# ANTIDIARRHEAL AND ANTISPASMODIC ACTIVITY OF *WRIGHTIA TINCTORIA* BARK AND ITS STEROIDAL ALKALOID FRACTION

Papiya Bigoniya*[1], Avtar Chand Rana[2]

[1]Radharaman College of Pharmacy, Radharaman Group of Institutions, Bhadbada Road, Ratibad, Bhopal-462002, Madhya Pradesh, INDIA.
[2]Rayat College of Pharmacy, Rail Majra, Ropar-144553, Punjab, INDIA

**Summary**

*Wrightia tinctoria* Roxb. R.Br. (family Apocynaceae) bark ethanol extract and isolated steroidal alkaloid fraction (WTSA) was investigated on different experimentally induced diarrhea models of rats, isolated rat ileum, and on enteric bacterium to establish the therapeutic potential. The extract at 500 and 1000 mg/kg dose, and WTSA at 50 and 100 mg/kg dose significantly inhibited the frequency and wetness of faecal droppings in castor oil-induced diarrhea. Extract and WTSA decreased propulsion of charcoal meal and also reduced prostaglandin $E_2$-induced enteropooling. WTSA reduced amplitude, frequency, and tone of spontaneous gut movement. Alkaloid fraction also inhibited acetylcholine (Ach)-induced contraction of rat ileum. *W. tinctoria* alkaloid has antisecretory, spasmolytic, antienteropooling, antimotility, and antiperistaltic activity. These results substantiate good antidiarrheal activity of *W. tinctoria* against secretory, osmotic, motility related, and inflammatory diarrhea.

**Key words:** Anticholinergic, antidiarrheal, antispasmodic, enteropooling, steroidal alkaloid, *Wrightia tinctoria*.

***Corresponding Author**
**Address for Correspondence:**
Dr. Papiya Bigoniya,
Principal, Radharaman College of Pharmacy, Radharaman Group of Institutions,
Bhadbada Road, Ratibad, Bhopal-462002, Madhya Pradesh, INDIA.
Phone: +91-755-2477941, 09827011258, Fax; +91-755-2896663
Email: p_bigoniya2@hotmail.com

## Introduction

Diarrhoea refers to the frequent passage of loose or watery unformed stools. Diarrhoea kills over 1 million children every year through dehydration and malnutrition. Diarrhoea becomes life threatening in the high-risk groups like young children, elderly, and immune suppressed people[1]. Diarrhoea is a major public health problem in developing countries and is said to be endemic in many regions of Asia and is the leading cause of high degree of morbidity and mortality[2].
The use of medicinal plants in the world and especially in India, contributes significantly to primary health care. From the fast array of the Materia Medica of the Indian indigenous system, many plants have been reported to have antidiarrheal activity thus act as very useful remedy for the alleviation of human suffering.

Multiple drug resistance among enteropathogens in various geographic regions presents a major threat in the control of diarrhoea. Therefore indigenous medicinal plants as an alternative to synthetic antidiarrheal play a significant role in traditional therapy based on folklore .

*Wrightia tinctoria* Roxb. (R.Br.) is a small deciduous tree of the family Apocynaceae distributed in Central India, Burma, and Timor. This plant is extensively used in the Indian system of medicine. Fresh leaves are pungent and are chewed for relief from toothache[3,4]. Bark and seeds are antidysenteric, carminative, astringent, aphrodisiac, and diuretic, used in flatulence, stomach pain, and bilious affections. The plant is very useful as stomachic, in the treatment of abdominal pain, skin diseases, as antidiarrheal and antihaemorrhagic[5,6]. Oil emulsion of *W. tinctoria* pods is used to treat psoriasis and also has fungicidal activity against *Pityrosporum ovale* recovered from dandruff[7-9]. Ethyl acetate, acetone, and methanol extracts of *W. tinctoria* bark showed antinociceptive activity in mice[10]. *W. tinctoria* is commonly used as adulterant of an important antidysenteric drug *Holarrhena antidysentrica* another apocynaceae plant[11]. The therapeutic properties of *W. tinctoria* are similar to that of *Holarrhena antidysentrica*, which contains several steroidal alkaloids[12].

*Wrightia tinctoria* bark ethanol extract showed immunomodulatory activity and protective activity against experimentally induced acute gastric ulcers in rats[13,14]. Till date no pharmacological evidence of *W. tinctoria* for the effectiveness in gastrointestinal disorder has been reported. The present study was performed to assess the antidiarrheal efficacy of *W. tinctoria* bark along with probable mechanism of action of isolated steroidal alkaloid fraction of *W. tinctoria* bark (WTSA). The antidiarrheal activity was assessed on a battery of tests to investigated spectrum of efficacy i.e. against castor oil-induced diarrhea, effect on gastrointestinal motility, prostaglandin (PG) $E_2$-induced enteropooling, and GIT secretory activity. *W. tinctoria* bark extract as well as WTSA has been tested employing all the above said models and effect of WTSA on isolated rat ileum along with antimicrobial sensitivity.

## Methods

### *Collection and extraction of plant material*

The *W. tinctoria* (R.Br.) bark was collected from Hoshangabad district of Madhya Pradesh, India in September, 2004. The plant was identified with the help of available literature and authenticated by Dr. A. P., Shrivastava, Principal, P K S Govt. Ayurveda College and Institute, Bhopal, India. A voucher specimen was deposited in the herbarium of department (Herbarium No. 1084).

