International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.3, pp 2038-2043, July-Sept 2010

Study of Analgesic, Antipyretic and Diuretic Activities of various Extracts of *Diospyros melonoxylon*

P.Devi[1]*, R.Meera[2] N.J.Merlin[3] D.Dinesh Babu[1]

[1]Department of Pharmacognosy, [2]Department of Pharmaceutical Chemistry, K.M.College of Pharmacy, Uthangudi, Madurai –625107, Tamilnadu, India,

3.Department of Pharmacy, Annamalai, University,Chithambaram,Tamil Nadu,India

Corres.author: devipmpharma@yahoo.co.in. Phone no: 09865377764

Abstract: To evaluate the leaves of *Diospyros melonoxylon* for Tail Immersion Method, Acetic Acid Induced Writhing Test, Eddy's Hot Plate Method for analgesic activity and Yeast induced hyperpyrexia method for antipyretic activity and diuretic activity.The leaf extracts of *Diospyros melonoxylon* were tested for analgesic activity in Tail immersion method Asprin as standard and Propyelene glycol as the control, Eddys hot plate method Morphine sulphate as the standard and Propyelene glycol as the control and Acetic acid induced writhing test Acetyl salicylic acid as the standard. For antipyretic activity yeast induced hyperpyrexia method Paracetamol as the standard and Propyelene glycol as the control.Chloroform, ethanol, petroleum ether and aqueous extracts of leaves of *Diospyros melonoxylon* were studied for diuretic activity. Furosimide as the standard and normal saline as the control .The 6 hours acute study of successive chloroform and aqueous extracts of the leaf showed increase in urine volume and K+ ion excretion as compared to normal saline. Urinary levels of sodium, potassium (by flame photometry) and chloride (by titrimetry) were estimated.On the basis of observed results it is concluded that chloroform and ethanol extracts ($P<0.001$) have good analgesic activity while successive chloroform and aqueous extract ($P<0.001$) exhibited evidence of diuretic potentials in normal rat in our experimental model. Petroleum ether and ethanol extract ($P< 0.001$) have exhibited good antipyretic activity. *D.melonoxylon* possess significant analgesic, antipyretic and diuretic activities.

Keywords : *Diospyros melonoxylon* , Analgesic , Antipyretic , Diuretic.

Introduction :

Diospyros melonoxylon is moderated to large sized tree, belonging to family *Ebenaceae* . It is distributed in the Indian peninsula. The plant is useful in the treatment of Carminative and Laxative. The leaves are Diuretic and Styptic. The bark is astringent and its decoction is used in Diarrhoea and Dyspepsia. Dried flowers are useful in urinary, skin and blood diseases. Literature survey reveals that the plant bark contains tannins, ceryl alcohol, lupcol, betulin. Leaves contains hentriacontane , amyrin , ursolic acid.Seed oil contains fatty acids like malvic acid and sterulic acid, 9-Keto-cis-13-octadacenoic acid[1-4]. But adequate characterization of its analgesic, antipyretic and diuretic activity has not been yet confirmed. The present study was undertaken for scientific evaluation of analgesic activity using Tail immersion Method , Acetic Acid Induced Writhing Method and Eddy's Hot Plate Method, antipyretic activity using yeast induced pyrexia in rats and diuretic activity in normal healthy rats.

Materials and Methods :

Plant material

The entire herb of *Diospyros melonoxylon* has been collected in the month of June from Tirunelveli, authenticated. A voucher specimen has been deposited

in the herbarium of same department. The plant was shade dried and pulverized.

Animals

Male albino rats of wistar strain, obtained from Raja institute (150 – 175 gm) were maintained under standard husbandry conditions throughout the study. The animals were allowed to access to standard laboratory feed and water *ad libitum* and they were divided into 7 groups each having 6 animals. Aspirin (25mg/kg), Paracetamol (200mg/kg) and Furosemide (4mg/kg) were used as standard drugs for comparison of analgesic, antipyretic and diuretic activity respectively. Standard methods[5,6] were used for preliminary phytochemical screening of the extracts to know the nature of phyto constituents present in it. The institutional Animal Ethical Committee approved all experimental protocols.

