ISSN–2230-7346
Journal of Global Trends in Pharmaceutical Sciences

EVALUATION OF ANTI-NOCICEPTIVE AND ANT DIARRHEAL ACTIVITIES OF *VACCARIA PYRAMIDATA* ETHANOLIC ROOT EXTRACT ON ALBINO MICE MODELS

Rohtash Singh[1*], Mangal Sain Hooda[1], and Krishna Mohan[2]

[1]Department of Pharmacology, Janta College of Pharmacy, Butana, Sonepat-131301(Haryana), India
[2] Department of Pharmacology, Teerthanker Mahaveer College of Pharmacy, TMU, Moradabad-244001 (U.P)

*Corresponding author E-mail: rohtasrajput@gmail.com

ARTICLE INFO

Key Words

Vaccaria Pyramidata, Loperamide, Anti-nociceptive, Anti-diarrheal, Morphine

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Vaccaria Pyramidata ((Family: Caryophyllaceae)) is an indigenous medicinal plant India and mostly in Uttar Pradesh and Maharashtra. The root extract has been used traditionally as folk remedies for the treatment of many diseases including diarrhea, dysentery, and potent diuretics. The root extract of the plant is used in menstruation cycle problems. It is also used as a tonic, anti-periodic. However, there was no study on whole plant of *Vaccaria Pyramidata*. The present study designed to investigate the anti-nociceptive and anti-diarrheal activities of *Vaccaria Pyramidata* on animal models at different doses such as 250 and 500 ml/kg respectively. Various methods also employed for investigating these activities such as castor-oil induced diarrhea, tail immersion, tail flick, hot plate and also carrageenen induced paw edema models. The diarrheal episode was inhibited by 31.88% and 40.95% for ethanolic root extract at the doses of 250 and 500 mg/kg respectively. The extract significantly ($p<0.05$) lessened the intestinal volume (0.51 ± 0.04 ml for 250 mg/kg) and (0.46 ± 0.02 ml for 500ml/kg) for ethanolic root extract compared to control (0.63±0.02 ml) in castor-oil induced diarrhea and also decreased intestinal transit (54.58 – 60.12%) for ethanolic root extract comparable with standard (loperamide 5 mg/kg). The root extract of *Vaccaria Pyramidata*, increased latency in tail flick and tail immersion model and elevated the mean basal reaction time in hot plate model and also basal reaction time on carragennen induced paw edema. The results of root extract of *Vaccaria Pyramidata* showed highly significant but dose dependent on anti-diarrheal and anti-nociceptive activities, which supports its use in traditional herbal medicine.

INTRODUCTION

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of pain and arthritic conditions. Pain is an unpleasant sensation localized to a part of the body. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distention, or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system[1]. Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important

symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause[2]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency. Now-a-days, opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side effects and low potency[3]. In case of morphine, acute morphine poisoning, hypotension, drug dependence, etc. Diarrheal disease is the second leading cause of death in less than five children in world wide. According to latest data every year diarrhea kills around 760 000 children under five. There are nearly 1.7 billion cases of diarrhea disease year, globally[4]. Worldwide, diarrhea accounts for more than 5-8 million deaths in infants and small children less than 5 years each year. According to World Health Organization (WHO) estimation for the year 1998, there were about 7.1 million deaths due to diarrhea[5]. In India, one third of the total child death burden is due to diarrhea[6]. WHO organized a Diarrhea Control Program where they emphasized use of traditional medicines to combat the episodes of diarrhea.[7] Diarrhea appeared by several mechanism such as increasing the gut motility, along with increased secretion of ions and a decrease in the absorption of fluid, and thus a loss of electrolytes, particularly Na+ and water[8]. Synthetic drugs as well as conventional treatments are being failed to fulfil their objectives, due to their toxic and adverse side effects. For this reason it becomes necessary to search other alternatives such as plants. For these consequences herbal medicine has made a comeback to improve the fulfilment of our present and future health needs[9].

