Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(4): 341-344

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Anti-flatulent Studies of Traditional Medicinal Plant *Vitex negundo* Linn. In Rats

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ABSTRACT

Vitex negundo is a shrub from Verbenaceae family and is traditionally used in treatment of various disease and disorders. Oil prepared with the juice of leaves of *Vitex-negundo* is reported to have very useful medicinal properties like wonderful cures of sloughing wounds and ulcers. In Ayurveda; roots of *Vitex negundo* is reported to have anti-flatulent properties. Philippines peoples used to make tea from fruits of this plant, which was considered very useful in relieving stomach gas which, we refer to flatulence. Here we studied the anti flatulent activity of different essential oils and extracts of *Vitex negundo* Linn. The standard drug used was simethicone (10 mg/10 g of flatulent diet, *p.o.*), which inhibited gas production up to 90 % as compared to control. Addition of test drugs (essential oils/ethanolic extracts) to the chickpea diet (5 %) decreased the amount of gas production significantly up to 69% by root and leaves extracts while dry fruit oil inhibited gas formation to 81%. The anti-flatulent activity in this plant may due to combined effect of flavonoids and triterpenoids constituents. As the safety evaluation study indicates that *Vitex negundo* is well tolerated at very high doses without any toxic effects. Thus, *Vitex negundo* has a high potential for the development of modern medicine for the treatment of various diseases.

Keywords: Antiflatulent, essential oil, extract, *Vitex negundo*.

INTRODUCTION

Flatus is a physical condition of gastrointestinal tract with a large amount of gases like carbon dioxide, hydrogen gas and methane along with small amount of the inflammable gases like hydrogen sulfide. The main causes of flatulence may be intestinal infections, excess of certain enzymes, or certain foods like legumes containing certain oligosaccharides especially raffinose, stachyose and verbascose are main contributors to the flatulence problem. [1-2]

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Received: 03 September, 2014; **Accepted:** 06 September, 2014

Vitex negundo is a large aromatic shrub from the family Verbanaceae, found throughout India mainly at warmer zones and ascending to an altitude of 1500 m in the outer western Himalayas. It is widely used in the indigenous system of medicine for its many medicinal properties. [3-4] Dried leaves when smoked are said to relieve catarrh and headache. Its oil is used as bathing oil, for rubbing on the head and in cervical lymphadenitis. Also the oil is found to be useful for sloughing wounds and ulcers. It has been used in bloating of stomach, entrails and bowels. The folks of India have been using various parts of this plant as anti-flatulent in treatment of flatus/botulism. Seeds are used in bloating of stomach, entrails and are taken as a tea to remove wind, which we refer to flatulence or gas. [5-6] Philippines peoples used to make tea from fruits of

this plant, which was considered very useful in relieving stomach gas and mental stress. [7-8] In Ayurveda root is reported to have anti-flatulent properties. The tribal of Northern India have been using various parts of this plant as anti-flatulent. One of the ancient uses of *V. negundo* documented in Ayurveda is to provide mental peace. [5]

The main constituents reported in the leaves and fruits of *Vitex negundo* are pentacyclic triterpenoids, iridoids and flavonoids. These constituents are reported to have anti-microbial, anti-inflammatory, and antioxidant activity. As the safety evaluation study indicates that it is well tolerated at very high doses without any toxic effects. [9-10] Thus, *Vitex negundo* has a high potential for the development of modern medicine for the treatment of various diseases. Against this background information, an effort was made to study anti-flatulence activity of essential oils and some other ethanolic extracts.

Table 1: Essential oils collected with percentage yield

Essential oils	Code	% Yield
Essential oil of fresh leaves	LO	0.06
Essential oil of fresh flowers	FLO	0.08
Essential oil of fresh green fruits	GFO	0.05
Essential oil of dry ripe fruits	DFO	0.05

Table 2: Ethanolic extracts collected with percentage yield

Name of extracts	Code	% Yield
Ethanolic extract of leaves	EEL	11.5
Ethanolic extract of flowers	EEFL	12.5
Ethanolic extract of fruits	EEF	13.0
Ethanolic extract of root	EER	13.0

MATERIAL AND METHODS

Plant Material

The different parts of *V. negundo* were collected in the months of October to December, from local areas of Kurukshetra, Haryana (India). The plant parts got identified and authenticated by FRI, Dehradun and a voucher specimen of the sample (Sr. No. 160/Flora of Haryana) has deposited in the NWFP Herbarium collection at Forest Research Institute and College, Dehradun, India. The same was also identified and authenticated by Department of Botany, Kurukshetra University, Kurukshetra.