Preparation of Extracts :

250gm powdered plant material was subjected to continuous hot exhaustive extraction with 95% ethanol in Soxhlet extractor. After each extraction, the solvent was recovered and the extract was concentrated at room temperature, then it was fractionated using petroleum ether (60-80 ^{0}C), chloroform, ethanol and aqueous. These extracts were used for the study of analgesic, antipyretic and diuretic activities in rats.

Preliminary Phytochemical Investigation

The qualitative chemical test of various extracts of *Diospyros melonoxylon* was carried out using standard procedure[7,8,9]. Carbohydrates, proteins and amino acids are present in chloroform, ethanol and aqueous extract. Saponin, flavanoids and glycosides are present in ethyl acetate and ethanol extract. Phyto sterols, and phenolic compounds are present in chloroform , ethyl acetate, ethanol and aqueous extract.

Toxicity Studies [10]

Healthy adult male albino swiss mice weighing between 150-175 gm were used in the present investigation. The samples were tested for 50-1500 mg/Kg body weight (as suspension 0.5% CMC) in group of 6 animals by intraperitoneal administration. The control group of animals received only the vechicle (0.5%CMC).The animals were observed for 48 hrs from the time of administration of test compound to record the mortality.

Analgesic Activity :

Tail Immersion Method

Tail immersion method was used to determine the analgesic activity[11,12,13]. Rats of wistar strain were randomly divided into 7 groups having 6 animals each and they were fasted overnight but during the experiment had free access to water. All the extracts were administrated orally (300mg/kg) 60 minutes prior to the commencement of the estimation of reaction time. The temperature of water in the organ bath was set at 55 ± 0.5 ^{0}C with the help of a thermostat. The reaction time was determined by immersing the tail in hot water and the time taken by the rat to withdraw its tail clearly out of water was noted. Observations were repeated at an interval of 30 minutes up to 678 180 minutes. The animals which showed flicking response with in 3-5 sec, were collected for the study. A cut off period 15 sec is observed to avoid damage to the tail. The response of the treated groups were compared to the animals in control group (0.3 ml of saline only). (Table 1)

Acetic Acid Induced Writhing Test [14,15,16,17,18]

The non narcotic analgesic activity was evaluated by acetic acid induced writhing in mice. In this method, mice of either sex of weight between 20-25 g were randomly distributed in four groups each consisting of six animals. The first group served as control and the animals were administered intraperitoneally with 0.5 ml of 1% acetic acid dissolved in 0.9% saline. The number of writhnes were counted during at 30 min period following the injection of acetic acid. The animals of second and third group were administered with plant extract orally and 15 min later the animals of these groups were administered with acetic acid as before .The fourth group was administered with aspirin, intraperitoneally and then acetic acid was given for induction of writhing, which was recorded as described for the groups above.

Percentage of protection against acetic acid induced writhing was calculated using the formula

$$\text{Percent protection} = \frac{Wc - Wt \times 100}{Wc}$$

Where Wc = Control group; Wt = the mean values of number of writhing in the test groups . (Table 2)

Eddy's Hot Plate Method [19,20]

Healthy male albino mice weighing 25-30 g divided into 4 groups each consist of 6 animals.

Group-1 considered as control , Group-2 received morphine sulphate (5 mg/kg) served as positive control while group 3,4,5,6 received petroleum ether , chloroform, ethanol and aqueous extract(300 mg/kg) respectively. The animals were placed on the hot plate maintained 55 to 56 ^{0} temperature and the time until either paw licking or jumping was noted. The average basal reaction time was calculated using student t- test. The results were presented in (Table 3).

Antipyretic Activity :

The antipyretic activity was screened by using yeast induced hyperpyrexia method [21,22,23]. Male rats (150 – 175gm) were selected and divided into 7 groups each having 6 animals. They were maintained at constant temperature of 24-25^0 for 24 hours before pyrexia was induced by subcutaneous injection of 2ml of 15 % brewer's yeast suspension in saline solution. After 18 hours of yeast injection, the extracts at a dose of 300mg/kg were administered orally to each group as a suspension in propylene glycol. Rectal temperatures were noted using telethermometer at 30 minute interval up to 180 minutes. The reduction of the temperature is calculated as below:

$$\text{The percentage reduction} = \frac{\text{A-B} \times 100}{\text{A-C}}$$

A represent temperature after 18 hours of yeast injection. B represent group of different time intervals. C represent normal temperature. (Table 4)

Diuretic Activity :

