



Evaluation of Analgesic, Anti-Inflammatory and Anti-Ulcer Activity of *Diospyros melanoxyton* (Roxb). Methanolic Leaf Extract in Rats and Mice

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ABSTRACT

The aim of the present study was to evaluate the analgesic and anti-inflammatory and anti-ulcer activities of the *Diospyros melanoxyton* Roxb. Methanolic leaf extract (DMMLE) by using the acetic acid induced writhing test for peripheral pharmacological action, carragenan induced paw oedema and the pyloric ligation methods respectively. The results showed that DMMLE has significantly ($p < 0.001$) reduced inflammation at 100, 200 mg/kg. body weight (p.o) (i.e 35%,42%) when compared with control group, standard drug Diclofenac sodium(10mg/kg) has maximum anti-inflammatory effect (99%) and DMMLE also possessed significant ($p < 0.001$) analgesic activity in mice in dose dependent manner at 100, 200 mg/kg.body weight (p.o). in pyloric ligation method for anti-ulcer activity the DMMLE showed significant ($p < 0.05$) anti-ulcer activity at dose 200mg/kg. body weight. at the dose 100mg/kg showed non- significant with compared to control. The standard drug ranitidine (50mg/kg) showed significant anti-ulcer activity (i.e 85%).

Keywords: Analgesic, Anti-inflammatory and anti-ulcer activity, *Diospyros melanoxyton* Roxb.

INTRODUCTION

Diospyros melanoxyton(Roxb) is native and endemic tree of India and widely found forests of central, western and northern India. This is the most common species of forests of Madhya Pradesh, Bihar, Jharkhand, Chhattisgarh, Rajasthan, Gujarat, Orissa, Andhra Pradesh and Tamil Nadu. In English it is called Ebony and in telugu Tendu. It is a middle sized tree, height up to 10-15 ft., branchlets, young leaves, inflorescence clothed with soft grey or tawny tomentum. Leaves mostly sub-opposite, coriaceous, 3-6 inches long but sometimes much longer up to 12 inches, when full grown glabrous above, tomentose or pubescent beneath. The chemical constituents present in this plants are fruits contains flavonoids, saponins, tannins, terpenoids, alkaloids.¹ Leaves having valuable flavones, pentacyclic triterpines. Leaves possess antimicrobial activity, i.e ceryl alcohol, lupeol, betulin, sitosterol, triterpine alcohol, triterpine carboxylic acid i.e diospyric acid. Bark contains s tannins, flavonoids, terpinoids.

MATERIALS AND METHODS

Plant material

The leaves of the *Diospyros melanoxyton* Roxb. Was collected from forest of Thirumalgiri (Mdl), Nalgonda (Dist), Andhra Pradesh, India was taken authentication from Dr.V.S.Raju, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh.

Preparation of Extract

The leaves of *Diospyros melanoxyton* were collected and shade dried completely. The dried leaves were reduced to coarse powder by mechanical grinding. The powdered

material 500g was subjected to continue hot extraction in soxhlet by using methanol (95% v/v).the extraction was continued until the thimble became clear. After complete extraction, the extract was stored in deep freezer at 20°C for experimental use.

Experimental Animals

Wister rats (150-200g) and Swiss Albino Mice (25-40g) of either sex were used for this study. The animals were obtained from Sainath agencies, CPCSEA Reg. No: 282, DATE: 24-11-197/45/D, Bapuji nagar, Musheerabad, Hyderabad-48. Andhra Pradesh. India. The animals were maintained under standard environmental condition and had free access to food and water before administration of plant extract.

Acute Toxicity Studies

The acute oral toxicity studies were performed as per OECD-423 guidelines. Swiss albino mice of either sex selected by random sampling technique were used for acute toxicity study. the animal were kept fasting overnight providing only water, after which the extract (50% alcoholic extract) was administered orally at the dose of level of 5mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in two of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose would be repeated again to confirm the toxic dose. if mortality was not observed, the procedure was repeated for further higher doses such as 50,300 and 2000 mg/kg body weight. According to the results of the acute toxicity test, the doses were chosen for experiments.

MATERIALS AND METHODS

Anti- Inflammatory Activity: Carrageenan induced paw edema method

In this study the animals were divided in to four groups (n=6).

Group I: Served as toxic control group received carrageenan (1%) of 0.1ml in sub plantar region.