Plant Profile

The plant *Vaccaria Pyramidata* (Family: Caryophyllaceae) is grown as a wild flower. It has a medium greyish hairless annual with a regularly branching stem, oval leaves and small pink flower. It is found on arable fields, often on lime. It is found throughout India, as a weed. It is often grown in gardens, dry sandy plains near lakes, meadows, clay-solonetz places in steppes and solonetz meadows, marshes, ditches and wet grassy places. *Vaccaria Pyramidata* is an annual plant growing to 0.6 m (2ft) flowers appear in July to August and the seeds ripe between Aug to September .The flower are hermaphrodite (have both male and female organs) and are self-pollinated by Lepidoptera. The plant is self-fertile. The root is used for cough, asthma and other respiratory disorders, for jaundice, liver and spleen diseases (increases the bile flow). Mucilaginous sap is used in scabies. Saponins of the root showed haemolytic activity. Some others treditionals uses i.e. Anthelmintics, Appetizer, Depurative, Diuretic, Vermifuge. It is used with other herbs in the treatment of venereal infections, liver complaints and oedema. The main chemical constituents i.e. Lanostenol, stigmasterol, beta-sitosterol, diosgenin, vaccaxanthone[10],

MATERIAL AND METHODS

The roots of the plant *Vaccaria Pyramidata* were collected from Uttar Pradesh in the month of November 2018. The plant specimen was authenticated by Dr. Alok Lahri of National Botanical Research institute (Council of scientific and industrial research), Lucknow with reference no. CIF-RB-4-391dt., specification-NBRI-SOP-202.Qualitative chemical investigations were conducted in order to identify various phytochemical constituents present in different extracts. The aspects of only positive tests were taken into consideration, (Alkaloid, Flavonoids, Cardice Glycosides, Saponin, Steroids, Tannins, Phenolics, Proteins and Amino Acids). The collected roots of "*V. Pyramidata"* was ground to get coarse powder and then subjected to successive extraction using petroleum ether, chloroform, and ethanol with the help of soxhlet apparatus. After extraction with chloroform the coarse powder of the roots of *V. Pyramidata* was dried and extracted with ethanol for 24 hrs. The liquid extract was then cooled and then placed on the water bath at temp. 40-50ºC until the entire solvent evaporated. The dried extract was weighed and the percentage yield was calculated with reference to the crude drug. The dried ethanolic extract was stored in a desiccator and subjected to various chemical tests to detect the presence of different phyto constituents like alkaloids,

tannins, cardiac glycosides and traces of flavonoids etc.

Evaluation of Experimental Animals: Healthy adult Wister Albino mice were selected for the study. They were fed with standard pellet diet and water ad libitum. All animal protocols were approved by Institutional Animal Ethical committee (IAEC). (The Institutional Animal Ethical Committee 667/02/c/CPCSEA) approved the studies. All animals were maintained under standard conditions of humidity (50±10 %), temperature (22±20^{0}c) & light (12 hours light & 12 hours dark).

Castor oil induced diarrhea: This experiment was carried out by the method described by Awouters *et al*[11]. The experimental mice were kept fasting condition for 18 hours. Four groups of mice were taken for this experiment. Group I treated as control (saline 2 ml/kg body weight orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p.) and Group III-IV received ethanolic root extract of *V. Pyramidata* (250 and 500 mg/kg by oral route) respectively. Then 1 h later, castor oil (0.4 ml/mice) was administered orally. The mice were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

Anti-nociceptive activities

Eddy' Hot plate (Techno) method: The anti-nociceptive activity of the ethanolic root extract of *V. Pyramidata* was measured by hot-plate method[12]. The analgesic effect of hot plate model was explained in the following steps.

1. Mice were divided into four groups of five animals each. Group 1 received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV ethanolic root extract of *V. Pyramidata* (250 & 500 mg/kg) per oral route respectively.

2. take the basal reaction time by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55^{0}C). Normally animals show such response in 6-8 sec. A cut of period of 15 sec is observed to avoid damage to the paws.

3. Inject morphine (5 mg/kg) or ethanolic root extract of *V. Pyramidata*. (250 & 500 mg/kg) to animals and the reaction time of animals on the hot plate at 15, 30, 60, and 120 min after the drug administration. As the reaction time with morphine or ethanolic root extract of *V. Pyramidata*, 15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to the paws. And calculate basal reaction time at each time interval[13].

Tail immersion method: Mice were divided into four groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV ethanolic root extract of *V. Pyramidata*(250&500 mg/kg bw) respectively. Latency of mice tail with-drawing from hot water was noted as the basal reaction time. The lower 3 cm portion of the tail of mice was dipped in a water bath maintaining at temperature of 55 ± 0.5°C. The reaction time was noted at 0, 30, 60, and 90 min. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animals[14].