The method of Lipschitz et al[24,25] was employed for the assessment of diuretic activity. The animals were fasted and deprived of water for 18 hrs prior to the experiment, were divided into 7 groups having 6 rats each. The first group of animals serving as control received normal saline, the second group received furosemide as standard drug, and the remaining groups received the test extracts at a dose of 300mg/kg. Immediately after dosing, the animals were separately placed in metabolic cages (3 in each cage) suitable for collection of urine in graduated measuring cylinders at 25±0.5^0 through out the experiment. Urine samples were collected for 3 hours, while animals were deprived of food and water. During this period, no water or food was made available to animals. Na^+, K^+ concentrations were measured by Flame photometry[26]and Cl^- concentration was estimated by titration[27] with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20 mg/kg of furosemide per kg body weight in series of supportive experiments. (Table 5)

Statistical methods

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by student 't' test [28] at a probability level of $P < 0.001$.

Table 1 : Effect of different extracts of *Diospyros melonoxylon* on analgesic activity (Tail Immersion Method) in rats

Time (min)	Control	Standard	PE	CF	ET	AQ
0	5.1±0.11	6.1±0.22	7.3±0.31	6.6±0.21	6.1±0.15	5.1±0.09
30	5.1±0.14	8.2±0.11	8.1±0.23	8.2±0.20	7.7±0.12	5.3±0.27
60	6.1±0.11	9.2±0.11	9.5±0.22	10.2±0.20	12.2±0.22	6.3±0.21
90	6.9±0.11	13.3±0.12	9.6±0.26	13.2±0.18	14.2±0.19	8.3±0.28
120	7.20.14	23.2±0.13	9.7±0.28	20.1±0.11	19.2±0.15	21.4±0.11
180	6.60.14	35.3±0.15	20.4±0.13	30.1±0.13	29.3±0.16	30.3±0.43

Table 1: shows the data obtained from the analgesic activity of *Diospyros melonoxylon* . PE is petroleum ether extract , CF is chloroform extract, ET is ethanol extract . AQ is aqueous extract . Aspirin was used as standard ; propylene glycol was used in control group . Each value is a mean± standard error for group of 6 animals (n = 6). Values are expressed as mean ± SEM *P<0.001 as compared to control indicates the significant analgesic activity.

Table 2 : Effect of different extracts of *Diospyros melonoxylon* on analgesic activity (Eddy's Hot Plate Method) in rats

Time (min)	Control	Standard	PE	CF	ET	AQ
0	4.53±0.11	4.80±0.13	4.32±0.16	4.46±0.1	4.77±0.32	5.13±1.5
30	5.12±0.13	7.15±0.12	5.10±0.36	6.24±0.14	6.76±0.12	6.14±0.3
60	5.02±0.05	9.10±0.12	6.39±0.41	8.21±0.13	9.31±0.14	7.21±1.3
90	4.67±0.13	11.01±0.42	8.91±0.61	9.31±0.17	10.42±1.31	9.34±3.2
120	5.01±0.18	8.74±0.12	6.81±0.36	7.91±0.23	8.31±2.32	6.32±1.4
180	6.11±0.34	7.52±0.03	5.31±1.22	6.28±1.32	7.63±1.54	6.14±2.5

Table 2: shows the data obtained from the analgesic activity of *Diospyros melonoxylon* . PE is petroleum ether extract , CF is chloroform extract, ET is ethanol extract . AQ is aqueous extract . Morphinesulphate was used as standard ; propylene glycol was used in control group . Each value is a mean± standard error for group of 6 animals (n = 6). Values are expressed as mean ± SEM *P<0.001 as compared to control indicates the significant analgesic activity.

Table 3 : Effect of different extracts of *Diospyros melonoxylon* on analgesic activity (Acetic Acid Induced Writhing) in rats

Group	Treatment	Dose (mg/kg)	Avg.no.of.Writhing	Percentage Inhibition
1	Control	10ml/kg	34 ±0.45	-
2	ASA	100	10 ±1.04	71.42
3	PE	300	14 ±0.12	60.01
4	CF	300	11 ±0.23	70.87
5	EA	300	12 ±0.21	66.73
6	AQ	300	11 ±2.13	69.51

Table 3 shows the data obtained from the analgesic activity of *Diospyros melonoxylon*. PE is petroleum ether extract , CF is chloroform extract, ET is ethanol extract, AQ aqueous extract . ASA=Acetyl Salycilc Acid was used as standard. Each value is a mean ± standard error for group of 6 animals (n=6). Values are expressed as mean ± SEM *P<0.001 as compared to control indicates the significant analgesic activity.

