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DETERMINATION OF NON-TOXIC DOSE OF DIFFERENT FRACTIONS OF *LAWSONIA INERMIS* LEAVES IN ALBINO WISTAR RATS ON THE BASIS OF HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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Keywords:

L. inermis, SGOT, SGPT, Immune response, TLC

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ABSTRACT: Medicinal properties in *Lawsonia inermis* are due to the presence of bioactive compounds and can also show adverse effects at high concentrations. In the present study we have determined the nontoxic concentration of hexane, ethyl acetate and methanol fractions of plant leaves. 50 wistar albino rats of both sexes were used as experimental animal and were randomly divided into 4 groups *viz.* Group 1 (control), Group 2 (Hexane fraction), Group 3 (Ethyl acetate Fraction) and Group 4 (Methanol Fraction). Groups 2, 3 and 4 were further divided into following sub groups, Group 2A (H) (100mg/kg), Group 2B (H) (250mg/kg), Group 2C (H) (500mg/kg), Group 3A (H) (100mg/kg), Group 3B (H) (250mg/kg), Group 3C (H) (500mg/kg), Group 4A (H) (100mg/kg), Group 4B (H) (250mg/kg) and Group 4C (H) (500mg/kg). Rats in Group 1 (control) received 3% DMSO as solvent for dissolving fractions. All the doses were administered orally with the help of polythene cannula and were given once in a day, followed up to 21 days. After 21 days, blood was collected from orbital sinus of each rat and was utilized for Biochemical and Haematological tests. Data illustrates that body weight increased in dose dependent manner and also no death or toxic signs were observed in Hexane, ethyl acetate and methanol fractions at 100mg/kg, 250mg/kg and 500mg/kg concentration indicating no adverse effect of *L. inermis* leaves extract on wistar abino rats at these doses. Thus, this plant is safe and can be used as medicinal plant.

INTRODUCTION: Now a day, the use of traditional medicine is increasing and about 80% of world population uses these herbal medicines in primary health care as they do not have side effects like modern medicine, are easily available and cheap [1-4]. The effect of herbal medicines is mainly due to the phytoconstituents present in dose, but in some cases medicinal plants have showed undesirable effects like severe hepatic dysfunction [5-7].

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Thus, to know the pharmacological and medicinal values of any medicinal plant primarily it is important to determine the safe and nontoxic doses of these medicinal plants [8, 9].

Lawsonia inermis Linn. is the member of Lythraceae Family and is commonly known as Henna or Mehandi [10]. Despite medicinal values the dry leaves powder of this plant is also used as staining dye for hair, hands and beard [11]. Along with Lawsone (2-hydroxy-1,4 nepthaquinone), the main coloring pigment, other phytoconstituents such as quercetin, mannitol, flavonoids such as apigenin, mucilage, xanthone, luteolin, several phenolic glycosides, coumarin, quinoidsare also present [12].

L. inermis exhibits various therapeutic and medicinal properties like antimicrobial activity [13-15], hepatoprotective activity [16], antipyretic, analgesic and anti-inflammatory activities [17], cytotoxic activity, antioxidant, anti-diarrheal and also reduces the effect of free radicals [18, 19]. Keeping these points in view an attempt was made in the present study to determine the effect of different fractions of *L. inermis* plant leaves on the Haematological and Biochemical parameters of Wistar albino rats. We have also determined the nontoxic dose of different fractions by measuring some morphological characteristics like body weight. This study is important and necessary for *in-vivo* study and for investigating the immuno-modulatory effect of *Lawsonia inermis* Linn.

MATERIALS AND METHODS:

Collection of Plant Material and Authentication: *L. inermis* Linn. plant leaves were collected from G.L.A. University campus, Mathura and were authenticated by Dr. (Mrs.) A. S. Upadhye (Voucher no. L-081), Botany group, Plant Science Division, Agharkar Research Institute, Pune. Leaves were washed thoroughly with tap water then rinsed with distilled water three times. After it leaves were shade dried, coarsely powered and packed in airtight bottle for the preparation of extract.