Tail flick Method: Mice were divided into four groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV ethanolic root extract of *V. Pyrimidata*. (250 & 500 mg/kg) per oral route respectively.

1. Take basal reaction time radiant heat by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail-withdrawal from the heat (flicking response) is taken as the end point. Normally a mouse withdraws its tail within 3-5 sec. A cut of period of 10-12 sec is observed to prevent damage to tail. Any animal failing to withdraw its tail in 3-5 sec is rejected from the study. Take at least 3-5 basal reaction times for each mouse at a gap of 5 minutes to confirm normal behaviour of the animal.

2. Inject morphine or extract and noted the reaction time at 5, 15, 30, and after the drug administration. As the reaction time reaches 10 sec it is considered maximum analgesia and tail is removed from the source of heat to avoid tissue damage. And calculate basal reaction time at each time interval[15].

Carrageenan induced paw edema[16-17]

Procedure: Evaluation of Received *V. Pyramidata* ethanolic root extract was used against carrageenen induced paw edema model. Experimental animals were divided into following four groups: All the groups were pretreated according to their treatments, 1 hour before the administration of 0.1 ml of 1% carrageenan (suspended in sterile 0.9% normal sterilized saline) in subplanter region of right hind paw of rat. The initial paw volume (IPV) and final paw volume (FPV) was measured after 60, 120, 180, 240 & 300 minutes of carrageenan administration using plethysmometer. The difference initial and final paw volume was used to calculate the percentage inhibition using following equation:-

$$\text{Percentage inhibition} = \frac{(X-Y)}{X} \times 100$$

Where,

X= increase in paw volume of rats in the control group

Y= increase in paw volume of rat in the drug treated group

STATISTICAL ANALYSIS: The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard. The data were statically analyzed by Graph pad prism Software version (7.1). The ED_{50} value was determined best value fit regression line of a dose response curve.

RESULTS: The results are shown in tables and figure for illustration (Tables 1-6 and figures 1-3).

Castor oil induced diarrhea in mice

DISCUSSION

Preliminary phytochemical screening showed the presence of catechin, tannins, alkaloids and flavonoids in the *V. Pyramidata* ethanolic root extract, so the observed analgesic and anti-diarrheal activities may be attributed due to these compounds. The *V. Pyramidata* ethanolic root extract the maximum significant anti-diarrheal ($p < 0.05$) effect at the dose of 10 ml/kg and 20 mg/kg in compare to standard drug loperamide (5 mg/kg) and also possessed 31.88% and 40.95%, inhibitions of defecation respectively in the test of Castrol oil induced diarrhea. There are some mechanism are available to explain the diarrheal effect of castor oil include inhibition of intestinal Na+ K+ ATPase activity, thus reducing normal fluid absorption,[18] activation of adenylate cyclase or mucosal cAMP-mediated active secretion,[19] stimulation of prostaglandin formation and platelet activating factor,[20] However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hyper-secretory response.[21],[22] On the other hand, in castor oil induced method, the extract depicted significant ($p < 0.05$) effect at the dose of 250 and 500 ml/kg and also reduced the volume of intra-luminal contents respectively. These effects, which have direct consequences to reduced water and electrolytes secretion into the small intestine[23], suggest that the ethanolic root extract may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hyper-secretion. Hyper-motility characterizes diarrhea where the secretory component is not the causal factor.[24-25] Pre-treatment with the extract suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which significantly indicates that the extract may be able to reduce the frequency of stooling in diarrheal conditions. All these findings strongly suggested that *V. Pyramidata* ethanolic root extract should have anti-diarrheal activity and as per our best of knowledge which was never been explored before. On the other hand, in acetic acid induced writhing test the *V. Pyramidata* ethanolic root extract showed significant ($p < 0.05$) inhibition such as 31.88% and 40.95% at the dose of 250mg/kg & 500mg/kg respectively, Table 1. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels[26-27]. In hot plate method, the *V. Pyramidata* ethanolic root extract at a dose of 250mg/kg & 500mg/kg body weight showed significant anti-nociceptive activity.

