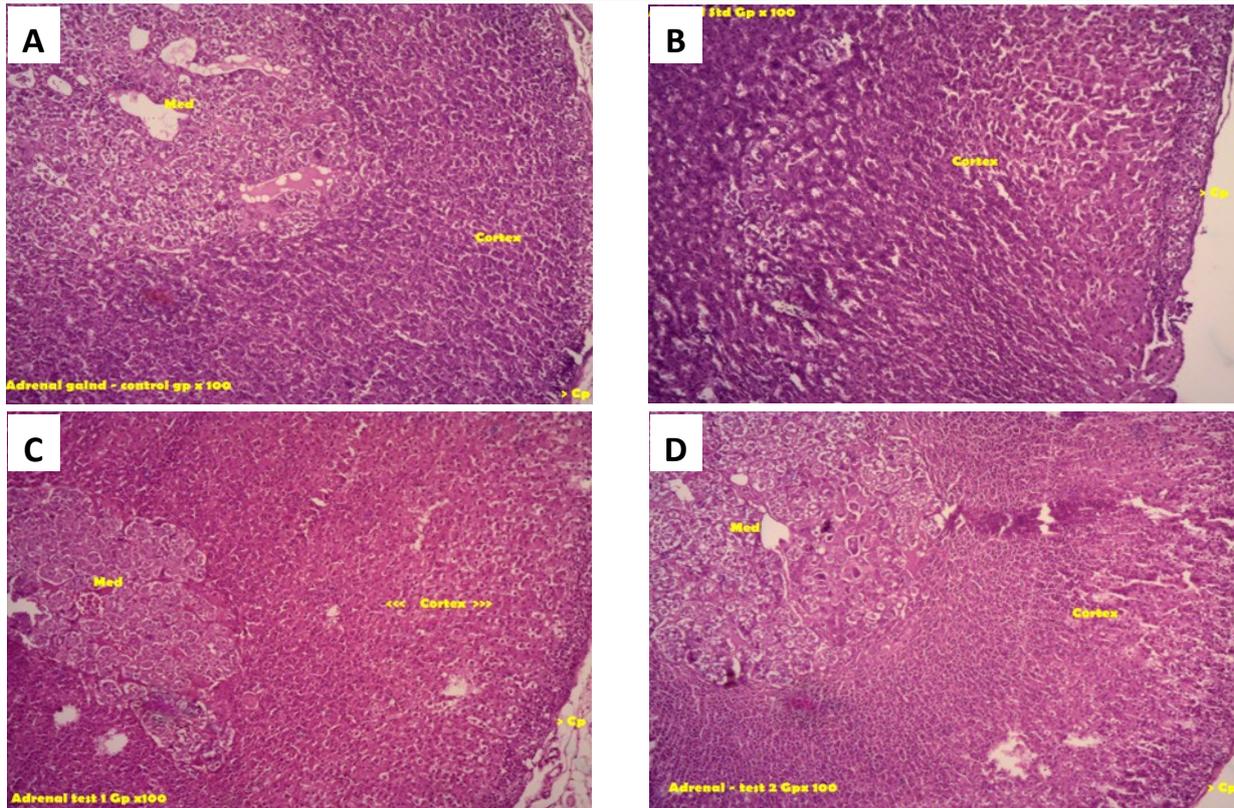
Table 5: Effect of *Antidesma menasu* on biochemical changes in cotton pellet induced granuloma test

Group (dose)	SGOT (unit/l)	% change	SGPT (unit/l)	% change	ALP (unit/dl)	% change	Total Protein (g/dl)	% change
Control (10 ml/kg)	154.66 ± 8.2	—	112.5 ± 5.28	—	448.33 ± 77.61	—	7.82 ± 0.34	—
Ibuprofen (100 mg/kg)	116.66 ± 9.51	24.57 ↓	69.33 ± 3.92	38.37 ↓	490.8 ± 65.16	9.47 ↑	7.69 ± 0.12	1.80 ↓
AAM (500 mg/kg)	138.33 ± 3.39	10.55 ↓	104.33 ± 5.15	7.26 ↓	359.5 ± 22.25	19.81 ↓	8.06 ± 0.03	3.06 ↑
AAM (1 g/kg)	52.33 ± 25.59	66.16 ↓	124.66 ± 28.59	10.80 ↑	560.8 ± 44.74	25.08 ↑	6.26 ± 0.15*	20.07 ↓

Values are represented in Mean ± SEM, *p < 0.01 vs. control group.