Extract Preparation: These fractionations were formed as per the method of Sharma *et al*., [20] with slight modifications. In this method 35 grams of leaves dry powder was placed in a porous cellulose thimble. The thimble was placed in an extraction chamber of Soxhlet apparatus, above a collection of flask containing the solvent (Hexane). The flask was heated and the solvent was allowed to evaporate. Temperature was adjusted according to boiling point of the solvent. The extraction process lasted 12- 15 cycles and after that solvent recovery was done. The extract formed was collected and was kept in oven for drying. Same thimble was then used for successive fractionation with ethyl acetate and methanol. All fractions isolated were dried and stored at 4 °C for further use. This fractionation was done on the basis of increasing order of polarity.

Animal Housing: Wistar albino rats (50) of both sexes having weight of 35-55 gram were used for the study. The animals were housed in animal cages at animal house of Department of Pharmaceuticals G.L.A. University Mathura. Rats were fed commercial pelleted food and water *ad libitum*. All the rats were accustomed for 10 days before starting the study and were exposed to 12:12 hr light: dark photoperiod (lights on at 06:00) until they were required. All experimental protocols and animal handling procedures were reviewed and approved by the Animal ethical committee of the institute with CPCSEA (GLAIPR/CPCSEAC/ IAEC/2016/Biotech/02) guideline.

Administration of the Different Fractions: All wistar albino rats were divided into groups *viz.* Group 1 (control), Group 2 (Hexane fraction), Group 3 (Ethyl acetate Fraction) and Group 4 (Methanol Fraction). Groups 2, 3 and 4 were further divided into following subgroups:

- **Group 2:** (Hexane fraction).
- **Group 2A (H):** Rats received 100mg/kg body weight Hexane Fraction.
- **Group 2B (H):** Rats received 250mg/kg body weight Hexane Fraction.
- **Group 2C (H):** Rats received 500mg/kg body weight Hexane Fraction.
- **Group 3** (Ethyl acetate Fraction).
- **Group 3A (E):** Rats received 100mg/kg body weight Ethyl acetate Fraction.
- **Group 3B (E):** Rats received 250mg/kg body weight Ethyl acetate Fraction.
- **Group 3C (E):** Rats received 500mg/kg body weight Ethyl acetate Fraction.
- **Group 4** (Methanol Fraction).
- **Group 4A (M):** Rats received 100mg/kg body weight Methanol Fraction.
- **Group 4B (M):** Rats received 250mg/kg body weight Methanol Fraction.
- **Group 4C (M):** Rats received 500mg/kg body weight Methanol Fraction.

Rats in Group 1 (control) received 3% DMSO as solvent for dissolving fractions. All the doses were administered orally with the help of polythene cannula and were given once in a day, followed up to 21 days [21]. Toxicity of all fractions was also checked by morphological characters and body activities like measurement of body weight after 7 days interval, body size, fur density, loss of appetite etc.

Blood Sample Collection: After the experimental period, blood was collected from orbital sinus of each rat with the help of heparinised micro-haematocrit tubes [22, 23]. Blood was utilized for Biochemical and Haematological tests while serum was used for biochemical test. Serum was isolated from blood by centrifugation at 3000 rpm for 5minutes [24].

Haematological and Biochemical Parameters: Haematological parameters conducted were Hb, TLC (Total leucocyte count), neutrophil, lymphocyte, eosinophil, monocyte, RBC (Red blood cell) and PCV (Packed cell volume) while Biochemical parameters included glucose, Serum creatinine, Serum cholesterol, SGOT/AST (Aspartate amino-transferase), SGPT/ALT (Alanine amino - transferase) and Serum protein. By these tests we determined the changes after the plant fraction feeds in comparison to the control group rats and also the non toxic dose concentration of each fraction.

Data Analysis: Data was calculated as Mean ± S.E. Effect of different fractions between the treatments groups were compared using student t test. The level of significance was recorded at the 5% level of confidence.