The barks free from adhering woods were air dried under shade and milled into coarse powder. 70% ethanol extract of bark powder was prepared by cold maceration, the macerated mixture was filtered through muslin cloth and evaporated under 40°C up to one third of initial volume, remaining solvent was completely evaporated at 40°C on 650 mm vaccum pressure using a rotary vacuum evaporator (Superfit, India). The brownish residue (yield 19.145% w/w) designated as ethanol extract was employed for the experimental studies. Crude plant extract was subjected to qualitative phytochemical investigation for alkaloid[15], steroidal saponin[16], reducing sugar[17], tannins[18], and flavonoid[19]. Extract give negative test for glycoside, fixed oil, gums and resins. Prior to use for pharmacological experiments, fresh suspension of dried crude bark extract was prepared with 2% (w/v) carboxy methyl cellulose in distilled water.

***Isolation of W. Tinctoria bark steroidal alkaloid fraction***

The powder plant material was defatted with Petroleum ether (40-60°C) in a continuous Soxhlet extractor. The dried defatted material was then packed in a percolator and maintained at 50-55°C by keeping in a big electric oven. Maceration was done with ethyl alcohol-chloroform mixture (1:4) containing 3% v/v ammonia and then continued with 95% alcohol containing 3% v/v ammonia till drug percolate ratio was 1:3. Both of the percolates were mixed and concentrated under reduced pressure until a syrupy residue. It was then dissolved in 2N HCl and shaken with chloroform to remove extraneous, and coloring matter, then cooled, and basified with cold 2N ammonium hydroxide solution. The liberated alkaloidal bases were extracted with five 50 ml portions of chloroform, washing each chloroform extract with 20 ml of distilled water[20]. The chloroform extract was concentrated under reduced pressure, and yield was 0.0452% (w/w). The alkaloidal residue showed positive result for Wagner's test (Dark brownish grey ppt), Mayer's test (Orange ppt), Dragendroff's test (Dark orangish brown ppt), Hager's test (Light yellowish ppt), and Libermann-Burchard test and negative result for Scheiblers test assuring presence of steroidal alkaloid (WTSA).

This WTSA was gradually eluted with benzene, mixture of benzene: chloroform: ethanol (1:2:4), ethanol, and methanol at a flow rate of 1.2 ml/min on silica gel column prepared by slurry method. Appropriate fractions were combined, filtered, concentrated, and chromatographed on silica gel G plates using benzene: chloroform: methanol (2:6:2) solvent system. Pure fractions having single spot on TLC are Fraction-1 (WTA-1) Rf value 0.923, Fraction-2 (WTA-2) Rf value 0.867 and Fraction-3 (WTA-3) Rf value 0.596. Spots showed orangish pink color on spraying with modified dragendroff's reagent and becomes violet after heating at 110°C for 10 min.

Fraction-2 (WTA-2) gives negative result for Salkowski test and positive result for Libermann Burchard, Dragendroff's, and Wagner's test indicating presence of steroidal alkaloid. The residue (32 mg, 0.0032%) obtained was carefully crystallized on chloroform gives a buffy yellow powder, m.p. 136.0°C; UV $\delta_{max}$ 254 nm; IR (KBr, $cm^{-1}$): 3430 (-N-H or -OH stretching), 2925 and 2854 (aliphatic -C-H or -$CH_3$ stretching), 1739 and 1625 (-C=O stretching), 1459 and 1402 (Methyl bending or -C-H def), 1265 (primary -$NH_2$ stretching), 516 (methylene rocking or 7$CH_2$ group)[21].

***Test animals***

Laboratory bred Wister albino rats of both sex (150-200 g) maintained under standard laboratory conditions at 22 ± 2°C, relative humidity 50 ± 15% and photoperiod (12 h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water was provided *ad libitum*. Animals were fasted over night before the experiment allowing free access to drinking water. In order to avoid diurnal variation all the experiments were carried out at same time of the day i.e. between 10 a.m. to 5 p.m. Approval was obtained from Institutional Animal Ethical Committee (approved body of "Committee for the Purpose of Control and Supervision of Experiments on Animals", Chenni, India) of Dr. H.S. Gour University, Sagar, before carrying out the experiments.

***Determination of $LD_{50}$***

$LD_{50}$ of the extract and WTSA were determined following the Organizations for Economic Co-operation and Development (OECD) guidelines. Acute toxicity Class method (OECD guideline No. 423) and revised Up and Down method (OECD guideline No. 425) was followed for the testing of chemicals. A Limit test was performed to categorize the toxicity class of the compound and then Main test was performed to estimate the exact $LD_{50}$. The test procedure is of value in minimizing the number of animals required to estimate the acute oral toxicity of a substance.

The animals (nulliparous and non-pregnant female Wistar albino rats) were fasted overnight with free access to water and weighed before dosing with the test substance. After drug administration, food was withheld for 3-4 h. Animals were observed individually during first 30 min after dosing, periodically during 24 h with special attention given during first 4 h and daily thereafter for total of 14 days. All the animals survived at Limit test on 2000 mg/kg and subsequently on 5000 mg/kg, so there is no need to do the Main test, as this is the upper limit of testing. The *W. tinctoria* bark extract showed $LD_{50}$ more than 5000 mg/kg which is practically a nontoxic class of compound. A dose of 500 and 1000 mg/kg were selected for further studies. In case of WTSA $LD_{50}$ was found greater then 1000 mg/kg in *limit test* and 1075.25 mg/kg from main test, doses selected for further studies were 50 and 100 mg/kg[22,23].

Animals were divided into five groups of 6 rats in each. Group 1 served as control received 2% CMS (0.5 ml/100 g) only, group 2 and 3 were treated with *W. tinctoria* extract 500 and 1000 mg/kg respectively. Group 4 and 5 were treated with WTSA 50 and 100 mg/kg respectively. Treatments of group 1 to 5 were same for all the four experimental protocols. Group 6 animals were treated with appropriate standard drug in every set of experiment.