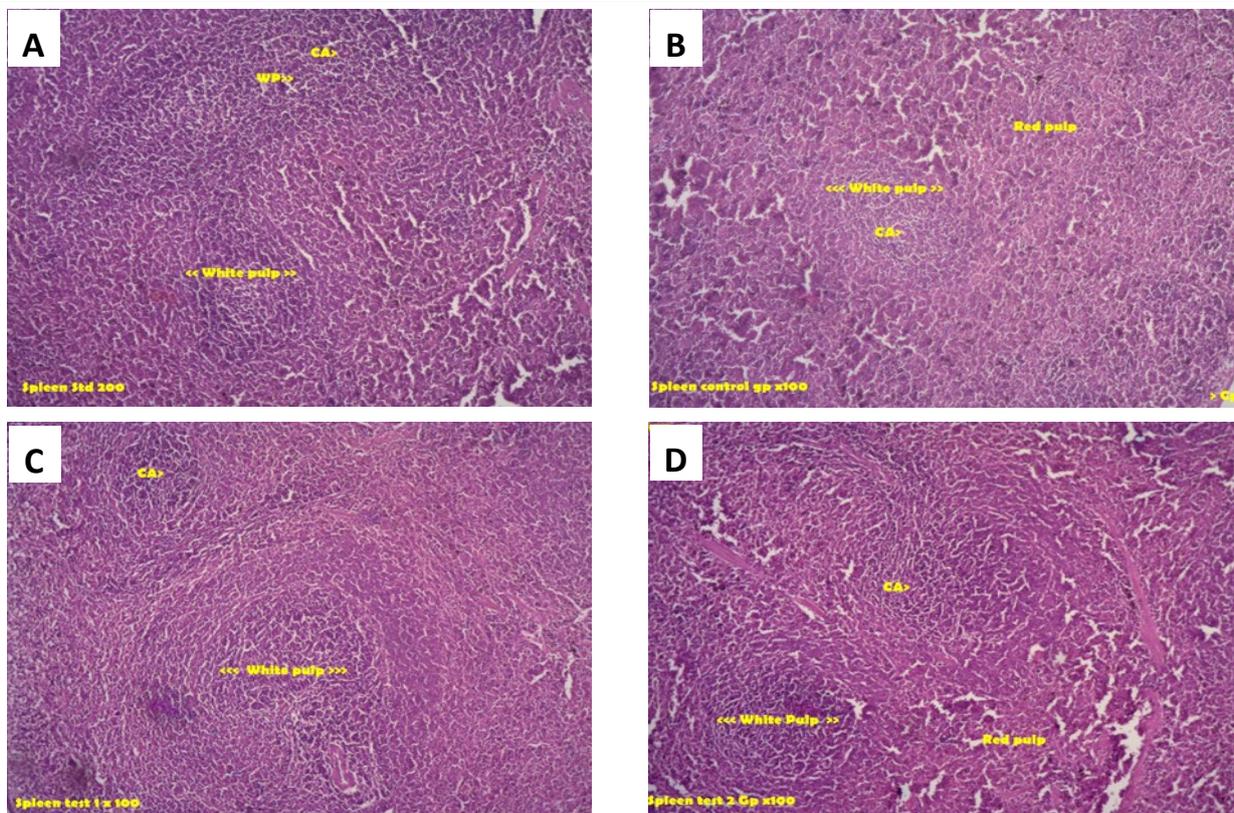
AAM - aqueous extract of *Antidesma menasu* leaves

Figure 1: Histology of adrenal gland after 7 days treatment with aqueous extract of *Antidesma menasu* leaves in rats.