Table 4 : Effect of different extracts of *Diospyros melonoxylon* on yeast induced On yeast induced pyrexia in rats

Time (min)	Control	Standard	PE	CF	ET	AQ
0	38.4±0.15	38.1±0.22	29.4±0.22	30.6±0.31	34.3±0.15	37.3±0.23
30	38.4±0.11	38.1±0.12	26.3±0.23	31.6±0.28	37.4±0.25	38.3±0.43
60	38.6±0.21	38.1±0.32	24.3±0.27	32.6±0.26	37.5±0.15	30.3±0.11
90	38.3±0.13	39.3±0.23	25.3±0.15	33.6±0.15	36.9±0.27	37.3±0.21
120	38.1±0.11	37.5±0.26	29.3±0.23	30.6±0.12	37.5±0.10	37.4±0.28
180	39.9±0.11	37.1±0.36	30.3±0.17	33.3±0.18	35.1±0.26	36.1±0.21

Table 4 shows the data obtained from the antipyretic activity of *Diospyros melonoxylon*. PE is petroleum ether extract , CF is chloroform extract, ET is ethanol extract, AQ aqueous extract . Paracetamol IP was used as standard, propylene glycol was used in control group. Each value is a mean ± standard error for group of 6 animals (n=6). Values are expressed as mean ± SEM *P<0.001 as compared to control indicates the significant antipyretic activity.

Table 5: Effect of different extracts of *Diospyros melonoxylon* on Urine Excretion Paramaters in Rats.

Treatment	Urine volume (ml)	Concentration of ions (mEq/lit)			
		Na^+	K^+	Cl^-	Na^+/K^+
Control	1.8±0.1	91.2±0.93	95.1±1.01	93.0±0.98	1.44
Standard	5.1±0.17	127±0.42	95.2±0.11	98.4±0.25	1.37
PE	2.1±0.10	91.1±0.53	74.3±0.77	94.2±0.84	1.27
CF	3.3±0.11	120.4±0.63	93.2±0.11	97.1±0.22	1.36
AQ	3.6±0.20	110.0±0.36	85.1±0.29	95.0±0.41	1.22
ET	3.1±0.5	105.3±0.23	80.1±0.5	94.0±0.17	1.48

Table 5 shows the data obtained from diuretic activity of *Diospyros melonoxylon*. PE is petroleum ether extract, CF is chloroform extract, ET is ethanol extract, AQ aqueous extract. Furosemide IP was used as standard drug and normal saline was used in control group. Each value is a mean ± standard error for group of 6 animals (n=6). Values are expressed as mean ± SEM *P<0.001 as compared to control indicates the significant diuretic activity as compared to standard.

Results and Discussion

Toxicological study

Neither mortality nor any gross behavioral changes were observed during and after the treatment. The petroleum ether, chloroform, ethanol and the aqueous extracts were found to be safe upto 1000 mg/kg.

Analgesic activity

In tail immersion method the chloroform and ethanol extracts (300mg/Kg) showed significant analgesic activity as shown in (Table1). In hot plate method the analgesic activity produced by all extracts of *Diospyros melonoxylon* (300 mg/kg) oral administration of the extract results in significant and dose dependent prolongation of the response tendency in hot plate method (Table 2). In acetic acid induced writhing method the non narcotic analgesic activity was evaluated. The activity was found to be more in chloroform extract (70.87% inhibition) and aqueous extract (69.51% inhibition) as shown in (Table 3)

Antipyretic activity

The petroleum ether and ethanol extracts have exhibited the significant antipyretic activity as shown in (Table 4), whereas chloroform and aqueous extracts did not show significant activity.

