

EVALUATION OF ANXIOLYTIC ACTIVITY OF HYDRO-ALCOHOLIC LEAVES EXTRACT OF *LAWSONIA INERMIS*

Vimal Kumar Singh

Assistant Professor, Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly (U.P.)

Email ID-vimalsingh1087@gmail.com

ABSTRACT

Anxiolytic activity of hydro alcoholic extract of *Lawsonia inermis* Leaves were evaluated in albino wistar rats. The experimental model used was Elevated Plus maze and Light dark box activity. Diazepam (4mg/kg, i.p.) was used as standard. Extracts were given orally in the dosages of 100 mg/kg, P.O and 200 mg/kg, PO. All the two extracts were found to have significant Anxiolytic activity. The hydroalcoholic extracts of *Lawsonia inermis* leaves was found to improves the anxiolytic related behavior in rats.

Key Words - *Lawsonia inermis*, Leaves, Anxiolytic Activity.

INTRODUCTION

The Plant *Lawsonia inermis* belongs to family Lythraceae plant are the rich source of Lawsone (2-hydroxy-1:4 naphthaquinone) Which is responsible for Colouring properties and it also Contain other Compound Coumarins, xanthone, flavonoids, terpenoids, Steroids, gallic acids, glucose, mannitol, resin, tanins, mucilage, alkaloid, olive oil and palmitic acid. The mineral elements of this plant are used for the treatment of rheumatism, in beri beri, jaundice, Skin disorders like ecezema, Scabies, fungal infections & burns, gonorrhoea, herpes infection, hysteria, nervous disorder. The plant shows many pharmacological activities like spermicidal, antimicrobial, antiulcer, abortifacient, hepatoprotective, anticonvulsant, antiarthritic, analgesic, diuretic, hypoglycemic, antifungal, wound healing, antifertility, anticancer, antioxidant and antibacterial activity. The present work is an effort to show that the leaves of *Lawsonia inermis* has anxiolytic activity.

MATERIALS AND METHODS

The drug was taken from local garden of Bareilly (U.P.). firstly clean the extra material was present in the leaves, after that leaves will dry under shade for 20 to 25 days. Plant Leaves was authenticated by department of Plant Science, M.J.P. Rohilkhand University Bareilly (U.P). The dried leaves will converted in the powder form with the help of mechanical support. About 300gm drug powder take and then suspended into 600 ml of aqueous ethanol at room temperature. After 96 hour solution was filter with the help of Buchner funnel filtration process to separate the coarse particles. A solution obtained after filtration was permit to dry in darkness at room temperature.

The 24 albino wistar rats (150-200gm) were weighed and divided into four groups, each containing six rats.

Group – I – It was treated with 5 ml/kg P.O. of normal Saline on day 1 to 15 before elevated plus maze method and light dark box method.

Group – II – It was treated with 100ml/kg, P.O. of Leaves extract on day 1 to 15 before elevated plus maze method and light dark box method.

Group – III – It was treated with 200 ml/kg, P.O. of leaves extract on day 1 to 15 before elevated plus maze method and light dark box method.

Group – IV – It was treated with 4mg/kg, i.p. of Diazepam on day 1 to 15 before elevated plus maze method and light dark box method.

All results were expressed as mean \pm SEM. Data was analyzed by using one-way ANOVA.

RESULTS

1. Effect of Normal Saline and Diazepam for number of entry on anxiety

Diazepam (4 mg/kg) administered group was found to be significant increase ($P < 0.22$) in number of entry in open arm. Where as in close arm diazepam (4mg/kg) administered group was found to be significant increase ($P < 0.015$ in number of entry as compared to normal saline (5ml/kg) administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Open	4.83 \pm 0.59	5.5 \pm 0.42	3.87 \pm 0.47
	Close	7.16 \pm 0.59	6.83 \pm 0.70	5.83 \pm 0.59
Diazepam	Open	7.83 \pm 0.24	4.83 \pm 0.59	5.83 \pm 0.59
	Close	8.83 \pm 0.59	9.83 \pm 0.59	8.33 \pm 0.41