Fig. 1: Shown the V*accaria Pyramidata* plant

Fig. 2: shown the V*accaria Pyramidata* root

Groups	**Treatments**
Group 1 (Control**)**	Received vehicle
Group 2 (Standard**)**	Received morphine dose of (50 mg/kg)
Group 3 (Test-1**)**	Received *V. Pyramidata* root extract dose of (250 mg/kg)
Group 4 (Test-2**)**	Received *V. Pyramidata* root extract dose of (500 mg/kg)

Table 1: Effect of *V. Pyramidata* ethanolic root extract on castor oil induced diarrhea in mice

S.No	Treatment	Dose (mg/kg)	No. of watery diarrhea	% Inhibition
1.	Control	-	22.33 ± 0.33	-
2.	Castor oil + Ethanolic. root extract ($test_1$)	10 ml/kg	15.21±0.44*	31.88
3.	Castor oil + Ethanolic. root extract ($test_2$)	20 ml/kg	13.18 ± 0.35*	40.95
4.	Loperamide	5 mg/kg	5.6 ± 0.26*	74.92

Values are mean ± SEM (n = 6) *P < 0.05, the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard.

Table 2: Analgesic activity of *V. Pyramidata* ethanolic root extract in mice by using Hot Plate model

Groups	Drugs	Dose mg/kg	Reaction time in seconds				
			0 min	30 min	60 min	90 min	120 min
I	Control	-	3.74±0.24	3.86±0.33	4.04±0.19	4.05±0.24	3.76±0.28
II	Morphine	5	3.62±0.21	7.12±0.23*	10.15±0.18*	11.11±0.17*	9.22±0.17*
III	Morphine + Ethanolic. root extract ($Test_1$)	10	3.54±0.23	4.87±0.34*	5.04±0.27*	5.66±0.25*	5.14±0.26*
IV	Morphine + Ethanolic. root extract ($Test_2$)	20	3.80±0.11	5.94±0.12*	6.36±0.15*	6.58±0.14*	6.40±0.14*

All values in terms of Mean ± SEM, (n=5) in each group. *P <0.05 statistically highly significant as compared with control group.

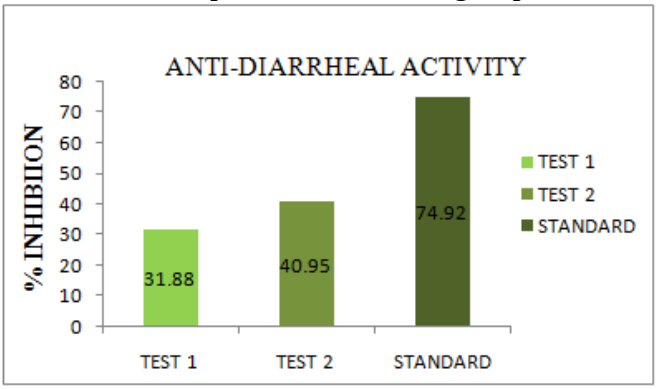

Fig 3: Graph representing percentage inhibition curve in various groups on castor oil induced diarrhea

Table 3: Analgesic activity of *V. Pyramidata* ethanolic root extract in mice by using Tail immersion model

Groups	Drugs	Dose mg/kg	Reaction time in seconds				
			0 min	30 min	60 min	90 min	120 min
I	Control	-	3.78±0.14	3.96±0.33	4.04±0.19	4.05±0.24	3.76±0.28
II	Morphine	5	4.99±0.27	8.12±0.23*	10.25±0.18*	12.11±0.17*	11.22±0.17*
III	Morphine + Ethanolic. root extract ($Test_1$)	10	3.54±0.23	4.87±0.34*	5.14±0.27*	6.67±0.25*	5.45±0.26*
IV	Morphine + Ethanolic. root extract ($Test_2$)	20	3.89±0.11	4.94±0.12*	6.86±0.15*	7.88±0.18*	6.40±0.15*

All values in terms of Mean ± SEM, (n=5) in each group. *P <0.05 statistically highly significant as compared with control group.

Table 4: Analgesic activity of *V. Pyramidata* ethanolic root extract in mice by using Tail-Flick model

Groups	**Drugs**	**Dose mg/kg**	**Basal reaction time in seconds**				
			0 min	**30 min**	**60 min**	**90 min**	**120 min**
I	Control	-	2.78±0.14	2.96±0.33	4.04±0.14	4.10±0.34	3.76±0.27
II	Morphine	5	4.66±0.27	8.80±0.23*	9.25±0.18*	9.95±0.17*	9.22±0.17*
III	Morphine + Ethanolic. root extract ($Test_1$)	10	3.50±0.23	4.77±0.34*	5.14±0.27*	5.67±0.25*	5.39±0.25*
IV	Morphine + Ethanolic. root extract ($Test_2$)	20	3.89±0.14	4.94±0.16*	6.36±0.15*	6.88±0.18*	5.40±0.15*

All values in terms of Mean ± SEM, n=5 in each group. *P <0.05 statistically highly significant as compared with control group.

