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SCREENING OF GASTROPROTECTIVE ACTION OF DIOSPYROS VIRGINIANA BERRY EXTRACTS AGAINST PYLORIC LIGATION PROCESS IN WISTAR ALBINORATS

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ABSTRACT

Medicinal plants are the background for the traditional system of medicine throughout the world. They play a vital role in curing various ailments in the form of medicines. The objective of present study is to screen the gastroprotective action of *Diospyros virginiana* berry extracts. The motivation behind this study was to highlight the importance of traditional usage of the *Diospyros virginiana.* Phytochemical screening of the berry extracts showed the presence of various phytoconstituents responsible for pharmacological actions. To validate the traditional use with scientific evidence this study has been conducted.

KEYWORDS: Diospyros virginiana, gastro protective action, chloroform extract, flavonoids.

INTRODUCTION

The use of natural medicine is a very tradition particularly among the people of India, China, Egypt, and Brazil.[1] The traditional medicinal system includes Ayurveda, Siddha, Homeopathy, and Unani which are based on the medicinal plants.[2] Diospyros Virginian range includes the eastern part of India belongs to Ebenaceae family.[3] The Diospyros virginiana is of great practical interest for fruit growing, since ancient times it was used in folk medicine, it is also called as persimmon fruit consisting of high nutritional value because of strong antioxidant capacity induced by a high content of flavonoids, vitamin-c, beta-carotene.[4] The fruits of persimmon are excellent dietary products that are used in fresh condition and from them pastes, jams, syrups, marinades were prepared. Fruits were used to make wine, brandy, white wine, and beer, additionally; these are used in animal nutrition as a source of bioactive compounds there by improve the performance of farm animals.[5] Medicinally Diospyros species are used as anthelmintic, anti-inflammatory, antibacterial, antifungal, antioxidant, molluscidal and termite resistant activities.[6] But there is no scientific evidence available hence with this objective we aimed to screen gastro protective action of Diospyros virginiana berry extracts.

MATERIAL AND METHODOLOGY

Plant materials: *D.Virginana* berries were collected in the months of October 2016 from the market of Missouri and were authenticated by Prof D. Ramakanth Raju retired botanist Acharya Nagarjuna University, a voucher specimen (Snvl/jntu/2017-05) has been deposited in the Viswabharati college of pharmacy, Guntur, A.P.

Preparation of plant extracts: Obtained plant material has been dried under shade and made into coarse powder passed though sieve# 20 and has been successively soxhelated using solvents like petroleum ether, chloroform, and ethanol for 72 hours. Obtained extracts were made solvent free using rota evaporator and stored in a vacuum desiccators. Yield was found to be 0.4%, 2.5% and 3.72% respectively. Obtained extracts were tested for preliminary phytochemical screening.[7] Oral suspensions of the extracts were prepared at a dose of 200mg/ml and 100 mg/ml using 5% aqueous gum acacia.[8]

Acute toxicity studies:[9] Adult Swiss albino mice 20-25g were taken for acute toxicity tests. The mice were divided into control and tests groups containing 6 animals each. The control group received vehicle (5% of normal saline) test group received graded doses of extracts. Animals observed carefully up to 4 hours then occasionally up to 48 hours for a sign of any behavioral changes and motility and LD50 values were calculated.

Determination of Gastro protective activity:[10] The experimental protocol was approved by the institutional animal ethical committee (Reg.No.1963/po/Re/s/17/CPCSEA).

Selection of Animals: Wister albino rats about 150-200g were chosen for study. The animals were fed with a

balanced diet and tap water adlibitum. The animals were maintained at room temperature and 40-70% RH with 12 hrs light/12 h dark cycle. They were allowed free access to a standard dry pellet diet. The food was withdrawn 18h before the experiment but allowed free access to water.

Drug protocol: Animals were divided into eight groups; each group consists of six animals.