Group II: Served as standard group received Diclofenac sodium (10mg/kg) in sodium CMC (1%) (p.o) + carrageenan (1%) of 0.1ml in sub plantar region.

Group III: Served as test group received 100 mg /kg. Body weight. *Diospyros melanoxylon* leaves methanolic extract in sodium CMC (1%) (p.o)+ carrageenan (1%).

Group IV: Served as test group received 200 mg /kg. Body weight. *Diospyros melanoxylon* leaves methanolic extract in sodium CMC (1%) (p.o)+ carrageenan (1%).

Procedure

Wister rats of either sex were taken and divided in to four groups, each group contain six rats. All four Groups pre-treated with the standard and test drugs as per study design. After half an hour carrageenan (0.1 ml of 1 %) administration in the sub plantar region of left hind paw of each rat. They were deprived of water during experiment to ensure uniform hydration and to minimize variability in oedematous response². The measurement of the paw volume (cm³) was done on the principle of volume displacement using digital Plethysmometer. The readings were taken at 1hr intervals after the injection of carrageenan for a period of 6 hrs. The edema at each time was calculated in relation to the paw volume before the injection of the carrageenan. The anti- inflammatory activity was determined as the percentage of inhibition of inflammation after it was induced by carrageenan by taking volume of inflammation in control group as 100%. The percentage inhibition was calculated by using the formula:

$$\% \text{ inhibition} = \frac{\text{Control group mean} - \text{Test group mean}}{\text{Control group mean}} \times 100$$

Analgesic Activity

Acetic acid- induce writhing in mice

In this study the animals (mice) divided in to four groups (n=6).

Group I: Served as toxic control group received acetic acid (1%) of 0.1ml/10g, (i.p).

Group II: Served as standard group received Aspirin (100mg/kg) in sodium CMC (1%) per oral.

Group III: Served as test groups received 100, 200 mg /kg. Body weight. *Diospyros melanoxylon* methanolic leaf extract in sodium CMC (1%), (p.o)

Group IV: Served as test group received 200 mg /kg. Body weight. *Diospyros melanoxylon* methanolic leaf extract (DMMLE) in sodium CMC (1%) (p.o)

Procedure

The analgesic activity of *Diospyros melanoxylon* methanolic leaf extract was carried out according to the method. The mice were divided into four different groups (of six mice each). They were pre-treated as per Study design. After 30 min the treatment, 1% acetic acid solution was administered to the mice (1ml/100gm, i.p). They were placed in a transparent observation box. Five minutes after the administration of acetic acid the number of abdominal constrictions (writhes) and stretching of the hind limbs made within 20 minutes of every mouse was counted cumulatively. The results of the treatment groups were compared with those of normal saline pre-treated control. The percentage of the writhes was calculated as:

$$\% \text{ inhibition} = \frac{\text{Avg writhes in Control group} - \text{Avg writhes in Test group}}{\text{Avg writhes in Control group}} \times 100$$

Anti - Ulcer Activity

Pyloric ligation Method

In this study the animals (Rats) divided in to four groups (n=6)

Group I: Served as control group received normal saline (10ml/kg) (p.o).

Group II: Served as standard group received Ranitidine (50mg/kg) i.p. route.

Group III & IV: Served as test groups received 100, 200 mg /kg. Body weight. *Diospyros melanoxylon* leaves methanolic extract in sodium CMC (1%), per oral.

Procedure

Wister rats weighing between 150 – 250 g were selected for pyloric ligation ulcer model as described by Njar et al., (1995). Rats were divided into 4 groups, each group consisting of five animals. The animals were fasted for 24 hrs before the operation procedure. However they given free access to water. Prevent the cannabolism and caprophagy. After 24 hrs fasting, all the groups were treated with standard and test drugs as per the study design. At 25th hr under ether anesthesia pyloric ligation carried out by a one-inch middle abdominal incision was given below the xiphoid process. The pylorus is carefully lifted out with a minimal handling and traction and ligated without damaging its blood supply. Animals were sacrificed 4-6 h later by cervical dislocation. The stomach was opened to collect the gastric contents. Scoring of gastric ulceration was done as described by Kunchandy (1985). Ulceration in the stomach was accessed by means of a scoring technique whereby macroscopic examination of the stomach was made using a hand (10 xs) and ulcers were scored using the method and criteria.^{3,4}

Normal gastric mucosa was scored - -0

- Punctuate hemorrhage, pinpoint ulcer was scored-- 0.5.