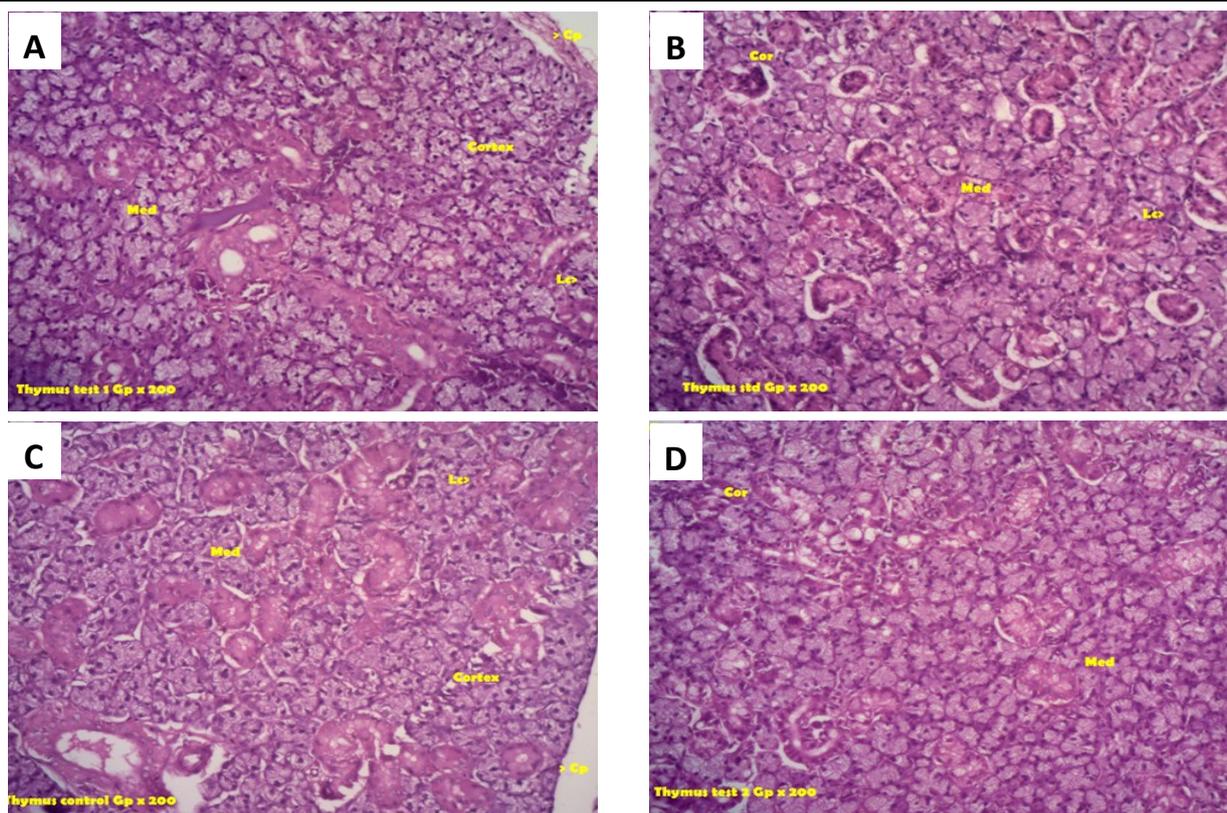


A - control group, B - ibuprofen (100mg/kg) treated group, C - aqueous extract of *Antidesma menasu* 500mg/kg D - aqueous extract of *Antidesma menasu* 1g/kg. All pictures show normal cytoarchitecture, there is no difference among the groups. Med - Medulla, CP - Capsule.

Figure 2: Histology of spleen after 7 days treatment with aqueous extract of *Antidesma menasu* leaves in rats



A - control group shows normal cytoarchitecture, B - ibuprofen (100mg/kg) treated group has moderate increase in white pulp, C - aqueous extract of *Antidesma menasu* 500mg/kg and D - aqueous extract of *Antidesma menasu* 1g/kg show significant increase in white pulp. CA - central artery, CP - capsule.

Figure 1: Histology of thymus after 7 days treatment with aqueous extract of *Antidesma menasu* leaves in rats

A - control group shows normal cytoarchitecture, B - ibuprofen (100mg/kg) treated group has moderate increase in white pulp, C - aqueous extract of *Antidesma menasu* 500mg/kg and D - aqueous extract of *Antidesma menasu* 1g/kg. All pictures show normal cytoarchitecture, there is no difference among the groups. Cor - cortex, Med - Medulla, CP - Capsule, Lc - lobule.

Chronic inflammation causes tissue destruction brought by activated macrophages by release of variety of biological substances. The control group rats has been showed enlargement of white pulp of spleen. It might be due to the increase in the macrophage and lymphocyte activities. But AAM extract has suppressive effect on these events, showed normal cytoarchitecture of spleen; the preliminary phytochemical constituents such as flavonoids, steroids may be responsible for its anti-inflammatory activity. The present study on aqueous extract of *A. menasu* suggested that this plant has significant analgesic and anti-inflammatory properties and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

The result suggests that the aqueous extract of leaves of plant *A. menasu* have moderate analgesic activity. The extract has significant anti-inflammatory activity against acute inflammation and significant dose dependant

anti-inflammatory activity against chronic inflammation. The data obtained supports the traditional folklore therapeutic claim about its anti-inflammatory and analgesic activity. Scientific investigation is required to establish its analgesic and anti-inflammatory property in other experimental models and clinical settings.

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