RESULTS:

Physical Characteristics and Percentage Recovery: The physical characteristics of hexane, ethyl acetate and methanol fractions of *L. inermis* leaves extract and percentage recovery are shown in **Table 1**. The hexane fraction was green in colour, oily in texture and percentage recovery was 5% while ethyl acetate fraction was dark brown in colour, powder in texture and percentage recovery was 6.7%. Furthermore, methanol fraction was dark brown in colour, sticky in texture and percentage recovery was 20%.

TABLE 1: PHYSICAL CHARACTERISTICS OF HEXANE, ETHYL ACETATE AND METHANOL FRACTIONS OF *L. INERMIS* LEAF EXTRACT

Characteristics	Hexane	Ethyl Acetate	Methanol
Color	Green	Dark Brown	Dark Brown
Texture	Oily	Powder	Sticky
Recovery (%)	5	6.7	20

Effect of Different Fractions of *L. inermis* Linn. Leaves on Body Weight of Rats: The effect of different fractions of *L. inermis* Linn. leaves on body weight of rats is shown in **Table 2**. Body weight gain observed in group 1, group 2A (H), group 2B (H), group 2C (H), group 3A (E), group 3B (E), group 3C (E), group 4A (M), group 4B (M) and group 4C is 18.11%, 20.32%, 20.62%, 21.42%, 21.74%, 22.18%, 22.61%, 18.32%, 20.35% and 20.81%, respectively. Data also illustrates that body weight increased in dose dependent manner.

TABLE 2: EFFECT OF DIFFERENT FRACTIONS OF *L. INERMIS* LINN. LEAVES ON BODY WEIGHT OF RATS

Groups		0 Day (gm)	7th Day (gm)	14th Day (gm)	21th Day (gm)	Weight Gain (%)
Group 1	(Control)	47.93 ± 1.8	55.17 ± 1.8	60.42 ± 1.8	66.04 ± 2.00	18.11
	Group A(H)	38.25 ± 3.17	46.32 ± 2.70	53.69 ± 3.57	58.57 ± 2.91	20.32
Group 2	Group B(H)	48.75 ± 1.35	55.11 ± 1.47	60.62 ± 3.70	69.37 ± 1.87	20.62
	Group C(H)	53.36 ± 1.56	61.46 ± 2.47	67.98 ± 2.96	74.78 ± 2.34	21.42
	Group A(E)	48.16 ± 1.08	57.48 ± 0.6	64.04 ± 0.9	69.9 ± 1.1	21.74
Group 3	Group B E)	39.72 ± 2.2	47.07 ± 1.3	54.91 ± 1.4	61.9 ± 0.8	22.18
	Group C(E)	43.15 ± 3.2	51.67 ± 2.7	58.13 ± 1.8	65.76 ± 1.7	22.61
	Group A(M)	42.25 ± 3.17	49.32 ± 2.70	53.69 ± 2.70	60.57 ± 2.91	18.32
Group 4	Group B(M)	52.75 ± 1.70	60.11 ± 1.74	70.62 ± 4.78	73.10 ± 1.74	20.35
	Group C(M)	52.61 ± 1.65	60.64 ± 3.74	66.89 ± 3.69	73.42 ± 3.43	20.81

Haematological and Biochemical Parameters: The effect of different fractions of *L. inermis* Linn. leaves on Haematological parameters (Hb, TLC (Total leucocyte count), neutrophil, lymphocyte, eosinophil, monocyte, RBC (Red blood cell) and PCV (Packed cell volume)) is shown in **Table 3**. Biochemical parameters which are affected by different fractions of plant leaves result are shown in **Table 4**.

