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Anti-Motility and Anti-Diarrheal Activities of *Lippia Nodiflora* and *Withania Somnifera* Against Castor Oil and Magnesium Sulphate-Induced Diarrhea in Mice

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ABSTRACT

Objective: The ongoing exploration is planned to narrate anti-motility and anti-diarrheal potential of *L. nodiflora* (*LN.Cr*) and *W. somnifera* (*WS.Cr*) hydro-alcoholic extract and their flavonoids and alkaloids rich fractions in case of castor oil and $MgSO_4$-mediated diarrhea in mice.

Methodology: Locally collected whole plant of *Lippia nodiflora* and roots of *Withania somnifera* were dried, pulverized and soaked into aqueous methanol to get hydro-alcoholic extracts. Alkaloids and flavonoids were separated from dried sample of each plant.

Result: *L. nodiflora* and *W. somnifera* showed significant inhibition (56.2 and 52%) of intestinal transport of charcoal like atropine (63%) and intestinal fluid accumulation (68 and 66%) in mice, respectively in dose-dependent manner with dosage range at 100-300 mg/kg. LN.Cr and WS.Cr showed dose-dependent safety in case of castor oil (67% and 63.3%) and magnesium sulphate-mediated diarrhea (69.2 % & 67%) at the highest tested dose of 300 and 200mg/kg, respectively in mice, like loperamide (73%). Isolated alkaloids and flavonoids also brought significant blockage of charcoal meal transit and safety against castor oil-mediated diarrhea.

Conclusion: This work suggested that *L. nodiflora* and *W. somnifera* extracts and their isolated alkaloids and flavonoids showed antidiarrheal activity by inhibiting intestinal motility, secretion of fluid and possibly inhibition of autacoids and prostaglandin. Antidiarrheal effects of flavonoids were more pronounced than alkaloids and crude extracts of both plants.

Keywords: *Lippia nodiflora, Withania somnifera, anti-motility, anti-diarrheal, flavonoids and alkaloids*

INTRODUCTION

Diarrhea is defined as enhance gastrointestinal motility resulting in rapidity in fluid passage, water and electrolyte loss from individuals, it is normally characterized with thrice or extra episodes of loose or liquid feces in twenty-four hours [1]. Normally, most of the gut fluids are reabsorbed prior to enter distal small bowel and very few enter the colon [2]. The absorption of electrolytes and water is governed *via* Na^+/K^+ ATPase pump located on basolateral membrane of intestine [3]. Thus disturbance or irregularity in synchronized flux of electrolytes (including sodium, chloride, potassium or calcium), water and/or motility can lead to diarrhea.

The anti-diarrheal therapy includes the agents, which have anti-secretory effects, decrease intestinal motility and modify stool consistency. These agents include adsorbents (kaolin, pectin, chalk, charcoal, methylcellulose), bulk-forming agents (psyllium, polycarbophil and methyl cellulose), probiotics [4]. Otherintraluminal agents including bismuth subsalicylate, bile acid resins (cholestyramine), glucocorticoids (budesonide), [5] opiate like medication (loperamide, diphenoxylate, [6] enkephalinase inhibitors (racecadotril) [7] and anticholinergics or spasmolytic (atropine) [8] are used in anti-diarrheal therapy. These agents may cause different side effects like headache, nausea or vomiting, dry mouth and constipation. On the other hand, herbal treatments have been reported to cause fewer untoward effects and thus are safer to use. From literature, it is revealed that tannins, flavonoids, alkaloids and terpenes of plants possess anti-diarrheal potential. It is examined that tannins and flavonoids from active natural extract exhibited anti-diarrheal potential *via* enhancing the water and electrolyte re-absorption in colon, whilst the later display these effects *via* blocking the intestinal motility [9]. In spite of the availability of multiple treatment options for diarrhea, novel and cost-effective anti-diarrheal therapy is still awaited [10].