***Experimental Models for antidiarrheal study***

***Castor oil-induced diarrhea on rats****:* The method of Awouters *et al*[24] was followed with minor modification. 24 h fasted animals were randomized into different groups. Group 1-5 were treated as mentioned above and group 6 received loperamide (Reckitt and Colman) suspended in 2% carboxy methyl cellulose (CMC) orally in a dose of 0.5 mg/kg as standard drug for comparison. After 1 h each animal received 1 ml of castor oil orally by gavage and was then observed for defecation placing them in separate cages up to 6 h after the castor oil challenge. The consistency and frequency of faecal matter, and the number of respondents were noted on filter papers placed beneath the perforated metal cages. Purging index (PI) was calculated as follows.

Purging index (PI) = % respondent × average no of stools/average latent period

***Effect on gastrointestinal motility****:* Rats were fasted for 18 h and divided into different groups. Group 1-5 animals were given vehicle, extracts and WTSA. The sixth group received atropine sulphate (0.1 mg/kg, i.p) the standard drug for comparison. After 30 min of above treatment each animal was administered orally with 1 ml charcoal meal (3% deactivated charcoal in 10% aqueous tragacanth). After further 30 min of this treatment animals were sacrificed and the intestinal distance moved by the charcoal meal from the pylorus was measured and expressed as a percentage of distance from the pylorus to the caecum[25].

***Effect on $PGE_2$-induced enteropooling****:* For this experiment rats were kept deprived of food and water for 18 h. The first five groups of animals were treated as mentioned previously, while the sixth group was kept as $PGE_2$ control. Immediately afterwards $PGE_2$ (100 µg/kg in 5% v/v ethanol in normal saline, i.m) was administered to each rat except the fifth group which received only 1 ml of 5% (v/v) ethanol in normal saline intraperitonially served as vehicle control. After 30 min animals were sacrificed, the whole length of the intestine from the pylorus to the caecum dissected out and volume measured[25].

***Effect on electrolyte mainly potassium secretion:*** Glibenclamide a potassium channel blocker was used for this study following the method of Awouters *et al*[24] with slight modification. 24 h fasted animals were randomized into six different groups and all the animals were treated with 1 mg/kg of glibenclamide (Ranbaxy, India) suspended in 2% carboxy methyl cellulose (CMC) orally. After 30 min, groups 1-4 were treated with different doses of extracts and WTSA, and group 5 with vehicle only. After further 30 min each animal received 1 ml of castor oil orally by gavage and using the same parameters as castor oil-induced diarrhea, effect of glibenclamide on antidiarrheal activity of *W. tinctoria* was assessed.

***Effect on isolated rat ileum****:* A rat ileum preparation was set up as described by Magnus[26]. Rat of either sex weighing 150-200 g was selected and fasted over night, being allowed water *ad libitum*. The animals were lightly anaesthetized with ether and sacrificed by head blow. Lower portion of the ileum was isolated and transferred to a dissecting dish containing continuously aerated (90% $O_2$ and 5% $CO_2$) warm (37°C) physiological salt solution (Tyrode; PSS) of following composition (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgCl_2.H_2O$ 0.5, $NaH_2PO_4$ 1.0, $NaHCO_3$ 25, glucose 11.1. The preparation (20-28 mm) was mounted in an organ bath filled with PSS, maintained at 37°C. The preparation was well aerated and allowed to stabilize for 30 min during which the bath medium was changed at every 10 min. The tissue response was recorded on a slow moving Kymograph using a frontal righting lever with 6-8-fold magnification. The effect of *W. tinctoria* steroidal alkaloid fraction was studied at different bath concentrations ranging from 1 to 2 mg/ml. The spasmogens used to observe the antagonism behavior were acetylcholine (10 µg/ml), histamine (10 µg/ml), and barium chloride (20 µg/ml). The response of the ileum to WTSA was expressed as a percentage of the maximum relaxation to papaverine (500 µg/ml) that was added at the end of the experiment[27].

***Antimicrobial studies:*** Effect of *W. tinctoria* alkaloid fraction against enteric bacteriae like *Escherichia coli* (ATCC 25922), *Salmonella typhi* (MTCC 98), and on *Staphylococcus aureus* (ATCC 25923) was carried out by agar well diffusion method[28]. The broth culture in log phase was diluted to 0.5 McFarland standard and swab streaked over Muller Hinton agar. Wells were drilled (7 mm dia.) and the dilutions of drug (100 to 5000 µg/ml) added in 50 µl amount. The extract was dissolved in 1% di-methly sulfoxide in double distilled water. The positive control was cefotaxime (10 µg/ml). The plates were kept on the bench for 30 min before incubation at 37°C for 24 h. All the results were taken from triplicate set of experiments.

**Statistical analysis**

All data were presented as Mean ± SEM. Experimental data was analyzed using one way ANOVA followed by Turkey-Kramer multiple comparison test. P value less than 0.05 was considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

## Results

Ethanol (70%) extract, dark brown in color (yield 19.145% w/w) was employed for the experimental studies. Qualitative phytochemical investigation of crude plant extract revealed the presence of steroidal saponin, alkaloid, reducing sugar, tannins, flavonoids and absence of glycoside.

*W. tinctoria* extract inhibited castor oil-induced diarrhea by reducing the number of deformed faeces, frequency, and consistency of diarrhea in dose-dependent manner. The negative control group had diarrhea with mucus. *W. tinctoria* extract at a dose of 1000 mg/kg significantly ($P < 0.001$) reduced the number of wet faeces and WTSA at 100 mg/kg dose was found to be equipotent with the standard drug as shown in Table 1. Loperamide showed significant reduction in frequency of defecation and number of wet faeces at 0.5 mg/kg dose.