Table No-1 Effect of Normal Saline and Diazepam on anxiety

2. Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for number of entry on anxiety

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group was found to be significant increase ($P < 0.03, 0.04$) in number of entry in open arm as compared to normal saline administered group. Where as in close arm *Lawsonia inermis* (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. was found to be significant decrease ($P < 0.03, 0.02$) in number of entry in open arm as compared to normal saline administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Open	3.43 ± 0.52	3.5 ± 0.54	4.87 ± 0.56
	Close	3.93 ± 0.48	3.83 ± 0.49	4.30 ± 0.52
<i>Lawsonia inermis</i> (100 mg/lg)	Open	5 ± 0.57	5.4 ± 0.56	6.4 ± 0.57
	Close	3.2 ± 0.42	3.5 ± 0.45	3.5 ± 0.49
<i>Lawsonia inermis</i> (200 mg/kg)	Open	5.5 ± 0.57	5.16 ± 0.56	5.66 ± 0.59
	Close	3.1 ± 0.43	3.4 ± 0.46	3.6 ± 0.47

Table no-2-Effect of Normal Saline, *Lawsonia inermis* (10 mg/kg), *Lawsonia inermis* (200 mg/kg)

3. Effect of Diazepam, *Lawsonia inermis* (100 mg/kg) and *Lawsoia inermis* (200 mg/kg) for number of entry of anxiety

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group were found to be significant decrease ($P < 0.06$, 0.03) in number of entry in open arm as compared to diazepam (4 mg/kg), i.p. administered group. Where as in close arm *Lawsonia inermis* (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group was found to be significant decrease ($P < 0.03$, 0.23) in number of entry as compared to diazepam (4 mg/kg), i.p. administered group.

Treatment		Day First	Day Seven	Day Fifteen
Diazepam	Open	5.83 ± 0.44	6.83 ± 0.47	7.83 ± 0.50
	Close	4.83 ± 0.43	3.83 ± 0.42	5.33 ± 0.44
<i>Lawsonia inermis</i> (100 mg/lg)	Open	4.33 ± 0.37	4.5 ± 0.40	5.33 ± 0.48
	Close	3.33 ± 0.46	2.83 ± 0.3	3.33 ± 0.35
<i>Lawsonia inermis</i> (200 mg/kg)	Open	5.5 ± 0.41	3.16 ± 0.47	4.66 ± 0.49
	Close	3.5 ± 0.35	3.65 ± 0.36	4.5 ± 0.39

Table no-3-Effect of Dizapem, *Lawsonia inermis* (100 mg/kg) *Lawsonia inermis* (200 mg/kg) on anxiety

4. Effect of Normal Saline and Diazepam for number of time Spent on anxiety

Diazepam (4 mg/kg), i.p. administered group was found to be significant increase ($P < 0.02$) in time spent in open arm as compared to normal saline (5 ml/kg), p.o. administered group. Where as in close arm diazepam (4 mg/kg) administered group was found to significant increase ($P < 0.01$) in time spent as compared to a normal saline (5 ml/kg) administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Open	53 ± 1.42	55.5 ± 1.44	59.2 ± 1.46
	Close	40 ± 1.41	45 ± 1.43	46.5 ± 1.44
Diazepam	Open	65 ± 1.50	70.2 ± 1.55	78.6 ± 1.58

	Close	55±1.35	65.5±1.40	67.5±1.42
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Table no-4-Effect of Normal Saline, Diazepam on anxiety.

5. Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for time Spent on anxiety

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered both was found to be significant increase ($P < 0.001$, 0.001) in time spent in open arm as compared to normal saline (5 ml/kg) administered group. Where as in close arm *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) administered group were found to be significant increase ($P < 0.001$, 0.001) in time spent as compared to normal saline (5 ml/kg) administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Open	65.8± 1.46	70.2± 1.52	75.3 ± 1.73
	Close	55.3 ±1.35	60.1±1.40	65.2 ±1.51
<i>Lawsonia inermis</i> (100 mg/kg)	Open	120.3 ±1.94	121.5±1.98	125.6±1.99
	Close	105.2±1.75	109.2±1.80	115.3±1.83
<i>Lawsonia inermis</i> (200 mg/kg)	Open	122.4±2.11	125.5±2.21	128.2±2.33
	Close	110.2±1.55	116.3±1.79	119±2.14

Table no. 5 Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg)

6. Effect of Diazepam, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for time spent on anxiety

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. were found to be significant decrease (0.79, 0.85) in time spent in open arm as compared to diazepam (4 mg/kg), i.p. Where as in close arm *Lawsonia inermis* (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. was found significant decrease (0.98, 0.99) in time spent as compared to diazepam, i.p. administered group (4 mg/kg).

Treatment		Day First	Day Seven	Day Fifteen
Diazepam	Open	192.66±7.90	201.66± 8.39	218 ±10.91
	Close	126.66 ± 7.51	99.66±2.90	80.5 ±2.97
<i>Lawsonia inermis</i> (100 mg/kg)	Open	225±8.39	175.83±10.23	179.83±9.94
	Close	125.83±5.08	94±4.21	76.16±3.86
<i>Lawsonia inermis</i> (200mg/kg)	Open	222.5±9.44	179.5±6.31	185 ±6.06
	Close	139.66±4.15	88.16±0.79	74.83±2.64

Table no. 6 Effect of Diazepam, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg) on anxiety

7. Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for Light and Dark Area by using light dark box apparatus

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group was found to be significant increase ($P < 0.73, 0.24$) in number of entry in light area as compared to normal saline administered group. Where as in dark area *Lawsonia inermis* (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. was found to be significant decrease ($P < 0.01, 0.04$) as compared to normal saline (5 ml/kg) administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Light	5.23±0.43	5.33±0.47	5.53 ±0.49
	Dark	4.5 ± 0.50	4.76±0.52	4.93 ±0.54
<i>Lawsonia inermis</i> (100 mg/kg)	Light	5.16±0.49	5.53±0.56	5.73±0.57
	Dark	5.15±0.51	5.20±0.53	5.35±0.50
<i>Lawsonia inermis</i> (200mg/kg)	Light	5.53±0.52	5.63±0.54	5.74 ±0.55
	Dark	5.25±0.56	5.33±0.57	5.45±0.42

Table no-7-Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg) on anxiety

8. Effect of Diazepam, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for light and dark area by using light dark box apparatus

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group were found to be significant decrease ($P < 0.04, 0.14$) in number of entry in light area as compared to diazepam (4 mg/kg), i.p. administered group. Whereas in (4 mg/kg) dark area *Lawsonia inermis* (100 mg/kg), p.o. & *Lawsonia inermis* (200 mg/kg), p.o. significant decreases (0.006, 0.29) as compared to diazepam (4 mg/kg), i.p. administered group.

Treatment		Day First	Day Seven	Day Fifteen
Diazepam	Light	5.66±0.55	6.33±0.56	7.5 ±0.59
	Dark	6.16 ± 0.42	6.5±0.46	6.83 ±0.51
<i>Lawsonia inermis</i> (100 mg/kg)	Light	4.56±0.59	4.83±0.59	5.43±0.47
	Dark	3.35±0.43	3.46±0.46	4.5±0.42
<i>Lawsonia inermis</i> (200mg/kg)	Light	4.83±0.59	5.83±0.47	5.46 ±0.59
	Dark	6.5±0.76	5.83±0.47	4.5±0.42

Table no. 8 Effect of Diazepam, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg) on anxiety

9. Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for light and dark Area By Using Light dark box apparatus

Lawsonia inermis (100 mg/kg), p.o. *Lawsonia inermis* (200 mg/kg), p.o. administered group was found to be significant increases (0.006, 0.29) in time spent in light area as compared to normal saline (5 ml/kg), p.o. administered group. Where as *Lawsonia inermis* (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group were found to be significant decreases ($P < 0.008$, 0.017) as compared to normal saline (5 ml/kg), p.o. administered group.