Lippia nodiflora (LN), family Verbenaceae, is an ornamental plant. This is extensively distributed in South Asia. LN contains various phytochemicals such as alkaloids, flavonoids, tannins, triterpenoids, and steroids. It possess gastro-protective effects and useful in gastrointestinal disturbances, while also used in heart diseases, ulcer, asthma, loss of consciousness, fever and joint pain [11]. This plant possesses anti-diarrheal, [12] anti-bacterial, anti-oxidant and hepato-protective, [13] anti-hyperuricemic, [14] anti-inflammatory, [15] anti-ulcer, [16] anti-diabeticdue to γ-sitosterol, [17] anti-tumor [18] andanti-fungal [19] activities.

Withania somnifera (WS)*,* family Solanaceaeis well distributed in South Asia. It is frequently studied as ashwagandhaor winter cherry, which contains different phytochemicals including alkaloids, tannins, flavonoids, starch and with anolides [20]. It is extensively used in diarrhea, constipation, in CNS disorders as analeptic, nervine tonic, anti-stress, memory enhancer or in case of cognitive deficits, insomnia and anxiety. It is also effective in infections, infertility and rheumatoid/ gouty arthritis over thousands of years [21]. Previous reports showed that WS have anti-diabetic, [22] useful in neuro /cognitive dysfunctions, [23] anti-fertility, [24] anti-bacterial, anti-proliferative and anti-oxidant, [25] nephron-protective [26] and hepato-protective [27] activities.

On the basis of traditional use of *Lippia nodiflora* (*LN*) and *Withania somnifera* (*WS*) in gastrointestinal ailments like diarrhea [11, 21] and to further study the lack in current literature for anti-diarrheal effects of *Lippia nodiflora* as mentioned by Begum, [12] where it showed the anti-diarrheal potential of LN leaves aqueous extract in the rat model with castor oil-mediated diarrhea and anti-secretary potential in castor oil-mediated enteropooling. The primary focus of instant study is to examine scientifically the anti-motility and anti-diarrheal potential of these aforementioned plants in castor oil-mediated and $MgSO_4$-mediated diarrhea in mouse model. Further the studies were extended to the alkaloid and flavonoids rich fractions of LN and WS, which were separated and were investigated for anti-diarrheal activities by using standard pharmacological protocols.

METHODOLOGY

Plant assemblage and devising of crude extracts

The whole plant of *Lippia nodiflora* and roots of *Withania somnifera* were assembled from a small Town namely “Nehang” located at Tehsil Sahiwal District Sargodha, Punjab-Pakistan in May, 2015. The whole plant and roots were unsoiled under running tap water thrice. The plant samples were substantiated by Dr. Amin (Taxonomist) Department of Botany, University of Sargodha Sargodha, Punjab-Pakistan. Little sections of *Lippia nodiflora* (L.) L.C.

Rich. And *Withania somnifera* (L.) Dunal (voucher no. SA-6036 and SA-6035), respectively were amassed in the herbaria of Department of Botany (SARGU) of same University for ready reference. The thorough material of whole plant of *Lippia nodiflora* and roots of *Withania somnifera* were kept under shady open area and dried. The parched plant matter was pulverized, using big size mortar and pestle. 500 gm of each plant powder was soaked in two liters of aqueous methanol (70% methanol: 30% distilled water) for 72 hours. First the sludge was transferred over a cotton cloth and then over the Whatman filter- paper No- 25. The filtrate was amassed in round shape rotary flask, and then the flask was attached to rotary evaporator, maintained at under reduced pressure and at 40 ºC and eventually a paste like material was obtained. The solutions from extracts were always freshly prepared for each study, by dissolving an appropriate required quantity in 1% tween 80 in water under normal laboratory environment.