**Table 1.** Antidiarrheal activity of *W. tinctoria* bark extract and WTSA against castor oil-induced diarrhea on rat.

| Group and dose (mg/kg) | Mean defecation/gr. (upto 6 hrs) | Number of wet faeces/gr. | % Respon-dent | Mean latent period in mins | Purging index | % Protec -tion |
|---|---|---|---|---|---|---|
| Vehicle control | 36.43±4.27 | 24.49±4.18 | 67.21 | 12.14 ± 2.05 | 201.63 | 0.00 |
| Loperamide (0.5) | 18.99±3.03** | 3.57±0.02*** | 18.80 | 48.12±5.63*** | 07.41 | 85.42 |
| W.t (500) | 24.23±3.24$^{ns}$ | 12.03±1.06** | 49.66 | 38.51±5.16** | 31.23 | 50.88 |
| W.t (1000) | 18.56±2.64** | 6.10± 0.95*** | 32.86 | 40.02±6.39** | 15.23 | 75.09 |
| WTSA (50) | 18.03±2.57** | 4.85±1.31*** | 26.85 | 41.56±4.42** | 11.64 | 80.19 |
| WTSA (100) | 16.01±2.01** | 3.64±0.93*** | 22.73 | 44.32±4.15*** | 8.21 | 85.14 |

Purging index (PI) = % respondents × average no of stools / average latent period.
% Protection = C – T / C × 100.
The values are expressed as Mean ± SEM (standard error of mean), n = 6 in each group. One-way ANOVA followed by Turkey-Kramer multiple comparison tests. ***P<0.001 and **P<0.01 when compared to control group. W.t, *W. tinctoria* bark alcoholic extract; WTSA, *W. tinctoria* bark steroidal alkaloid fraction.

There was dose-dependant reduction in the propulsion of charcoal meal through the gastrointestinal track by *W. tinctoria* extract as compared to the vehicle treated group. Moreover, as shown in Table 2 the propulsion of charcoal meal through the gastrointestinal tract was decreased significantly from 76.44 to 23.63% (control vs. WTSA 100 mg/kg; $P < 0.001$). Atropine sulphate significantly reduced the intestinal motility.

**Table 2.** Effect of *W. tinctoria* bark extract and WTSA on gastrointestinal motility of rats after charcoal meal.

| Group and dose (mg/kg) | % Intestinal transit | % Protection |
|---|---|---|
| Vehicle control | 76.44 ± 7.98 | ---- |
| Atropine sulphate (5, i.m) | 29.07 ± 3.45*** | 61.97 |
| W.t (500) | 61.73 ± 6.22 | 19.24 |
| W.t (1000) | 48.15 ± 5.52* | 37.00 |
| WTSA (50) | 53.30 ± 4.14*** | 30.27 |
| WTSA (100) | 23.63 ± 2.94*** | 69.08 |

% intestinal transit = distance traveled by charcoal / total length of intestine × 100.
The values are expressed as Mean ± SEM (standard error of mean), n = 6 in each group. One-way ANOVA followed by Turkey-Kramer multiple comparison tests. ***P<0.001 and *P<0.05 when compared to control group. W.t, *W. tinctoria* bark alcoholic extract; WTSA, *W. tinctoria* bark steroidal alkaloid fraction.

$PGE_2$ treatment induced a significant increase in fluid volume of the rat intestine when compared with the control animals receiving only ethanol in normal saline. *W. tinctoria* extract (1000 mg/kg) produced 39.87% inhibition of intestinal content volume. The extract dose-dependently reversed the effect of $PGE_2$ on fluid accumulation in intestinal loop. However, 50 and 100 mg/kg dose of WTSA produced a significant ($P < 0.001$) inhibition of volume of intestinal content, 58.17 and 70.59% respectively (Table 3).

**Table 3.** Effect of *W. tinctoria* bark extract and WTSA against prostaglandin $E_2$-induced intestinal fluid accumulation on rat.

| Group and dose (mg/kg) | Volume of intestinal fluid (ml/100 gm of body wt.) | % Protection |
|---|---|---|
| Vehicle control | 1.53 ± 0.13 | ---- |
| Prostaglandin $E_2$ (100µg, i.m) | 2.56 ± 0.24*** | - 67.32 |
| W.t (500) | 1.06 ± 0.09 | 30.72 |
| W.t (1000) | 0.92 ± 0.05* | 39.87 |
| WTSA (50) | 0.64 ± 0.03*** | 58.17 |
| WTSA (100) | 0.45 ± 0.02*** | 70.59 |

The values are expressed as Mean ± SEM (standard error of mean), n = 6 in each group. One-way ANOVA followed by Turkey-Kramer multiple comparison test. ***P<0.001 and *P<0.05 when compared to control group. W.t, *W. tinctoria* bark alcoholic extract; WTSA, *W. tinctoria* bark steroidal alkaloid fraction.

Glibenclamide administered with castor oil showed purging index more than that produced by castor oil alone. *W. tinctoria* extract was devoid of having significant effect on diarrhea induced by simultaneous administration of glibenclamide with castor oil. Fig. 1a,b and 2 showed that WTSA at 50 and 100 mg/kg dose produced 10.60 and 29.39% protection when given with glibenclamide compared to 60.05 and 66.18% diarrhea reduction in castor oil alone induced diarrhea.