- One or two small hemorrhages ulcer was scored-- 1.0
- Ulcers greater than 3mm in diameter were scored -- 2.0.
- Ulcer index and percentage inhibition of ulceration were calculated as earlier described⁵ thus;

Ulcer Index:

$$\frac{\text{Mean degree of ulceration} \times \text{Percentage group of ulceration}}{100}$$

% Inhibition of ulceration=

$$\frac{\text{Ulcer index in Control} - \text{Ulcer index in Test}}{\text{Ulcer index in Control}} \times 100$$

The total volume of gastric content was measured. The gastric contents were centrifuged at 100 rpm for 10 min. 1ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The

volume of NaOH required was noted and was taken as corresponding to the total acidity.

Acidity was expressed as

$$\text{Acidity} = \frac{\text{Vol.of NaOH} \times \text{Normality} \times 100}{0.1} \text{mEq/L}$$

Statistical Analysis

The Results were exposed as mean \pm SD. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. Values less than 0.05 ($P < 0.05$) were considered as indicative of significance.

RESULTS

In the acetic acid induced writhing method, pre treatment with DMMLE at the doses of 100,200 mg/kg body weight exhibited very significant ($P < 0.001$) dose dependant analgesic activity comparison to control group. The maximum analgesic activity showed aspirin 100mg/kg (78%), followed by extract 200mg/kg (58%), 100 mg/kg extract showed lowest analgesic effect. which is represented in Table 1.

Table1: Effect of crude extract of diospyros melanoxylon (roxb).on acetic acid induced writhing response

Group	Treatment	No .of writhing within 15 min	% of inhibition
I	Normal saline + acetic acid (1%) of 0.1ml	50 \pm 9.58	----
II	Aspirin (100mg/kg) + acetic acid (1%)	11 \pm 2.31 ^{***}	78%
III	DMMLE (100mg/kg) + acetic acid (1%)	30 \pm 5.68 ^{***}	40%
IV	DMMLE (200mg/kg) + acetic acid (1%)	25 \pm 3.34 ^{***}	58%

Values are expressed as mean \pm SD, (n=6), *** $p < 0.001$ Percentage Inhibition, Compared to Control group.

Table 2: Effect of DMMLE on pyloric ligation induced gastric ulcer

Group	Treatment (mg/kg)	Gastric volume (ml)	p ^H	Ulcer index (mean \pm SD)	Free acidity	Total acidity	% of protection
I	Normal saline (10ml/kg)	9ml	2.36	3.4 \pm 1.36	51.6	63.3	—
II	Ranitidine (50mg/kg)	4ml	4.02	0.56 \pm 0.456 ^{***}	38.6	67.6	85%
III	DMMLE (100mg/kg)	6.9ml	3.35	2 \pm 0.790 ^{ns}	61.33	62.3	32%
IV	DMMLE (200mg/kg)	4.1ml	3.03	1.6 \pm 0.741 [*]	42.66	60.6	58%

Values are expressed as Mean \pm SD, *** $P < 0.001$, * $P < 0.05$ (n = 6), Percentage protection compared to Control, *Ns* =non significance.

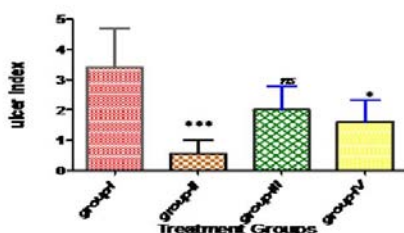
Pre treatment with DMMLE exhibit anti-ulcer activity at the dose of 200mg/kg body weight plant extract significantly ($P < 0.05$) decreased ulcer index (78%) comparison to control group .The extract 100 mg/kg, showed non-significant (*ns*) anti ulcer activity. The standard drug Ranitidine (50mg/kg) showed maximum significant ($P < 0.001$) anti-ulcer activity. This is represented in Table 2 & Figure 1. The figure 2 shows that A, B were control Group; C, D Ranitidine(50mg/kg) treated Group; E and F plant Extracts 100,200 mg/kg, in

treatment group it is dose dependent controlled the pyloric ligation ulcer .