Segregation of alkaloids and flavonoids

Alkaloids separation

The plant sample of about five gm was taken in 200 mL ethanol-acetic acid solution (80:20), which was then placed separately for approximately four hours. Afterwards it was filtered to remove the debris. The liquid was placed in water bath to concentrate until the quarter of original volume evaporated. Then to get the precipitations, the concentrated ammonia hydroxide was spilled drop by drop to the filtrate. Now this solvent was settled down and the precipitations were segregated *via* the process of filtration and placed on weighing balance to weigh [28].

Flavonoids separation

Almost ten gram of each plant dry powder was taken and mixed in 100 mL of mixture of 80 L water and 20 mL methanol, which was kept for two to four hours at room temperature. Then the slurry was passed *via* Whatsman filter-paper No-1. Then the solvent was spilled into petri-dish already placed in water-bath. This was allowed to evaporate until dryness, hence a constant weight achieved [28].

Animals

Healthy weighing 20-25 gm Swiss albino mice of either gender were acquired from animal house-rooms of University of Veterinary and Animal Sciences, Lahore, Punjab-Pakistan. These were retained individually in separate cages. These cages were put in well maintained-ventilated rooms at the University of Sargodha, Sargodha-Pakistan and given specific feed and water *adlabitum*. Total experimental work was done as per the University Research Policy guidelines.

Acute toxicity analysis

Almost forty mice (20-25 g) of either gender were selected for every plant. They were divided into four groups by the method of physical randomization ensuring ten animals per group. One group got saline (10 mL/kg), the second, third and fourth got increasing dosing at 3, 5 and 10 gm/kg of crude extract. Thereupon, these were put into specially designed cages with tap water *ad labitum*. These were investigated for regular intervals to check any sign of piloerection, variations in exploratory behavior, loco motor actions, eating behavior and/or visual impairment for 6 hours; however the lethal effects were noticed up to twenty-four hours for each plant [8].

Charcoal meal gut transit test

The method of Abdullahi [29] was taken to investigate the *L. nodiflora* and *W. somnifera* extracts in charcoal meal gut transit in mice. Animals starved for twelve hours prior to the assay with free water access. They were segregated into different groups, while every group had five mice. Group one received vehicle of 10 mL/kg (1% Tween 80 in distilled water), group two gained atropine sulfate (0.1 mg/kg) intraperitoneally, groups three, four and five received the LN.Cr (100,200 and 300 mg/kg) and groups six, seven and eight received the WS.Cr (100,150 and 200 mg/kg), orally. After thirty minutes, each mice received charcoal meal (1 mL) consisting of three percent deactivated charcoal suspension in 0.5 percent aqueous-methyl cellulose. Thereupon the thirty minutes later, the each mouse was euthanized. The total distance covered by the charcoal and total length of intestine for each animal was calculated. Now the percent charcoal movement was measured with total distance of intestine for each assay.

To establish the effects of the alkaloids and flavonoids of *L. nodiflora* and *W. somnifera* on gut motion, the alkaloids were administered at the dose of 40 and 26 mg/kg respectively to mice, while flavonoids were administered at the dose of 85 and 55 mg/kg. The other procedural method was kept same

Gut fluid accumulation

The intraluminal fluid accumulation procedure was done by pursuing the method of Robert [30]; mice were abstained from food for 12-18 hours and were segregated into different groups. Each group had five animals. Group one obtained vehicle (1% Tween 80 in distilled water) at 10 mL/kg orally, other group (two) received (loperamide) at 5 mg/kg *i.p.* groups three, four and five received the LN.Cr (100,200 and 300 mg/kg), groups six, seven and eight received the WS.Cr (100,150 and 200 mg/kg) orally. After one hour, castor oil was given *via* special needle to stimulate diarrhea. Two hours later, these animals were anaesthetized with chloroform overdose and sacrificed. Then the small intestine was ligated at the pyloric sphincter and ileocecal junctions regions and dissected out. The gut was weighed and intestinal matter was squeezed out by milking in a cylinder to measure the contents. The dissected slice of gut was re-weighted to note variations between full and empty intestines.