Diuretic activity

Furosemide treated rats showed a significant increase in volume of urine and urinary excretion of sodium, potassium and chloride ($p<0.001$) as compared to control. The successive chloroform and aqueous extracts produce significant actions in dose of 300 mg/kg and it showed significant increase in volume of urine and urinary excretion of sodium, potassium and chloride. The successive extracts have shown diuretic activity ($p< 0.001$) where in significant increase in K^+ but not in Na^+ excretion when compared to control was observed. Pet ether extract did not show remarkable increase in volume of urine, urinary sodium, potassium or chloride. The results are summarized in (Table 5)

References

1.S.N. Yoganarasimhan, Medicinal Plants of India , 2000,Vol 2, 198

2. R.N. Chopra, S.L .Nayar, I.C. Chopra .Glossary of Indian medicinal plants, National Institute of Science communication , Council of scientific and Industrial research, New Delhi, India, 1999, 189

3.The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, CSIR, NewDelhi , 1952,,81

4.R.J. Tallarida, D.J.Stone, J.D. Mc Cary and R.B. Raffa, Response surface analysis of synergism between morphine and clonidine, J. Pharmacol. Exp. Ther., 1999,289.

5. G.E .Trease, and W.C. Evans, Pharmacognosy,1989,171

6.J.B. Harbone, Phytochemical Methods : A Guide to Mordern Techniques of plant Analysis , 1984, 85

7. G.E. Trease and Evans M.C. Textbook of Pharmacognosy, 1983,343-383.

8.C.K. Kokate, Practical pharmacognosy ,1996, 107

9.H. Plaisted ,Philip Contributions from Boyee Thompron Institute , 1958,Vol 9: 231- 44.

10. M.N .Ghosh, Fundamentals of Experimental Pharmacology, 1986, 156 .
11. S.C. Pal and A .Nandy, Antiinflammatory, analgesic and antipyretic activity of *Achras Sapota*

13. R.A .Turner, In ; Screening Methods in Pharmacology, Academic Press, New York and London,1965, 112 .
14. G.C .Sonavane, V .Sarveiya, V.Kasture and B.S. Kasture , Indian J Pharmacol.2001,41.
15. S .Dewan, H .Sangraul and V.L. Kumar . J Ethnopharmacol.,2003,307 .
16. S.Ramaswamy, P.R.M.K. Reddy and D.G. Shewade, Clonidine induced antinociception: Biochemical and cellular evidences on the mechanism of action, 1998,30-33 .
17. C.A .Winter and C.C Poster, Effect of alteration in side chain on anti-inflammatory and liver glycogen activites in hydrocortisone ester. J.Amer.Pharmacol Soc, 1957,551-519 .
18. R.R .Rao, R.M. Balu, M.R.V.Rao and M.G.V .Babu, Studies on antipyretic, analgesic and hypoglycemic activites of root of *Gynandropsis gynandra* Linn. Indian Drugs,1997, 690 .
19. N.B. Eddy, Studies of morphine and codine and their derivaties. J. Pharmacol ,1932,339.
20. J.V.Kamant and A.C .Rana. Indian Drugs, 2003,292.
Linn. leaf extracts and its isolated compounds, Indian Drugs, 1999**,** 36,106
12. S.K. Kulkarni , Handbook of Experimental Pharmacology, Vallabh Prakashan, Delhi, edn 3,1993, 43 .
21. V.K .Pendse , A.P. Dadich, P.N. Mathur, M.S. Bal and B.R .Madan. Indian J Pharmaco, 1977,221.
22. P .Jeyasekar, P.V .Mohan and K.Rathinam. Hepatoprotective activity of ethylacetate extract of *Acacia catechu* . Indian J Pharmacol, 1997,426 .
23. J.J. Loux, P.D. Depalma and S.L. Yankell, Antipyretic testing of asprin in rats.Toxicol Appl Pharmacol,1972,672-673 .
24. W.L. Lipschitz, Z .Haddian and A .Kerpscar. Bioassay of diuretics. J.Pharmacol.Exp.Ther,1943,97-110.
25. T. Murugesan, L .Manikandan, K.B. Suresh, M .Pal and B.P. Saha. Evaluation of diuretic potential of *Jussiaea suffruticosa* Linn.extract in rats. Indian J.Pharm.Sci, 2000,150-151 .
26. G.H. Jeffery, J .Bassett, J.Mendham, R.C .Denny , Vogels Textbook of Quantitative Chemical Analysis, 1989, 801.
27. A.H .Beckette, J.B. Stenlake, Practical Pharmaceutical Chemistry ,Part I, 1997,197 .
28. P. Amritage ,Eds., In; Stastical Methods in Medical Research, 1971, 217 .

*****