In the carrageenan induced paw edema method, the oral administration of DMMLE in graded doses (100,200 mg/kg body weight) produced a significant ($P < 0.001$) reduction in paw edema in a dose dependant manner in comparison to control. The maximum effect was seen at the oral dose 200mg/kg that showed a very significant ($P < 0.001$) reduction as 42% in paw volume in comparison to control. The group-II received Diclofenac (100mg/kg) as

standard drug showed 53% reduction in paw volume, which is represented in Table 3.

FIG1:effect of DMMLE on pyloric ligation induced gastric ulcers



Values are expressed as Mean \pm SD, (n = 6) Percentage protection compared to control group. **** P<0.001= very significant; * P<0.05= significant; ^{ns} = non significance

Table 3: Effect of crude leaves extract of diospyros melanoxylon (roxb).on carrageenan induced paw edema in wister rats

Group	Treatment	Increase in paw volume (ml)					% of inhibition	Mean \pm SD
		1 hr	2 hr	3 hr	4 hr	6 hr		
I	Normal saline (10ml/kg)	2.170 \pm 0.31	2.813 \pm 0.23	3.310 \pm 0.44	3.041 \pm 0.44	2.848 \pm 0.42	—	2.8 \pm 0.42
II	Diclofenac sodium (10mg/kg)	1.678 ^{***} \pm 0.178	1.427 ^{***} \pm 0.122	1.257 ^{***} \pm 0.110	1.166 ^{***} \pm 0.150	1.105 ^{***} \pm 0.095	53%	1.3 \pm 0.23 ^{***}
III	DMMLE (100mg/kg)	2.0868 ^{***} \pm 0.15	1.890 ^{***} \pm 0.20	1.779 ^{***} \pm 0.343	1.6855 ^{***} \pm 0.337	1.64 ^{***} \pm 0.4059	35%	1.8 \pm 0.19 ^{***}
IV	DMMLE (200mg/kg)	1.896 ^{***} \pm 0.28	1.835 ^{***} \pm 0.188	1.549 ^{***} \pm 0.267	1.49 ^{***} \pm 0.160	1.424 ^{***} \pm 0.291	42%	1.6 \pm 0.21 ^{***}

Values are expressed as Mean \pm SD; Percentage Inhibition Compared to Control; ***p<0.001 very significant (n=6).