Castor oil-mediated diarrhea

The strategy, as reported by Mehmood [8] was pursued in instant protocol. Mice were segregated into eight groups with five in each and each was put in separate cage. Group one gained vehicle (1% Tween 80 in distilled water, 10 mL/kg) orally, as positive control. Second group was administered with loperamide (3 mg/kg) as negative control. Remaining groups gained crude extracts of *L*. *nodiflora* and *W*. *somnifera* at dosing range with 100, 200, 300 mg/kg and 100, 150, 200 mg/kg, respectively. Each cage was floored with blotting paper, which was kept changing after every hour during study. After the thirty minutes, each mouse gained half milliliter of castor oil. During the four hours of observational period, total figure of feces and total figure of particular diarrheal feces were computed and valued in terms of weight.

To establish the anti-diarrheal activity of the alkaloids and flavonoids of *L. nodiflora* and *W*. *somnifera*, the alkaloids were dispensed at the dosing range of 40 and 26 mg/kg respectively, whilst flavonoids were administered at the dosing range of 85 and 55 mg/kg to mice. The other procedure was kept same.

Magnesium sulfate-mediated diarrhea

The procedure as in earlier section 2.7 of castor oil-mediated diarrhea was pursued. However magnesium sulfate (2 g/kg) orally was given to each mouse instead of castor oil. Whilst group one gained vehicle (1% Tween 80 in water, 10 mL/kg), group two loperamide (3 mg/kg), groups three, four and five LN extract (100, 200 and 300 mg/kg) and groups six, seven and eight gained WS extract (100, 150 and 200 mg/kg) *via* oral route. Subsequently passage of four hours, total figure of all feces and total figure of particular diarrheal feces excreted, were computed and weighted [31].

Statistical methods

The data was manifested in mean ± standard error of the mean (SEM). Statistical scrutiny was done by employing Unpaired *t*-test/ and One-way analysis of variance (ANOVA), trailed by Dunnet's test for differentiation of data with controls and among groups. Whilst the statistical significance was fixed at $p < 0.05$.

RESULTS

Concentrations of alkaloids and flavonoids from *L. nodiflora* extract

The alkaloids and flavonoids were nearly 28 % and 14 % respectively, *i.e.* 1.4 g of alkaloids and 1.35 g of flavonoids from sample 5 and 10 g, were calculated. Thus the 85 mg of alkaloids and 40.5 mg of flavonoids dose was calculated *i.e.* equivalent to 300 mg dose of LN.Cr as given in table **1**.

Concentrations of alkaloids and flavonoids from *W. somnifera* extract

With same method, the concentration of alkaloids and flavonoids of *W. somnifera* from the WS sample of 5 and 10 g was nearly 27.4 % and 13 % respectively, *i.e.* 1.37 g of alkaloids and 1.3 g of flavonoids. Thus the 55 mg of alkaloids and 26 mg of flavonoids dose was calculated *i.e.* equivalent to 200 mg dose of WS. Cras given in table **2**.

Acute toxicity analysis

LN and WS extracts given to three groups of mice at dosing of 3, 5 and 10 g / kg. The rodents were keenly detected for acute toxicity or lethality insignia. LN and WS were established harmless and/ or lacking of any sign of toxicity and / or lethality for twenty four hours of assay.

Effect on gastrointestinal motility

Oral administration of LN.Cr and WS.Cr to mice at dosing range (100, 200 and 300 mg/kg) and (100, 150 and 200 mg/kg), respectively displayed a significant low down in intestinal transport of charcoal,

likewise to action of atropine sulphate, in dose-related manner. At the maximum experimental doses of both LN and WS extract instigated 56.2 % and 52 % inhibition in charcoal meal transit while positive control caused 62 % inhibition.