Treatment		Day First	Day Seven	Day Fifteen
Normal Saline	Light	138.66±3.18	138.±3.90	140.5 ±2.70
	Dark	124.66 ±2.17	127.2±2.25	130 ±2.09
Lawsonia inermis (100 mg/kg)	Light	136.16±3.80	144.66±3.54	154.5±2.80
	Dark	110±2.04	112±2.13	100.5±2.00
Lawsonia inermis (200mg/kg)	Light	148.16±2.83	151.16±3.52	163.16 ±1.49
	Dark	118.23±1.72	108.33±2.36	104.83±1.20

Table No. 9 Effect of Normal Saline, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg) on anxiety

10. Effect of Diazepam, *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) for light and dark area by using light dark box apparatus

Lawsonia inermis (100 mg/kg), p.o. and *Lawsonia inermis* (200 mg/kg), p.o. administered group was found to be significant decrease ($P < 0.22$, 0.44) time spent in light area as compared to diazepam (4 mg/kg), i.p. administered group. Where as *Lawsonia inermis* (100 mg/kg. p.o.) & *Lawsonia inermis* (200 mg/kg), p.o. administered group were found to be significantly increases ($P < 0.96$, 0.34) as compared to diazepam (4 mg/kg), i.p. administered group.

Treatment		Day First	Day Seven	Day Fifteen
Diazepam	Light	160.66±2.43	177.66±2.34	202.16 ±2.38
	Dark	126.33 ± 1.51	128.83±1.66	138 ±1.90
Lawsonia inermis (100 mg/kg)	Light	136.16±55.80	144.60±3.54	174.5±2.80
	Dark	182±6.04	122±2.13	103.5±2.89
Lawsonia inermis (200mg/kg)	Light	148.16±2.83	151.16±3.52	183.16 ±1.49
	Dark	181.83±3.72	118.33±2.36	104.83±3.00

Table no. 9 Effect of Diazepam, *Lawsonia inermis* (100 mg/kg), *Lawsonia inermis* (200 mg/kg) on anxiety.

DISCUSSION

Normal saline (5 ml/kg) administered group exhibit no significant differences in anxiety behaviour of rats. This group is administered from day first to day fifteen of experiment and results show that normal saline not affects the behaviour of rat.

Diazepam (4 mg/kg) administered group exhibits significant increases the number of entry and number of time spent in open arm as compared to normal saline administered group (5 ml/kg) and *Lawsonia inermis* extract (100 mg/kg), 200 mg/kg) administered group by using elevated plus maze apparatus and light dark box activity.

Lawsonia inermis extract (100 mg/kg, 200 mg/kg) administered group was found to be significantly increases the number of entry and time spent in open arm as compared to normal saline (5 ml/kg) administered group. But it decreases the number of entry and time spent in close arm as compared to normal saline (5 ml/kg) administered group. When *Lawsonia inermis* extract (100 mg/kg) and *Lawsonia inermis* extract (200 mg/kg) administered group compared to diazepam (4 mg/kg) it will significantly decreases the number of entry and number of time spent in open arm but it will mildly increases the number of entry and number of time spent in close arm by using elevated plus maze apparatus and light dark box activity.

CONCLUSION

The following findings are summarized on the basis of result obtained in the present research study.

Normal Saline administered group showed no significant difference in behavior of rats.

Diazepam (4 mg/kg) administered group shows a significant increase in open arm and decreases the number of entry and number of time spent in close arm as compared to normal saline administered group and *Lawsonia inermis* extract administered group. This result shows that diazepam improves the anxiety related behavior in rat.

Lawsonia inermis extract (100 mg/kg) and *Lawsonia inermis* extract (200 mg/kg) administered group were found to be significantly increases the number of time spent and number of entry in open arm as compared to normal saline (5 ml/kg) administered group. While it decreases the number of entry and time spent as compared to normal saline (5 ml/kg) administered group. This result stated that *Lawsonia inermis* extract improves the anxiety related behavior in rat.

Finally it may be concluded that *Lawsonia inermis* extract exerts a protective effect may attribute to its antianxiety effect.

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