TABLE 3: EFFECT OF DIFFERENT FRACTIONS OF *L. INERMIS* LINN. LEAVES ON HAEMATOLOGICAL PARAMETERS OF RATS

Tests	Group 1 (Control)	Group 2			Group 3			Group 4		
		Group A(H)	Group B(H)	Group C(H)	Group A(E)	Group B(E)	Group C(E)	Group A(M)	Group B(M)	Group C(M)
Hb	13.7 ±0.46	13.87 ±0.23	13.9 ±0.15	14.57 ±0.12	13.56 ±.19	14.32 ±0.12	14.9 ±.25*	13.53 ±0.22	14.67 ±0.26	15.23 ±0.22*
TLC	4283 ±216.67	5100 ±378.59	5359 ±225.15*	5663 ±69.36*	4820 ±340.20	5166 ±176.38*	5346 ±66.58*	5200 ±172.27*	6166 ±317.98*	6766 ±433*
Neutrophil	22.33 ±1.2	26.33 ±0.33*	28.33 ±0.67*	29 ±1.15*	21.67 ±0.88	22.67 ±1.45	25.33 ±1.45	30.33 ±1.22*	32.67 ±1.45*	29.67 ±3.18*
Lymphocyte	69.33 ±2.19	64.33 ±1.67	65.33 ±1.86	65.67 ±1.86	71 ±1.53	70 ±1.73	66.67 ±2.60	64.57 ±0.88	63 ±1.73	66.67 ±5.27
Eosinophyl	5.67 ±0.33	5.67 ±0.33	4.33 ±0.33	4.33 ±0.33	3.33 ±0.33	5 ±0.58	4.67 ±0.88	3.67 ±0.33	3.67 ±0.33	5.67 ±0.33
Monocyte	2.33 ±0.33	2.33 ±0.33	2.33 ±0.33	2.33 ±0.33	2 ±0	2 ±0	2 ±0	2.33 ±0.33	2.33 ±0.33	2.33 ±0.33
RBC	4.71 ±0.15	4.63 ±0.11	5.62 ±0.11*	6.32 ±0.06*	5.7 ±0.54	6.63 ±0.026*	6.74 ±0.09*	4.9 ±0.11	5.38 ±0.34	5.62 ±0.1*
PCV	29 ±1.98	29.0 ±.17	31.04 ±1.3	34.68 ±1.17	37.43* ±0.28	37.86* ±2.42	43.84* ±3.45	32.1 ±2.1	35.7 ±1.9*	36.43 ±1.96*

Hb- haemoglobin; PCV - packed cell volume; TLC- Total leucocyte count, RBC- red blood cell; *represents significant difference at $p < 0.05$.

TABLE 4: EFFECT OF DIFFERENT FRACTIONS OF *L. INERMIS* LINN. LEAVES ON BIOCHEMICAL PARAMETERS OF RATS

Tests	Group 1 (Control)	Group 2			Group 3			Group 4		
		Group A(H)	Group B(H)	Group C(H)	Group A(E)	Group B(E)	Group C(E)	Group A(M)	Group B(M)	Group C(M)
S. creatinine	0.717 ±0.14	0.57 ±0.03	0.62 ±0.02	0.57 ±0.02	0.52 ±0.07	0.59 ±0.03	0.67 ±0.2	0.58 ±0.08	0.30 ±0.06*	0.43 ±0.03
S. cholestrol	63 ±2.03	68.33 ±0.88	65 ±2.5	62.67 ±1.46	82.67 ±1.45	71 ±1.53	60 ±3.78	67.3 ±1.8	61.3 ±0.88	55 ±3.6
SGOT/AST	150 ±8.24	119 ±1.15*	97.67 ±2.03*	87 ±2.31*	136.67 ±2.24	102 ±3.22*	101.3 ±3.2*	89 ±1.53*	92 ±2.5*	71.67 ±2.19*
SGPT/ALT	58.33 ±1.46	52.33 ±3.76	48.33 ±1.2*	42.33 ±2.03*	40.33 ±1.3*	31.67 ±2.3*	27.67 ±1.45*	41.67 ±1.45*	32.3 ±1.45*	32.3 ±3.76*
S. protein	7.17 ±0.15	7.47 ±0.09	6.8 ±0.25	7.17 ±0.33	7.07 ±0.07	6.97 ±0.03	7.13 ±0.33	7.13 ±0.33	7.13 ±0.33	7.13 ±0.33

SP - Serum protein, AST - Aspartate amino-transferase, ALT -Alanine amino- transferase, *represents significant difference at $p < 0.05$.