Isolated flavonoids from LN and WS also showed high degree of inhibition (56.5 % & 32.3 % respectively) as compared to alkaloids with 18 % (LN) and 14 % (WS) inhibition in charcoal meal transit respectively (Table **3**).

Effect on intestinal fluid accumulation

Nursing of mice with *L. nodiflora* and *W. somnifera* extract at the dosing range (100, 200 and 300 mg/kg) and (100, 150 and 200 mg/kg), respectively presented a significant blockade of gut fluid accumulation in dose-related fashion likewise to loperamide actions against castor oil-induced enteropooling. *L. nodiflora* and *W. somnifera* at the highest tested dose of 300 mg/kg and 200 mg/kg exhibited 68 % and 66% inhibition, respectively while loperamide showed 73% of inhibition in intestinal contents as depicted in table **4**.

Anti-diarrheal potential in case of castor oil-mediated diarrhea

The mice treated with LN.Cr (200 & 300 mg/kg) and WS.Cr (150 & 200 mg/kg) extracts displayed a significant (P<0.01) lowering of total number and weight of feces for the observational period of four hours in dose-specific fashion likewise loperamide actions. The high doses of both LN.Cr and WS.Cr caused 67% and 63.3% protection respectively while positive control caused 73.3% protection from diarrhea as summarized in table **5**.

Animals treated with isolated alkaloids and flavonoids displayed very significant decrease (P< 0.01) in number and weight of diarrheal feces over the period of four hours when matched with control group. Isolated flavonoids from LN and WS showed high degree of diarrheal inhibition (69 % & 50 % respectively) as compared to alkaloids with 36 % (WS) and 48 % (LN) protection from diarrhea respectively (Table **5**).

Anti-diarrheal potential in magnesium sulphate-mediated diarrhea

The animals nourished with LN.Cr and WS.Cr at dosing range (100, 200 & 300 mg/kg) and (100, 150 & 200 mg/kg), respectively exhibited a significant (P< 0.05) alleviation in number and weight of feces like loperamide in dose-specific manner. The maximum experimental doses of both LN & WS hydro-alcoholic extracts caused 69.2 % & 67 % inhibition while positive control caused 72 % inhibition in diarrheal feces as summarized in table **6**.

Table 1. Concentrations of alkaloids and flavonoids in whole plant of *L. nodiflora.*

Phytochemicals	Sample quantity	Concentration found in the sample quantity	Approximate %age concentration in the sample quantity	Doses equivalent to 300 mg dose of crude extract
Alkaloids	5 g	1.4 g	28	85 mg
Flavonoids	10 g	1.34 g	13.4	40 mg

Table 2. Concentrations of alkaloids and flavonoids from the roots of *W. somnifera.*

Phytochemicals	Sample quantity	Concentration found in the sample quantity	%age concentration in the sample quantity	Doses equivalent to 200mg dose of crude extract
Alkaloids	5 g	1.37 g	27.4	55 mg
Flavonoids	10 g	1.3 g	13	26 mg

Table 3. Antidiarrheal effect of crude extracts and isolated alkaloids and flavonoids of *L. nodiflora* and *W. somnifera* on charcoal meal transit in mice.

Treatments	Dose(mg/kg) p.o	Total length of intestine (cm)	Distance travelled by charcoal meal (cm)	%age of inhibition
Control 1% Tween 80 in water	10mL/kg p.o)	60.0±2.23	58.6±1.8	-
Atropine sulphate (i.p)	0.1	56.8±2.6	21.1±1.8**	63

L. nodiflora (crude extract)	100	58.0±2.6	51.8 ± 0.73*	11
	200	57.2±2.5	46.6 ± 1.88**	19
	300	52.0±0.95	22.8± 0.95**	56.2
L. nodiflora (flavonoids)	40	56.9±2.2	25.4±2.5**	56.5
***L. nodiflora* (alkaloids)**	85	59.8±2.1	49.1±1.9**	18
W. somnifera (crude extract)	100	56.6±2.3	56.2± 1.1*	-
	150	55.0±1.7	34.1± 0.60**	38
	200	59.2±1.02	28.4 ± 1.11**	52
W. somnifera (flavonoids)	26	55.2±2.5	37.7±2.2**	32.3
W. somnifera (alkaloids)	55	56.0±1.6	48.4±1.5*	14

Results shown as means ± SEM. p.o (per oral), n= five mice per group, *p<0.05 and **p<0.01 show a comparison of group 2 -8 versus group 1 (One way ANOVA followed by Dunnet's test).