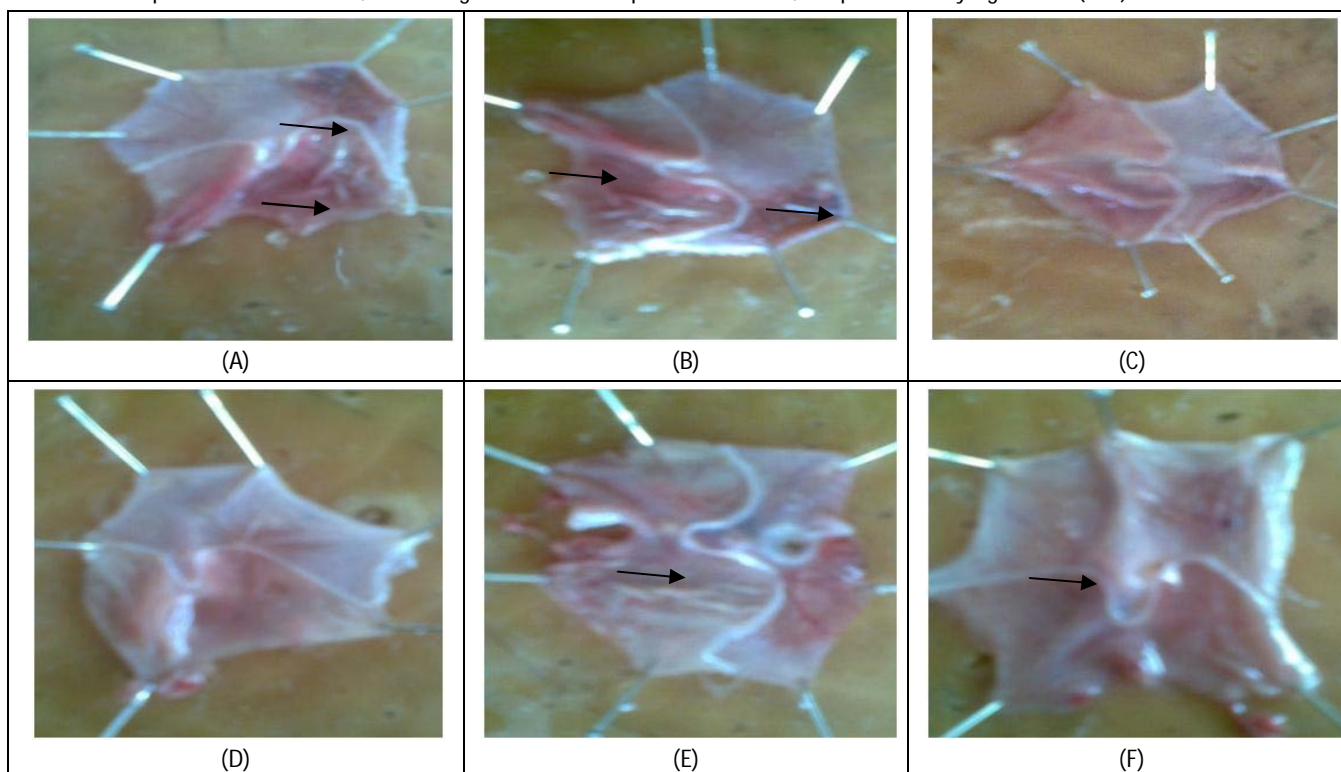


Figure 2: The effect of vehicle, Ranitidine, and extracts of *diospyros melanoxylon* on pylorus ligation induced ulcer model; The above figure shows **A, B** control Group; **C, D** Ranitidine (50mg/kg) treated Group; **E and F** plant Extracts 100, 200 mg/kg. Body weight treated group.

Non-steroidal anti-inflammatory drugs are known to inhibit cyclooxygenase (COX-2) enzyme involved in Prostaglandin synthesis.¹¹ Based on the reports the present study investigation revealed that the methanolic extract of *diospyros melanoxydon* (Roxb), extract¹² (100,200mg/kg), significantly (P<0.001) reduction in paw volume in carrageenan induced paw edema method. It could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. It may be containing flavonoids.¹³

Writhing response of the animals to an i.p injection of noxious chemical are used to screen peripheral acting analgesic activity. Acetic acid causes analgesia by liberating endogenous substances that excite the pain nerve endings.¹⁴ In the present study the DMMLE (100, 200mg/kg) significantly (P<0.001) reduce the number of abdominal writhings induced by acetic acid(1%) in mice . abdominal writhing induced by the acetic acid involves the process or release of Arachidonic acid metabolite via COX and prostaglandins biosynthesis.

Pyloric ligation method mainly used for the evaluation of anti-ulcer drugs. Pyloric ligation increased the acid secretion, which in turn caused increase in gastric volume, low P^H, increased free and total acidity resulting into increase in ulcer index.¹⁵ In the present study investigation, Methanolic leaf extract of *Diospyros melanoxydon* evaluated for its anti-ulcer activity against pylorus ligation induced gastric ulcer model. The results of study are tabulated in Table 3 and figure 3. The methanolic extract 200mg/ kg body weight reduces the ulcer index significantly (p< 0.05) comparing to control group this investigation supporting to that of Maruthappan1 et al., 2010, investigations.¹⁵ It also reduces the free Acidity, total Acidity and pH of the gastric content. It was showing protection index 32% and 58% at the dose of 100 mg/ kg and 200 mg/ kg. Respectively, the standard drug ranitidine (50mg/ kg) showed 85 % of ulcer protection. This activity of DMMLE may be due to the presence of flavonoids and terpenoid in its chemical constituents.

In pyloric ligation method, the histopathological study finds that normal saline treated (Group-I) rats gastric mucosa shows severe wound formation (figure 4: A, B) and Ranitidine (50mg/kg) treated (Group-II) rats gastric mucosa showed (figure 4:C,D) inhibition of the ulcer formation and DMMLE (100,200mg/kg body weight) treated (Group-III,IV) rats gastric mucosa showed (figure 4:E,F) inhibition of gastric ulcer index.

CONCLUSION

The present investigation concluded that, the flavonoids and pentacyclic triterpenoids of *Diospyros melanoxydon* showed significant Analgesic, anti-inflammatory and anti-ulcer activity in dose dependant manner. These effects could therefore, be a point for the use of the plant

traditionally as a remedy for inflammatory treatment. This suggests that, further work to be carried out on this plant extract with the view of characterizing the possible such activities.

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