- Group I: served as diseases control group subjected to pyloric ligation for induction of ulcer, received acacia suspension at a dose of 1mg/kg b.w, p.o
- Group II: Received standard ranitidine 50 mg/kg
- Group III: Received the petroleum ether extract of 100mg/kg b.w
- Group IV: Received the petroleum ether extract of 200mg/kg b.w
- Group V: Received the chloroform extract of 100mg/kg b.w
- Group VI: Received the chloroform extract of 200 mg/kg b.w
- Group VII: Received the ethanolic extract of 100mg/kg b.w
- Group VIII: Received the ethanolic extract of 200mg/kg b.w

All the groups received the respective doses twice daily for 5 days.

Pyloric ligation method[11]

On the 5th day after 45 mins of extracts and ranitidine treatment, pyloric ligation was done by ligating the pyloric end of the stomach of rats of respective groups under light ether anesthesia at a dose of 35mg/kg b.w. Ligation was done without causing any damage to the blood supply to the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during the postoperative period. After 4 hours of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretions studies were determined by titration with 0.01N NaOH using phenolphthalein as indicator.

Using the formula

$$\text{Acidity} = \frac{\text{Vol of NaoH x Normality of NaoH x 100mEq/L}}{0.1}$$

The pH of gastric juice was measured by using PH paper strips of varying ranges. The color of PH paper after the procedure was matched with the standard scale and pH was determined.

Scoring of ulcer was made as follows:

Normal stomach----0
Red coloration------ 0.5
Spot ulcer ----------1
Hemorrhagic streak----1.5
Ulcers ---------- 2
Perforation --------- 3

Mean ulcer score of each animal was determined by ulcer index.

The percentage ulcer protection was determined by using the formula[12]

$$\% \text{ protection} = \frac{\text{Control mean ulcer index—test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Values are represented as mean ± SEM for 6 rats.

RESULTS

Table I: Results of qualitative phytochemical evaluation of D.Virginiana berry extract.

S.no	Tests	*D.Virginiana*		
		P.E	C.E	E.E
1.	Alkaloids	-	+	-
2.	Aminoacids	+	+	+
3.	Carbohydrates	-	-	+
4.	Flavonoids	-	+	+
5.	Mucilage	-	+	+
6.	Proteins	-	-	-
7.	Starch	+	-	+
8.	Steroids & triterpenoids	+	-	-
9.	Glycosides	-	+	+

+= positive, - = Negative

Table: II: Results of D.Virginiana berry extracts on pyloric ligation model in Wister albino rats.

Groups	Dose	Gastric volume(ml/100g)	Free acidity (mEq/l)	Total acidity (mEq/l)	pH	Ulcerative index	% protection
I.CONTROL	Acacia- 1mg/kg	7.69±0.35	63.2±0.59	97.7±0.23	2.1±0.07	3.56±0.06	
II.STANDARD	Ranitidine - 50mg/kg	4.41±0.11	20.3±0.01	31.4±0.45	6.1±0.09	1.02±0.04	72
III.D.V.P.E-100	petroleum extract-100mg /kg	7.08±0.89	47.6±0.95	83.1±0.51	2.9±0.23	3.75±0.09	35
IV. D.V.P.E-200	petroleum extract-200 mg/kg	6.32±0.67*	38.5±0.86*	51.2±0.42*	3.7±0.04*	2.08±0.05*	40
V. D.V.C.E-100	chloroform extract-100mg/kg	4.71±0.11***	26.8±0.12***	44.4±0.05***	5.1±0.01***	1.25±0.21***	64
VI. D.V.C.E-200	chloroform extract-200mg/kg	4.12±0.12***	23.7±0.06***	34.3±0.02***	5.7±0.09***	1.06±0.35***	70
VII. D.V.E.E-100	Ethanolic extract-100mg/kg	5.61±0.54**	38.2±0.35**	34.2±0.26**	4.3±0.06**	1.54±0.21**	56
VIII D.V.E.E-200	Ethanolic extract - 200mg	5.01±0.64**	27.5±0.23**	42.1±0.15**	1.98±0.52**	4.9±0.64**	44

*** p<0.001, when compared with control Group,
**p<0.01, when compared with control Group,
* p<0.05, when compared with control Group