Table 5 Effects of *V. Pyramidata* ethanolic root extract on carrageenan induced paw volume

Paw edema volume (cm) measured						
Mean± SEM						
Groups	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control	1.205± .004	1.816± .026	2.231± .010	2.210± .007	2.523± .010	2.453± .016
Standard 5 mg/kg	1.055±. 014	1.196±. 014***	1.320± .024***	1.40± .022***	1.34± .023***	1.28± .020***
Test 1 Ethanolic root extract 250(mg/kg)	1.153± .003*	1.391± .011*	1.476± .016*	1.883± .044*	1.861± .039*	1.595± .037*
Test 2 Ethanolic root extract 500(mg/kg)	1.121± .009*	1.420± .022*	1.548± .035*	1.945± .029*	1.906± .020*	1.748± .022*

All valves were shown as mean ± SEM n=6 by one way ANOVA, *P<0.05, **P<0.01, ***P<0.001 vs control.

Table 6: % Inhibition of edema by *V. Pyramidata* ethanolic root extract at deferent time intervals

Percentage (%) Inhibition					
Groups	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Standard 5 mg/kg	34.14 %	35.00 %	36.65 %	44.69 %	47.81 %
Test 1 Ethanolic. root extract 250(mg/kg)	23.84 %	27.32 %	14.79 %	23.19 %	34.97 %
Test 2 Ethanolic. root extract 500(mg/kg)	21.8 %	23.78 %	11.99 %	21.33 %	28.74 %

The results were found to be statistically significant table 2. In tail immersion method, and tail- flick method the extend of activity shown by the ethanolic root extract are less than that of the standard drug morphine but many folds more than that of the control group, which justifies its activity. The results were found to be statistically significant, tables 2-3. This tail immersion method and carrageenan induced paw edema was used to evaluate the central mechanism of analgesic activity. The results were found to be statistically significant, tables 5-6. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. This ethanolic root extract inhibited both Narcotic analgesics inhibit both peripheral and central mechanism of pain.[28,29] Above observations suggest that the extract in graded doses reduce diarrhea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enter pooling and inhibit both peripheral and central mechanism of pain. Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, flavonoids, sterol and/or triterpenes and anti-nociceptive properties of medicinal plants due to alkaloid, flavonoids, steroids, glycoside etc. Hence, tannins, saponine, alkaloids, steroids and glycoside may be responsible for the mechanism of action of *V. Pyramidata* ethanolic root extract against diarrheal and nociception.

CONCLUSION

From the above investigation it is quite apparent that a *V. Pyramidata* ethanolic root extract possesses the analgesic effect against different stimuli in small animals. This is evidenced by a significant increase in the reaction time by stimuli in different experimental models. And also Along with wide range of traditional uses such as treating diarrhea, as a tonic etc., In present studies, *V. Pyramidata* ethanolic root extract showed dose dependent anti-diarrheal activity at the dose of 250mg/kg and the inhibition rate was found at 31.88% ; whether at the dose of 500 mg/kg, the inhibition rate increased up to 40.95%. It also depicted significant success rate ($P<0.05$). From the above point of view, it can be concluded that further investigation of *V. Pyramidata* ethanolic root extract will help to develop noble anti-diarrheal drug and pain killer based on natural resources.

Conflict of Interest: Authors have declared that no competing interests exist.

Acknowledgement: I would like to thank entire department of Pharmacy for their support and cooperation during the research.