Table 4. Effect of *L. nodiflora* and *W. somnifera* extract on castor oil-induced enteropooling.

Treatments	Dose (mg/kg) p.o	Volume of intestinal content (mL)	Weight of intestinal content (g)	% inhibition of volume of intestinal content
Control 1% Tween 80 in water(10mL/kg p.o)	_	0.68 ± 0.04	0.82 ± 0.06	_
Loperamide(i.p)	5	0.19 ± 0.10**	0.21± 0.02**	73
L. nodiflora	100	0.58 ± 0.04 ^{ns}	0.70 ± 0.05 ^{ns}	15
	200	0.34 ± 0.07**	0.42 ± 0.04**	49
	300	0.22 ± 0.05**	0.26 ± 0.02**	68
W. somnifera	100	0.60± 0.04 ^{ns}	0.72 ± 0.09 ^{ns}	12.2
	150	0.30 ± 0.04*	0.36 ± 0.04**	56.1
	200	0.23 ± 0.04**	0.28 ± 0.06**	66

Results are means± SEM. All groups are compared with control (n=5)
ns= non-significant, p.o= per oral, *P<0.05, ** P< 0.01.

Table 5. Summary and comparative antidiarrheal effect of crude extract and isolated alkaloids and flavonoids of *L. nodiflora* and *W. somnifera* against castor oil-induced diarrhea.

Treatments	Dose (mg/kg) p.o	Total number of faeces	Total number of diarrheal faeces	Weight of faeces (mg)	%age inhibition of diarrhea
Control 1% Tween 80 in water	10	9.0±0.55	8.4±0.51	0.82±0.06	-
Loperamide plus castor oil	3	2.3±0.24**	0.6±0.24**	0.22±0.04**	73.3
L. nodiflora (crude extract) **plus castor oil**	100	6.0±0.32^{ns}	5.2 ± 0.37^{ns}	0.66±0.05^{ns}	13.3
	200	4.6±0.4**	3.3 ± 0.37*	0.42±0.04**	30
	300	3±0.37**	1± 0.32**	0.30±0.04**	67
L. nodiflora (flavonoids) **plus castor oil**	40	2.8±0.37**	0.9±0.24**	0.26±0.02**	69
L. nodiflora (alkaloids) **plus castor oil**	85	4.8±0.37**	2.5±0.24**	0.48±0.04**	48
W. somnifera (crude extract) **plus castor oil**	100	5.8±0.37*	4.1±0.37*	0.56 ± 0.05*	30
	150	5.1±0.71**	2.7±0.58**	0.44±0.02**	47
	200	3.0±0.55**	1.1±0.58**	0.32±0.04**	63.3

W. somnifera (flavonoids) **plus castor oil**	26	4.4±0.51**	2.2±0.37**	0.34±0.05**	50
W. somnifera (alkaloids) **plus castor oil**	55	5.8±0.37**	3.8±0.51**	0.52±0.04**	36

Results are means± SEM. All groups are compared with control (n=5), ns= non-significant, p.o (per oral), *p<0.05 and **p<0.01 show a comparison of group 2 -8 versus group 1 (One way ANOVA followed by Dunnet's test).

Table 6. Effect of *L. nodiflora* and *W. somnifera* extracts on magnesium sulphate-in.duced diarrhea.