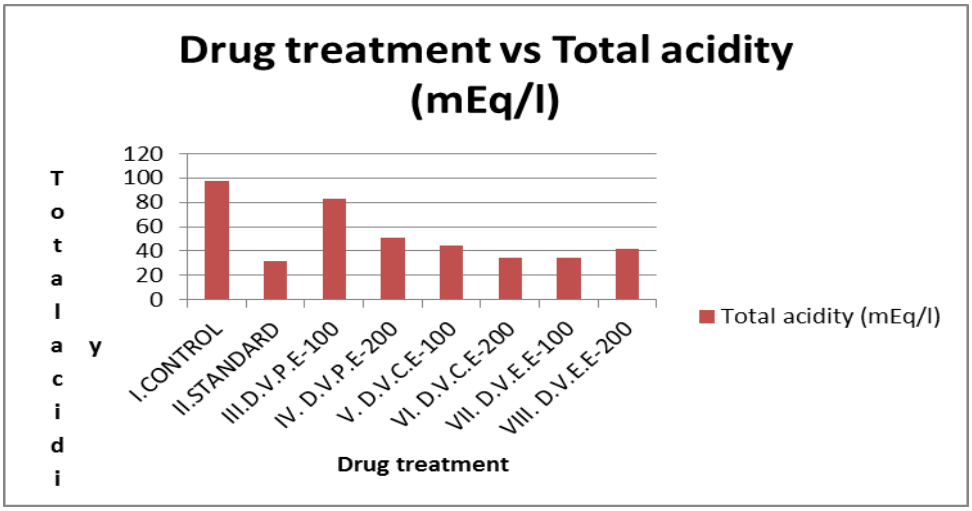

Figure: I: Effect of D.Virginiana berry extract on total acidity.

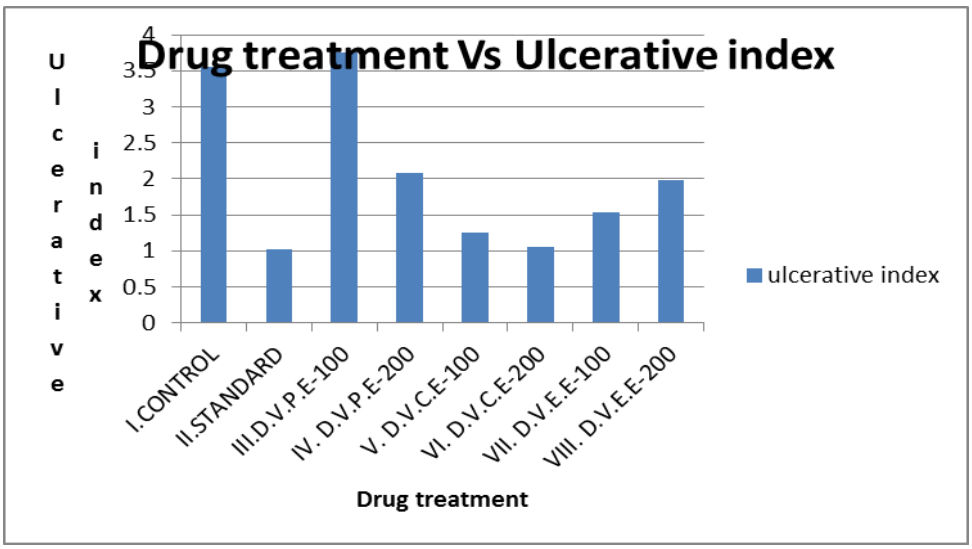

Figure II: Effect of D.Virginiana berry extract on ulcerative index.