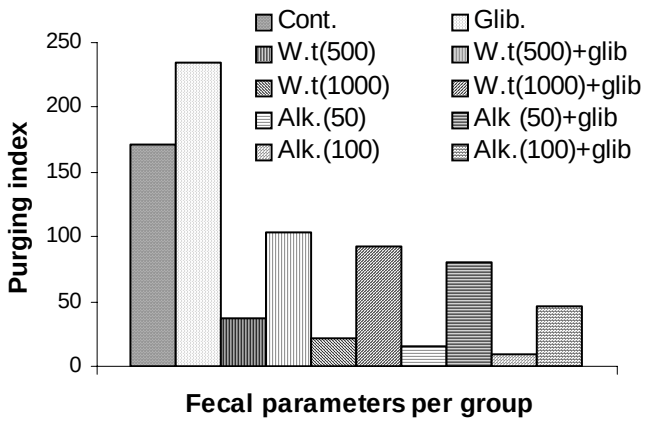


Fig. 1a

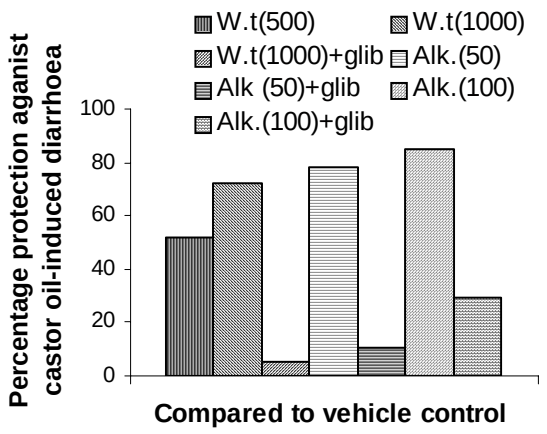


Fig. 1b

**Figure 1.** Effect of glibenclamide on antidiarrheal activity of *W. tinctoria* bark extract and WTSA against castor oil-induced diarrhea on rat (Purging index and percentage protection).
Purging index (PI) = % respondents × average no of stools / average latent period.
% protection = C – T / C × 100. W.t, *W. tinctoria* bark alcoholic extract; WTSA, *W. tinctoria* bark steroidal alkaloid fraction.

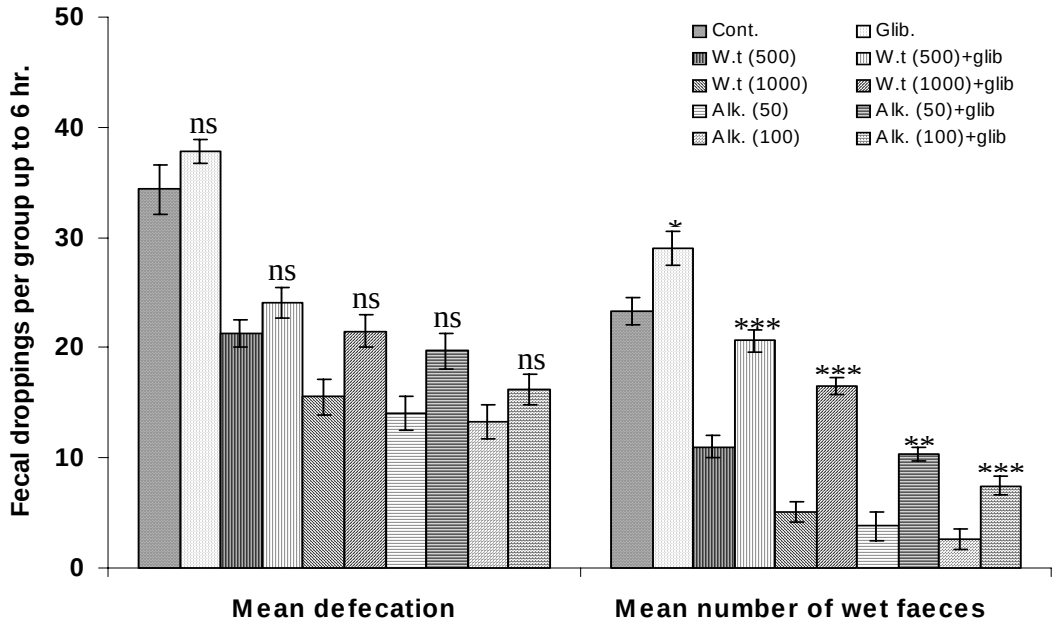


**Figure 2.** Effect of glibenclamide on antidiarrheal activity of *W. tinctoria* bark extract and WTSA against castor oil-induced diarrhea on rat (Defecation parameters).
Mean defecation and mean no. of wet faeces was observed per group upto 6 hrs after treatment. The values are expressed as Mean ± SEM (standard error of mean), n = 6 in each group. One-way ANOVA followed by Turkey-Kramer multiple comparison test. ***P<0.001, **P<0.01, *P<0.05 and NS = not significant when the glibenclamide along and glibenclamide with extract treated groups were compared with extract treatment alone. W.t, *W. tinctoria* bark alcoholic extract; WTSA, *W. tinctoria* bark steroidal alkaloid fraction.

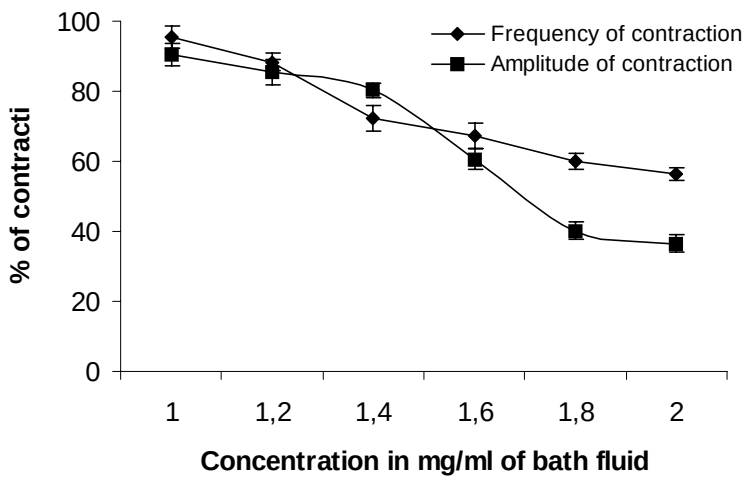


**Figure 3.** Concentration-effect curves of the inhibitory effects of *W. tinctoria* bark steroidal alkaloid fraction (WTSA) on frequency and amplitude of spontaneous contractions of isolated rat ileum.
Frequency of spontaneous contraction and amplitude of contraction of the ileum to alkaloidal extract were expressed as a percentage of the maximum contraction of control. Values are mean of five experiments and bars represent the Mean ± SEM.