Treatments	Dose (mg/kg) p.o	Total number of faeces	Total number of diarrheal faeces	Weight of faeces (mg)	%age inhibition of diarrhea
Control 1% Tween 80 in water	(10mL/kg)	7.6±0.24	7.4±0.37	0.74± 0.05	_
Loperamide plus $MgSO_4$(2 g/kg)	3	2.1±0.32**	0.6±0.32**	0.22±0.04**	72
L. nodiflora (crude extract) **plus $MgSO_4$(2 g/kg)**	100	6.4±0.32*	5.0±0.37*	0.56±0.05*	21
	200	5.6±0.24**	4±0.24**	0.42±0.04**	29
	300	2.7±0.37**	0.8±0.24**	0.24±0.02**	69.2
W. somnifera (crude extract) **plus $MgSO_4$(2 g/kg)**	100	6.5±0.37*	5.2±0.58*	0.58 ±0.04*	20
	150	5.9±0.37**	4.3±0.40**	0.44±0.04**	28.2
	200	2.8±0.40**	0.9±0.24**	0.26±0.04**	67

The data is represented as means ± SEM. n=5 animal / group, ns= non-significant, per oral (p.o), *p<0.05 and **p<0.01 exhibit difference between group 2 -4 versus group 1 (One wayANOVA followed by Dunnet's test).

DISCUSSION

A number of herbs have been studied, which showed their effects on gastrointestinal transit, water and electrolyte secretion, with antidiarrheal potential [8, 31, 32]. *Lippia nodiflora* (LN) and *Withania somnifera* (WS) have been given traditionally to treat gastrointestinal disorders like diarrhea [1, 21].The hydro-alcoholic extract of LN and WS and their isolated flavonoid and alkaloid rich fractions were studied for their anti-motility, anti-enteropooling and anti-diarrheal potential in case of castor oil and $MgSO_4$-mediated diarrhea of mouse model. The anti-propulsive and anti-enteropooling models were used to test their effects on gastrointestinal transit, water and electrolyte secretion, while castor oil and $MgSO_4$-induced diarrheal model were used for mechanistic insight and anti-diarrheal activity.

In gastrointestinal transit studies, atropine (an anticholinergic drug) inhibit the intestinal transit, is deployed as standard drug [33]. As LN.Cr and WS.Cr showed its effects on all parts of the gut, thus LN.Cr and WS.Cr at dosing range (100, 200 and 300 mg/kg) and (100, 150 and 200 mg/kg), respectively inhibit the gut propulsive activity in dose-related fashion in charcoal meal treated mice. Activated charcoal blocks the absorbing of drugs and/ or chemicals into the blood stream from the gut, as of its ability to adsorb them on to its particle surface. Commonly the activated charcoal is employed in experiments to examine the actions of test materials on peristaltic motions in the gastro-intestinal motility test [29]. The inhibition of gut propulsive activity by crude extracts was not prominent as of pure compound of atropine sulphate, because the extract is a mixture of many compounds and some of these compounds may have opposite activities as mostly observed in herbal remedies [10]. These assays suggested that the plants extracts and their fractions blocked the movement of charcoal meal in mouse model, so increase the time for retention of meal to absorb the water and electrolytes, likely to same actions of atropine sulphate. Hence, these investigations displayed the blockade in the propulsive motions of the small bowel, while nursing the mouse model with these aforementioned plants.

In case of castor oil-mediated enteropooling study, the intra-luminal fluid increase was inhibited significantly by nursing with hydro-alcoholic extract of these plants, in dose-related fashion. Literature

studies showed that castor oil produces its effects by increasing luminal osmolality, electrolytes secretion, reduction in electrolytes absorption, and abnormal intestinal motility and decrease in gut transit time [34].Our results showed that LN and WS produced anti-secretary actions, inhibit gut motility, and enhance the absorption of electrolytes and fluids, an effect same to that of loperamide effects, which block fecal mass production and slow down its evacuation *via* blockage of water secretion and intestinal motility [35].