Isolation of essential oils

The plant parts used for isolation of essential oils were fresh leaves, fresh flowers, fresh green fruits and dry ripe fruits. The freshly collected plant parts were cleaned from unwanted material and then washed with water to remove dust. Each plant part in sufficient amount was subjected to hydro distillation by putting with water in closed type Clevenger apparatus specially designed for isolation of essential oils lighter than water. The ratio of plant part and water was kept in the ratio of 1:3, so that maximum yield can be obtained. All necessary precautions were taken care to prevent any loses of oil. After refluxing 4 - 5 hours with water, a yellowish oil started to separate on the upper layer of water in Clevenger apparatus. Assembly was run for further 4-5 hours until the amount of oil in the upper layer became constant. The oil so obtained was isolated from the distillate (water) with the help of hexane and was dried over anhydrous magnesium sulfate. Percentage yield of oils were calculated (Table 1) and oils were stored in sealed glass bottles in a refrigerator (-10°C) for analysis. Before subjecting to hydro distillation, the leaves were treated with a 10% Sodium Chloride solution for 3 hours and then washed twice with fresh water.

Extraction of ethanolic extracts

The dried and coarsely powdered leaves, flower, fruit and root were packed in Soxhlet apparatus separately. A sufficient volume of solvent was added to the boiler and hot continuous extraction process in a Soxhlet extractor was started. The hot continuous extraction process was continued for about 48 hours or until extracting solvent (e.g. ethanol) coming down the siphoning tube became colorless. The excess of solvent was distilled under reduced pressure using rotatory vaccum evaporator (Heidolph Laborota 4011, digital) at temperature below 40±1°C. A crude mass (extracts) was recovered from flask and concentrated at room temperature and percentage yield was calculated (Table 2). The extracts were used for pharmacological studies by suspending a weighed amount of the extract in normal saline (95 ml): tween 80 (5 ml) ratio.

Animals

Albino rats (Wistar strain) of either sex, weighing (190-225 g) are used for anti-flatulent studies. The animals were procured from CCHAU, Hisar, Haryana. The clearance for the use of animals for experimental purpose was obtained from Institutional Ethical Committee constituted for the purpose. Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory condition with control environment of temperature 25 ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle (16.00–18.00) as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. They were provided standard rodent chow/feed and water ad-libitum. Before subjecting them to experimentation, the animals were given a week time to get acclimatized with laboratory conditions. The animals were fasted for 12 hrs before the experiment and deprived of water to ensure uniform hydration and to minimize variability in edematous response.

Drugs

All test and standard drugs were administered orally and dose selection of test drug was based on previously reported studies. Simethicone, 10 mg/10 g of flatulent diet, *p.o.* was used as standard.

Feeding regimen contained two types of diets:

Basal semi synthetic diet had the following percentage composition:

Ingredients	%	Ingredients	%
Casein	15	Refined groundnut oil	10

Vitamin mixture	01	Mineral mixture	02
Corn starch	72		

Flatulent diet had the following percentage composition:

Ingredients	%	Ingredients	%
Dehusked chickpea flour	87	Refined groundnut oil	10
Vitamin mixture	01	Mineral mixture	02

Procedure for anti-flatulent activity

Animals were divided into seven groups (I-VII) consisting of six animals per group. The animals were housed in individual cages. Initially all the animals were fed a basal semi synthetic diet. In the first 10 days duration, all animals were trained to consume 10 g diet in an hour time. On the 11th day group (I) was fed the basal semi synthetic diet and served as negative control while the remaining experimental groups (II-VII) received chickpea highly flatulent diet. Test drugs (essential oils / extracts) at 5 % levels were incorporated into flatulent diet of III-VI of the groups receiving chickpea flour and group (II) served as positive control. The group VII received standard anti-flatulent drug (Simethicone) 10 mg/10 g of flatulent diet, *p.o.* The diet cups were withdrawn from the cages after an hour of giving diet. Four hours later the animals were anaesthetized, and the volume of gas in the intestinal tract was determined as described by Hedin and Adachi. [11-14]