DISCUSSION: Medicinal plants are used from the beginning of human civilization after various trials. Those plants which were toxic were eliminated and nontoxic plants were used as herbal medicine. The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. In order to contribute further to the knowledge of Indian traditional plants, in the present study *L. inermis* Linn. was screened to determine thenontoxic dose of different fractions.

Effect of *L. inermis* Linn. Leaves on Body Weight of Rats: Determination of nontoxic dose of different fractions was measured by body weight increase. Toxicity was measured through various physiological changes and death of the animals. Any abnormalities and deaths were not recorded after 21 days treatment. The effect of different fractions of *L. inermis* Linn. leaves on body weight of rats is shown in **Table 1**. Data also illustrates that body weight increased in dose dependent manner indicating the non-toxic nature of *L. inermis* Linn. Leaves extract on rats [25].

Haematological Parameters: As per our results, haematological parameters hb, TLC, RBC and PCV increased in dose dependent manner *i.e.* group C(H), group C(E) and group C(M) showed highest

results. Furthermore, in case of hb, group C(E) and group C(M) were significantly higher ($p < 0.05$) while in TLC, group B(H), group C(H), group B(E), group C(E), group A(M), group B(M) and group C(M) were significantly higher ($p < 0.05$) than control. As per RBCs, group B(H), group C(H), group B(E), group C(E) and group C(M) were significantly higher ($p < 0.05$) and in case of PCV, no significant difference was observed in all the doses of hexane fraction while group A(E), group B(E), group C(E), group B(M) and group C(M) were significantly higher ($p < 0.05$) than control.

Suggesting that at this concentration, erythropoietin forming substances got activated and stimulated RBC formation [26]. However, in a previous study RBC count significantly decreased at 1000 mg/kg body weight [27] indicating the toxic effect of extracts at higher concentration while at lower concentration it is beneficial.

In case of neutrophils, hexane fraction group and ethyl acetate fraction group showed effect in dose dependent manner *i.e.* maximum results in group C(H) and group C(E) were observed while in methanol fraction group maximum results were recorded in group B(M). And also, in case of neutrophils, significant difference was observed in all doses of hexane fraction and methanol fraction while no significant difference was observed in ethyl acetate fraction. TLC and neutrophil are indicators of humoral or nonspecific immunity and significant increase in TLC enhances the humoral immune response as Neutrophil activates on entry of any pathogen (mainly bacteria) in the body with in 24 hrs. Our findings are supported by previous investigations [28-32] indicating the ability to stimulate the immune system. However, no significant difference was observed in all the groups for lymphocytes, eosinophils and monocytes.

Biochemical Parameters: In case of biochemical parameters, no significant difference was observed in S. creatinine, S. cholesterol and S. protein levels while ALT and AST were significantly lower ($p < 0.05$) than control. However this change is within normal range indicating plant extract frations have no toxic effect on the liver functions. The decrease in level could be due to the inhibitory effect of plant leaves extract on the synthesis of these enzymes. ALT and AST are indicator enzymes of liver and any alteration in the level of these enzymes affect the liver functions. Increase in ALT and AST level is more harmful as increase in the ALT level in blood indicates cell death [33]. Furthermore, level of serum creatinine in blood indicates the kidney function and no significant difference in any group indicates that these fractions do not affects kidney [34]. It also suggests no change in the carbohydrate metabolism as no significant difference was observed in any group.

CONCLUSION: In the present investigation, we determined the effect of hexane, ethyl acetate and methanol fractions of *L. inermis* leaves on body weight, Haematological and Biochemical parameters. No death and toxic signs were observed in Hexane, ethyl acetate and methanol fractions at 100 mg/kg, 250 mg/kg and 500 mg/kg concentration indicating no adverse effect of *L. inermis* leaves extract on wister albino rats at these doses. Thus, this plant is safe and can be used as medicinal plant.

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CONFLICT OF INTEREST: The Author(s) declare(s) that they have no conflicts of interest to disclose.

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