WTSA at 2 mg/ml of bath concentration caused relaxation of isolated rat ileum. The $EC_{50}$ value for WTSA on the amplitude of relaxation is 1.986 ± 0.82 mg/ml. WTSA at 2 mg/ml dose showed 88.82% reduction in frequency of spontaneous movement. The effect of *W. tinctoria* alkaloid fraction on amplitude and frequency of freshly isolated rat ileum is shown in Fig. 3. WTSA also inhibited Ach-induced contraction of rat ileum (Fig. 4).

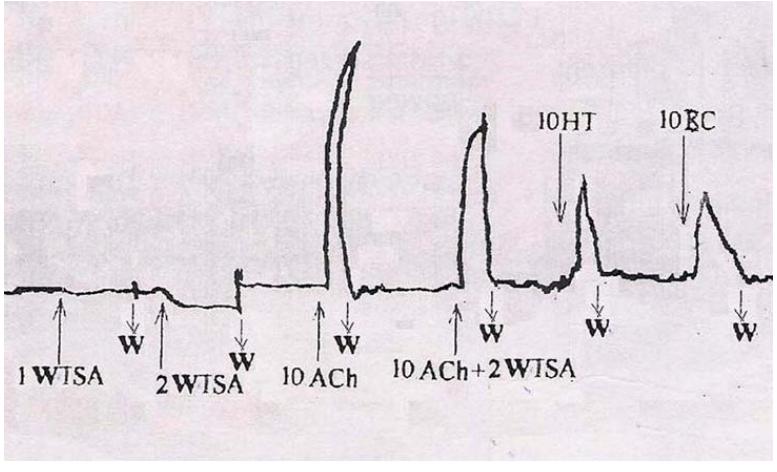


**Figure 4.** Representative tracing showing effect of *W. tinctoria* bark steroidal alkaloid fraction (WTSA) on isolated rat ileum challenged with acetylcholine, histamine and barium chloride.
WTSA in 1 mg/ml and 2 mg/ml, ACh; acetylcholine (10 μg/ml), HT: histamine (10 μg/ml), and BC: barium chloride (20 μg/ml) concentration, W signifies washing with physiological salt solution (Tyrode).

WTSA did not show zone of inhibition surrounding the well even at 5 mg/ml concentration (data not shown). The positive control cefotaxime showed 20-28 mm inhibition zone.

## Discussion

Diarrhea is an imbalance between absorptive and secretory mechanism of intestine resulting excess loss of fluid in faeces, but in some diarrheas hypermotility predominates the secretory parameters. Increased motility of the gastrointestinal tract and decreased absorption of fluid are major factors in diarrhea. Antidiarrheal drugs includes antimotility agents, adsorbents and drugs that modify fluid and electrolyte transport of GIT. Castor oil induces diarrhea by preventing reabsorption of NaCl and water thus increasing the volume of intestinal content. In castor oil-induced diarrhea model liberation of recinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandins, which stimulate motility and secretion[29].

One of the main causes of diarrhea is increase in intestinal motility, which decreases the time of absorption. Atropine produces a significant reduction in intestinal transit time due to its anticholinergic effect[30]. Anatomical, functional and receptor binding studies evidenced the role of Ach as a putative neurotransmitter directly involved in epithelial cell electrolyte transport. Human ileal mucosa receives a direct cholinergic innervation to all its epithelial cells and muscarinic $M_3$ receptors are located to the basolateral domain of epithelial cells[31]. Acetylcholine released from mucosal or submucosal nerves can stimulate colonic fluid secretion. Endogenous prostaglandins are possibly liberated as a consequence of the injury, and may be involved in the Ach release[32]. Prostaglandin increases the intestinal fluid volume and contents by modulating

activity of the cholinergic nervous system in the myenteric plexus, which is compatible with that found in acute diarrhoea[33-34]. The effect of prostaglandin $E_2$ probably mediated by duel way; neurotropic in myenteric plexus by increasing Ach release and musculotropic by sensitization of the smooth muscle[35-36]. Increased local synthesis of prostaglandins occurs in diarrhea associated with inflammatory bowel disease[37].

Castor oil-induced model was suggested for evaluation of prostaglandin biosynthesis inhibition[29]. *W. tinctoria* extract produced significant reduction in the severity and frequency of castor oil-induced diarrhea. Our results showed that intraluminal fluid accumulation induced by castor oil was inhibited by extract and WTSA in a dose-dependent manner. WTSA inhibited the frequency of defecation and the content of the fecal droppings like standard drug loperamide. The significant antidiarrheal activity shown by the *W. tinctoria* may be due to protective effect on mucosa, thus reducing accumulation of fluid in the intestinal lumen. The extract and WTSA decreased intestinal transit of charcoal meal probably by decreasing the intestinal propulsive movement. The extract also reduced gastrointestinal content as evident from its antienteropooling activity aganist $PGE_2$. The protection showed may be due to prostaglandin inhibition. Considering prostaglandins as a possible mediator of diarrhea, the inhibition of prostaglandin biosynthesis may be involved in the antidiarrheal action of WTSA. The results suggest that the extract and primarily the alkaloid fraction may have prostaglandin inhibitory effect on intestinal tract, resulting in inhibition of gastrointestinal motility and secretion.

Experimental and clinical observations indicate that non-steroidal anti-inflammatory agents (NSAIDS) such as aspirin and indomethacin are effective in controlling diarrhea. Mennie & Dalley[38] reported findings indicating usefulness of aspirin in the treatment of diarrhea induced by radiation. Prostaglandins are involved in radiation induced diarrhea. The treatment of cervical cancer by a combination of external radiation and radium often produces diarrhea, probably because of bowel loop damage in the path of X-rays. Indomethacin is an effective inhibitor of prostaglandin biosynthesis, showed prevention of cholera-induced diarrhea[39]. A US patent has been issued by the title “Antidiarrheal compositions and use thereof” on Sept 19, 1989. The invention includes a antidiarrheal compositions of enhanced antidiarrheal activity provided by compositions of a NSAID, selected from the group consisting of indomethacin and aspirin and a polymeric hydroabsorptive agent to treat patients needing preventative or remedial treatment of diarrhea symptoms[40]. Bismuth subsalicylate, used for travelers diarrhea, decreases fluid secretion in the bowel, its action may be due to its salicylate component[41].