Sacrificing procedure and gas sample collection

Four hour after removal of the diet from the cages, animals were sacrificed and the gastrointestinal tract exposed by a midline incision. Evident pockets of gases were then removed by a commercial gas-tight syringe and the volume was measured. For the experimental uniformity, the gases collected from the cecum were assumed to represent an average of all gases in the intestinal tract. [14]

Table 3: Anti-flatulent activity of essential oils

Group (n=08)	Treatment (10 g *p.o*)	Volume of gas collected (ml)	% inhibition in gas production
I.	Basal semi-synthetic diet (-ve Control)	0.05±0.01	---
II.	Diets with dehusked chickpea flour (+ve Control)	0.16±0.02	---
III.	Do + LO	0.04±0.07***	75
IV.	Do + FLO	0.06±0.07**	62
V.	Do + GFO	0.05±0.06**	69
VI.	Do + DFO	0.03±0.04***	81
VII.	Do + Simethicone (Standard)	0.01±0.02***	>90

Note: Value expressed as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 compared with control (one way ANOVA followed by Dunnett's't' test)

Do-, LO- Essential oil of fresh leaves, FLO- Essential oil of fresh flowers, GFO- Essential oil of fresh green fruits and DFO- Essential oil of dry ripe fruits

RESULTS

As shown in Table 3, the gas formed in the intestine of group (II) of rats i.e. positive control which fed chickpea diet (flatulent diet) was maximum 0.16 ml while its negative control counterpart had only 0.05 ml gas formed. All the test and standard groups (III-VII) decreased the gas production. Almost all essential oils inhibited the gas production induced by flatulent diet to a significant level (p<0.001) while ethanolic extracts exhibited moderate inhibition (p<0.01). Dry fruit oil showed maximum inhibition 81% while leaves oil showed 75 % inhibition. Other two oils and ethanolic extracts showed inhibition in gas production between 60 to 70 % only as presented in Table 4. The standard drug simethicone inhibited gas production up to 90 %.

Table 4: Anti-flatulent activity of ethanolic extracts

Group (n=08)	Treatment (10 g *p.o*)	Volume of gas collected (ml)	% inhibition in gas production
I.	Basal semi-synthetic diet (-ve Control)	0.05±0.01	---
II.	Diets with dehusked chickpea flour (+ve Control)	0.16±0.02	---
III.	Do + EEL	0.05±0.04***	69
IV.	Do + EEFL	0.06±0.08**	62
V.	Do + EEF	0.06±0.06**	62
VI.	Do + EER	0.05±0.04***	69
VII.	Do + Simethicone (Standard)	0.01±0.02***	>90

Note: Value expressed as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 compared with control (one way ANOVA followed by Dunnett's't' test)

Do-, EEL- Ethanolic extract of leaves, EEFL- Ethanolic extract of flower, EEF- Ethanolic extract of fruit, EER- Ethanolic extract of root

DISCUSSION

The main classes of constituents which have been discovered so far in leaves of *V. negundo* are flavonoids and pentacyclic triterpenoids. Fruits contain triterpenoids - 3β-Acetoxyolean-12-en-27-oic acid and its derivatives. Root contains triterpenoids and flavonoids like vitexin and isovitexin. The essential oils are mainly consisting of mono and sesqui-terpenoids. Pentacyclic triterpenoids, Ursolic acid and betulinic acid, isolated from seeds extract were found to be effective against *Bacillus subtilis* and *E. coli* and oleanoic acid derivatives are found to have anti-microbial and anti-inflammatory activity. [7-10]

Almost all essential oils inhibited the gas production induced by flatulent diet to a significant level (p<0.001) may be due to anti-microbial activity of terpenoids. Ethanolic extracts exhibited moderate inhibitions are reported to have pentacyclic teriterpenoids. We know flatus is mainly due to fermentation and microbial action so anti-flatulent activity reported in *Vitex negundo* may be due to the combined antimicrobial effects of triterpenoids and flavonoids or due to any one class of constituents, which needs further investigation.

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Source of Support: Nil, Conflict of Interest: None declared.