Hydro-alcoholic extracts of LN and WS exhibited dose-dependent anti-diarrheal actions in event of castor oil and magnesium sulphate-mediated diarrheal mouse. These LN and WS extracts showed antidiarrheal effects that was comparable with loperamide and significantly reduced the defecation frequency and fecal droppings. Isolated alkaloids and flavonoids also revealed prominent antidiarrheal actions in castor oil and magnesium sulphate-mediated diarrheal mice models.

Castor oil is converted to a hydroxylated fatty acid known as ricinoleic acid, by an enzyme 'lipases' in gut lumen. Ricinoleic acid produces irritation on intestinal mucosa and brings inflammation, which leads to the release of prostaglandins [34]. Thus causes an escalation in the permeability of the mucosaand altersthe electrolyte transference, consequently in a hyper-secretory response (decreasing Na^+ and K^+ absorption), stimulating peristaltic movements and diarrhea [8]. Prostaglandin inhibitors reverse the diarrhea mediated with castor oil [36]. *L. nodiflora* has been reported to reverse NO release *via* iNOS blockage, prostaglandin biosynthesis *via* PLA2 and COX-2 suppression [37]. *W. somnifera* also reported to inhibit the prostaglandins synthesis and COX-2 [38]. Thus this model suggests that the anti-diarrheal actions of LN and WS may be due to the suppression of prostaglandin biosynthesis.

Whilst, magnesium sulphate, in small bowel stimuli the release of cholecystokin in from its mucosa, increase the motor activity of gut and leads to loss of intestinal content. Hence the magnesium sulphate indirectly increase the motility of gut, prevent the reabsorption of salt and water and increases the secretions, thus leads to induction of diarrhea [39, 40]. Hence, mechanisms other than prostaglandin inhibition should be considered because the gut functions depend upon many other factors.

Lippia nodiflora contains triterpenoids, alkaloids, carbohydrates, tannins, flavonoids, phenols, steroids with strong antioxidant potential [41]. Flavanoids include hispidulin, eupafolin, nodifloretin within the whole plant with methanol extract, while lippiflorin, nodifloridin, jaceosidin, nepetin and batatifolin found with ethanol, on the other hand, flowers contain 6-hydroxyluteoli and luteolin-7-O-glucoside [42, 43]. While, *Withania somnifera* plant contains more than 35 chemical constituents including alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins (sitoindoside VII-VIII & sitonidoside XI- X), while other ingredients include ellagotannin, trigallayl glucose, phyllemblin, withaferin A and emblicol [44, 20]. It has already been established that plants containing tannins possess antidiarrheal activity [40, 9]. Tannins form a protein, tannates and make gastro-intestinal mucosa resilient [45]. Phenolic compounds and tannins were detected in LN and WS extract, which are most probably responsible for the anti-diarrheal activity.

Isolated alkaloids and flavonoids rich fractions also caused significant safety against castor oil-induced diarrheal like loperamide and also cause inhibition of charcoal meal transit.

It can be concluded that the *L. nodiflora* and *W. somnifera* extracts and their isolated alkaloids and flavonoids showed anti-diarrheal activity most likely because of inhibition in gut motility and secretion of fluid. These inhibitory actions of LN and WS extracts justify their use as a non-specific anti-diarrheal agent. Anti-diarrheal effects of flavonoids were more pronounced than alkaloids and crude extracts of both plants. The presence of flavonoid in plant extracts possibly cause interruption in prostaglandin release, thus inhibiting the movement and secretions prompted by castor oil [9]. More studies of LN and WS plant extracts may lead to the development of some novel antidiarrheal drugs.

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ETHICAL APPROVAL

This study was approved and got certificate from institutional research ethics committee faculty of pharmacy, university of Sargodha, Punjab Pakistan. Number of ethics committee approval: (20-IEC-32 UOS).

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