*W. tinctoria* alkaloid fraction inhibited the spontaneous contraction and Ach-induced spasmodic contraction of isolated rat intestine. WTSA acts as a relaxant of rat isolated ileum, by decreasing the amplitude, frequency, and tone of spontaneous contractions. The anticholinergic action of WTSA may contribute to its antisecretory and antimotility activity.

In acute secretory diarrhea the primary event driving fluid secretion is a transcellular electrogenic, serosal to mucosal transport of chloride ions. Electrogenic chloride transport by human ileal mucosa required the presence of basolateral $K^+$ channel[42]. Many antidiarrheal drugs act by reducing the loss of sodium and potassium as in diarrhea there is an increased loss of these ions predominantly potassium. To find out the effect of *W. tinctoria* extract and, WTSA on $K^+$ reabsorption the antidiarrheal activity was observed in presence of glibenclamide a potassium channel blocker. Glibenclamide, when given with castor oil showed diarrhea more than that produced by castor oil alone due to decrease potassium absorption along with chloride ion by mucosal cells. Co-administration of glibenclamide with extract and WTSA did not show significant protection on castor oil-induced diarrhea. Presence of glibenclamide prevented the antidiarrheal activity increasing the number of wet faeces dropping per group. The findings

suggest that *W. tinctoria* posses $K^+$ channel activating property, resulting increased reabsorption of potassium and thus reducing its loss in the watery stool.
The phytochemical tests carried out in *W. tinctoria* extract revealed presence of saponin, steroidal alkaloid, reducing sugar, tannins, flavonoids, and absence of glycoside. Of the numerous phytochemicals present in active extracts, tannins, and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption. Tannic acid and tannins, present in many plants denature proteins by formation of protein tannates, which makes the intestinal mucosa more resistant and reduces secretion. The antidiarrheal activity of *W. tinctoria* extract may be due to inhibition of increased watery secretion by the cumulative action of all its constituents like alkaloid, flavonoids, and tannins[43]. *W. tinctoria* may not be effective in infectious diarrhea or dysentery as alkaloid did not have antibacterial activity.

Studies can validate the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of such plant extracts for antispasmodic effect, delaying intestinal transit, suppressing gut motility, stimulating water adsorption or reducing electrolyte secretion. An antidiarrheal plant drug effective in non-infectious diarrhea should inhibit luminal secretion, promote reabsorption or may produce some decrease in intestinal motility to permit a longer contact time of luminal fluids with epithelial cells. *W. tinctoria* inhibited castor oil- induced secretory diarrhea may be by inhibiting prostaglandin biosynthesis or release, which intern is involved with Ach release. WTSA has spasmolytic and antienteropooling activity, alkaloid inhibited intestinal motility, and peristalsis. Anticholinergic activity may also contribute to its antisecretory effect promoting the $K^+$ and $Cl^-$ reabsorption. All these studies substantiate effectiveness of *W. tinctoria* in secretory, osmotic, motility related and inflammatory diarrhea. Thus this study provides a sound mechanistic base for the traditional uses of the plant in curing gastrointestinal disorder.

## Acknowledgements

The authors are grateful to All India Council of Technical Education, New Delhi for the financial assistance by awarding National Doctoral Fellowship to one of the authors (Dr. P. Bigoniya, grant no. FD/NDFS/2003-04).
The authors are also thankful to Ranbaxy Laboratories Ltd., Dewas for providing the gift sample of glibenclamide used for the study.

## References

1. Lawrence DR, Bennett PN, Brown MJ. Clinical Pharmacology. New York, Churchill Livingstone, 1997:583.
2. Schiller LR. Management of diarrhea in clinical practice: strategies for primary care physicians. Rev Gastroenterol Disord 2007; 7(Suppl 3):27-28.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. New Delhi, Jayyed Press, 1975:1581.
4. The Wealth of India: Raw Materials. New Delhi, Publications and Information directorate CSIR, 1976:588-590.
5. Nadkarni KM. Indian Materia Medica. Bombay, Popular Prakashan, 1976:1296.
6. Singh VP, Sharma SK, Kare VS. Medicinal plants from Ujjain District Madhya Pradesh: Part 2. Ind Drugs 1980; 17:7-12.