REFERENCES

1. Fields HL, Martin JB. Pain: Path physiology and Management. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL (eds). Harrison's Principles of internal medicine, 17th ed. New York, McGraw Hill, 2008: 81-86.
2. Mate, G.S., N.S. Naikwade, C.S.A.A. Chowki and S.B. Patil, Evaluation of anti-nociceptive activity of *Cissus quadrangular*ison in albino mice. Int. J. Green Pharm., 2008; 2: 118-121.
3. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S.. Antinocioceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J Ethnopharmacol 1998; 61: 229–235.
4. Coker MF, Berky S, Pandou C. New development in acute diarrhoea current problem. Paediatrics 1998; 24: 15-17.
5. Park K. Park's Text book of Peventive and Social Medicine. Jabalpur: Banarsidas Bharat publishers 2000; 122-175.
6. Victora CG, Huttly SR, Fuchs SC, et al. International differences in clinical patterns of diarrheal deaths: a comparison of children from Brazil, Senegal, Bangladesh, and India. J Diarrh Dis Res 1993; 11: 25-9.
7. Syder JD, Merson MH. The magnitude of the global problem of acute diarrheoal disease. A review of surveillance data. Bull World Health Organ 1982; 60: 605-13.
8. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, 5th ed. London: Churchill Livingstone. 2003; 375-378.
9. Rashid MH, Gafur MA, Sadik GM, Rahman MAA. Biological activities of

a new acrylamide derivative from *Ipomoea turpithum*. Pak J Biol Sci 2002; 5: 968- 969.
10. Mohan H.; The liver, biliary tract and exocrine pancreas in Text book of pathology; Jaypee Brothers Medical Publishers (P) Ltd; 2002; 22(4): 569-630.
11. Awouters F, Niemegeers CJE, Lenaerts FM, Janseen PAJ. Delay of castor oil diarrhea in rats; a new way to evaluate inhibitors of prostaglandin biosynthesis 1998; 30:41 -45.
12. Eddy NB, Liembach D. Synthetic analgesics II: Dithienylbuttenyl and dithiennylbulyl-amines. 1957; 107: 385–393.
13. S.K Kulkarni, "Handbook of experimental pharmacology" published by Vallabh Prakashan, 12 edition 2012, page no.139-141
14. Toma W, Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. J Ethnopharmacol 2003; 85: 19- 23.
15. S.K Kulkarni, "Handbook of experimental pharmacology" published by Vallabh Prakashan, 12 edition 2012, page no. 137-139
16. Vetriselvan, Subasini, Shankar Jothi"anti-inflammatory activity of cucumis sativus seed in carrageenan and xylene induced edema *model using albino Wistar rats"* International Journal of Biopharmaceutics, (2013) vol: 4(1); pp:- 34-37.
17. Neha Mohan, Suganthi, Gowri. "Evaluation of anti-inflammatory activity in ethanolic extract of Coriandrum sativum L. Using carrageenan induced paw oedema in albino rats" Scholars Research Library, (2013), vol:5(2); pp:-139-143
18. Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil-induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. 1994; 113:1127-1130.
19. Pinto A, Autore G, Mascolo N, Sorrentino R, Biondi A, Izzo AA, Capasso F. Time course of PAF formation by gastrointestinal tissue in rats after castor oil challenge. 1992; 44: 224-226.
20. Mascolo, Relationship between nitric oxide and platelet activating factor in castor oil-induced mucosal injury in the rat duodenum. Naunyn-Schmiedeberg's Arch Pharmacol 1996; 353: 680-684.
21. Racusen LC, Binder HJ. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. J Clin Invest 1979; 63: 743-749.
22. Vieira C, Effect of ricinoleic acid in acute and subchronic experimental models of inflammation. Med Inflam 2000; 9: 223-228.
23. Shah S. Evaluation of diarrhea: the challenge continues! Part-1. Indian J Med Sci 2004; 58: 75-78. 35.
24. Chitme HR, Chandra R, Kaushik S. Studies on antidiarrheal activity of *Calotropis gigantean* in experimental animals. J Pharmacol Pharm Sci 2004; 7: 70-75.
25. Hossain MM, Ali MS, Saha A, Alimuzzaman M. Antinocioceptive activity of whole plant extracts of *Paederia foetida*. 2006; 5: 67-69.
26. Ronaldo AR, Mariana LV, Sara MT, Adriana BPP, Steve P, Ferreira SH, Fernando QC. Involvement of resident macrophages and mastcells in the writhing nociceptive response induced by zymosan and acetic acid in mice. 2000; 387: 111-118.
27. Voilley N. Acid-Sensing Ion Channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-inflammatory Drugs (NSAIDs). Cur Drug Targ Inflam Allergy 2004; 3: 71-79.
28. Elisabetsky. Analgesic activity of *Psychotria colorata* (Wild. ex R. and S.) Muell. Arg. alkaloids. 1995; 48: 77-83.
29. Pal, Neuropsycho pharmacological profile of the methanolic fraction of *Bryophyllum pinnatum* leaf extract. 1999; 51: 313-518.