7. Clinical and Experimental Studies on the Efficacy of 777 oil, A Siddha Preparation in the Treatment of Kalanjagapadai (Psoriasis). New Delhi, Monograph Published by Central Council of Research in Ayurveda and Siddha, Ministry of Health and Family Welfare, Govt. of India, 1987:1-58.
8. Mitra SK, Seshadri SJ, Venkataranganna MV, Gopumadhvan S. Reversal of parakeratosis, a feature of psoriasis by *Wrightia tinctoria* (in emulsion) histological evaluation based on mouse tail. Ind J Dermatol 1998; 43:102-104.
9. Krishnamoorthy JR, Ranganathan S. Anti-*Pityrosporum ovale* activity of a herbal drug combination of *Wrightia tinctoria* and *Hisbiscus rosasinensis*. Ind J Dermatol 2000; 45:125-126.
10. Reddy YSR, Venkatesh S, Ravichandran T, Murugan V, Suresh B. Antinociceptive activity of *Wrightia tinctoria* bark. Fitoterapia 2000; 73:421-423.
11. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 1st ed. National Institute of Science Communication, New Delhi, Council of Scientific and Industrial Research publication, 1956:259.
12. Glasby JS. Encyclopedia of the Alkaloids. New York, Plenum Press, 1975:265-66.
13. BigoniyaP, Rana AC, Agrawal GP. Evaluation of the antiulcer activity of hydro-alcoholic extract of *Wrightia tinctoria* bark in experimentally induced acute gastric ulcers on rat. Nig J Nat Pro Med 2006; 10:36-40.
14. Bigoniya P, Rana AC, Lariya S. Immunomodulatory activity of *wrightia tinctoria* bark alcoholic extract on rats. Curr Pharm Res J 2007; 1(6):1-9.
15. Paech T, Tracy MV. Modern Methods in Plant Analysis. Berlin, Springer Verlog, 1955:387.
16. Finar IL. Organic Chemistry. 2nd ed. London, The English Language Book Society, 1959:280.
17. Wallis TE. Text Book of Pharmacognosy. 5th ed. New Delhi, CBS Publishers and Distributors, 1985:80.
18. Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy. 7th ed. India, Nirali Prakashan, 2001:133.
19. Shellard EJ. Practical Plant Chemistry for Pharmacy Students. London, Pitman Medical Publishing Co. Ltd., 1957:34.
20. Sethi PD. Separation of alkaloidal constituents of *Wrightia tinctoria* by TLC. Planta Med 1970; 18:26-29.
21. Silverstein RM, Webster FX. Spectroscopic Identification of Organic Compounds. 6th ed. New York, John Wiley and Sons Inc., 2005:71-143.
22. Lipniek RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP. Comparison of the Up and Down, Conventional $LD_{50}$ and Fixed Dose acute toxicity testing procedures. Food Chem Toxicol 1995; 33:223-231.
23. Organization for Economic Cooperation and Development (OECD): Guidance document on acute oral toxicity testing. Environment, Health and Safety Publication. OECD Series on Testing and Assessment, Number 24, 2001:12-23.
24. Awouters F, Niemeegers CJE, Lenaerts FM, Janssen PAJ. Delay of castor oil diarrhea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharm Pharmacol 1978; 30:41-45.
25. Lutterodt GD. Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible model of action of *Psidium guajava* leaf extracts in the treatment of acute diarrheal disease. J. Ethnopharmacol 1989; 25:235-247.
26. Magnus R. Versuche am überlebenden dünndarm von säugetieren I. Pflügers Arch Ges Physiol 1904; 102:123-151.
27. Afifi FU, Khalil E, Abdalla S. Effect of iso-orientin isolated from *Arum palaestinum* on uterine smooth muscle of rats and guinea pigs. J Ethnopharmacol 1999; 65:173-177.

28. Norrel SA, Messley KE. Microbiology Laboratory Manual Principles and Applications. New Jersey, Prentice Hall, 1997:85-90.
29. Pierce NF, Carpenter CCJ, Elliot HZ, Greenough WB. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterol 1971; 60:22-23.
30. Brown JA, Taylor P. Muscarinic receptor agonists and antagonist. In: Hardman JG, Limbird LE, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. New York, McGraw Hill, 2000:115-158.
31. Banks MR, Golder M, Farthing MJ, Burleigh DE. Intracellular potentiation between two second messenger systems may contribute to cholera toxin induced intestinal secretion in humans. Gut 2004; 53(1):50-57.
32. Gaginella TS, Grisham MB, Thomas DB, Walsh R, Moummi C. Oxidant-evoked release of acetylcholine from enteric neurons of the rat colon. J Pharmacol Exp Ther 1992; 263:1068-1073.
33. Ehrenpreis S, Greenberg J, Belman S. Prostaglandins reverse inhibition of electrically-induced contractions of guinea pig ileum by morphine, indomethacin and acetylsalicylic acid. Nature 1973; 245:280-282.
34. Milton-Thompson GJ, Cummings JH, Newman A, Billings JA, Misiewicz JJ. Colonic and small intestinal response to intravenous prostaglandin $F_{2\alpha}$ and $E_2$ in man. Gut 1975; 16:42-46.
35. Funaki H, Yagasaki O. Contribution of endogenous prostaglandin to the cholinergic nervous activity in Auerbach's plexus. Japan J Pharmacol 1977; 27:148-148.
36. Bennett A, Elly KG, Stockley HL. Modulation by prostsglandins of contractions in guinea-pig ileum. Prostaglandins 1975; 9:377-384.
37. Harvey RF, Read AE. Effect of cholecystokinin on colonic motility and symptoms in patients with irritable bowel syndrome. Lancet 1973; 1:1-3.
38. Mennie AT, Dalley V. Aspirin in radiation induced diarrhea. Lancet, 1973; 1:1131-1132.
39. Wald A, Gotterer GS, Rajendra GR, Turjman NA, Hendrix TR. Effect of indomethacin on cholera-induced fluid movement, unidirectional sodium fluxes and intestinal cAMP. Gastroenterol 1977; 72:106-110.
40. Sheth BB, Gilbert SA, Kinsel JF. Antidiarrheal compositions and use thereof. United States Patent 4867979. US Patent Issued on Sept 19th, 1989, Available at: http://www.freepatentsonline.com/4867979.html. Accessed on July 22nd, 2008.
41. Mycek MJ, Harvey RA, Champe PC, Fisher BD, Cooper M. Lippincott Illustrated Review: Pharmacology. 2nd ed. Philadelphia, Lippincott Williams and Wilkins, 2000:244.
42. Burleigh DE, Involvement of inwardly rectifying $K^+$ channels in secretory responses of human ileal mucosa. J Pharm Pharmacol 2003; 55:527-531.
43. Lulterodt GD. Inhibition of micro lax-induced experimental diarrhea with narcotic like extract of *Psidium guajava* leaf in rats. J Ethnopharmacol 1992; 37:151-157.