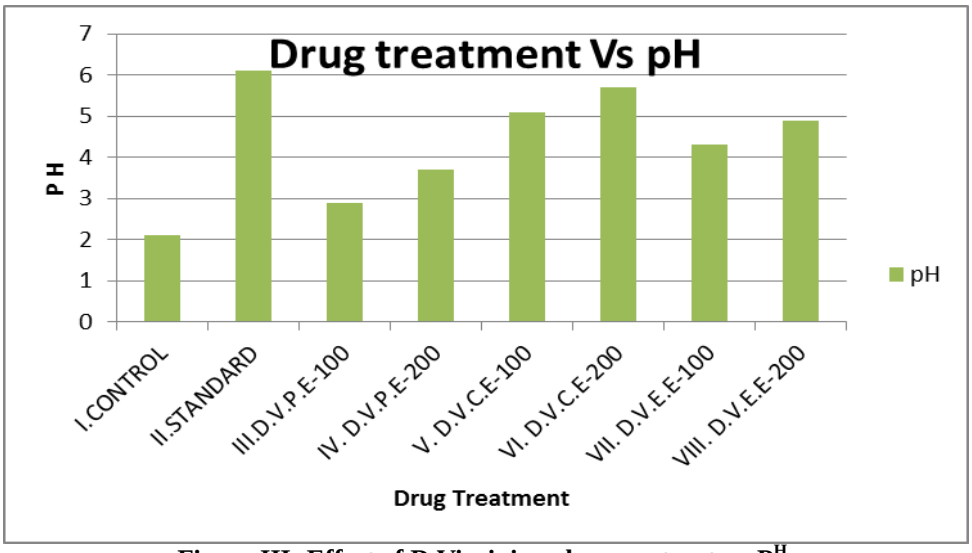

Figure III: Effect of D.Virginiana berry extract on P^H.

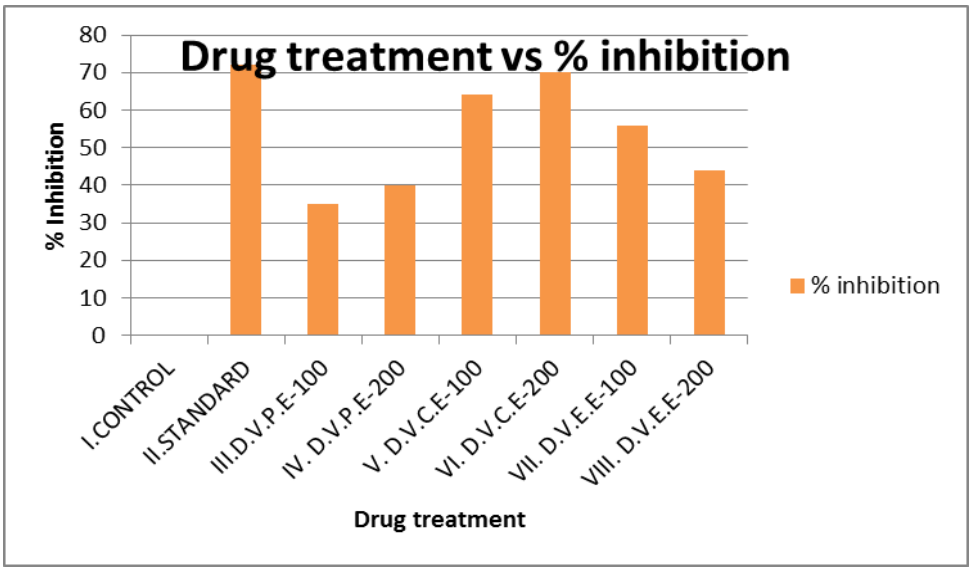

Figure IV: Effect of D.Virginiana berry extract on % inhibition.

From the results of acute toxicity studies done Swiss albino mice of either sex *D.Virginiana* berry extracts didn't show any behavioural changes, toxic reaction or mortality and found to be safe up to the dose of 2000mg/kg, LD50 was calculated >2000mg/kg $1/10^{th}$ of the LD50 is taken as therapeutic dose and $1/20^{th}$ of LD50 as graded dose.

The results of qualitative phytochemical tests given in (Table: I) reveal that petroleum ether extract contains amino acids, mucilage, steroids, and starch. Chloroform extract of *D.Virginiana* berry extract given positive to amino acids, flavonoids, mucilage, glycosides and alkaloid constituents and ethanolic berry extract showed positive to amino acid, carbohydrates, flavonoids, mucilage, starch and glycosides.

Effect of different D.Virginiana berry extract towards pyloric ligation induced ulcer model in Wister albino rats were given in Table: II. Pyloric ligation is an important procedure that shows the possible changes in parameters relative to the gastric content. The causes of gastric ulcer in pyloric ligation are believed to be due to increase in gastric hydrochloric acid secretion of acid, leading to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier.[13] These factors are associated with the development of upper gastro intestinal damage including lesions, ulcers and life threatening perforation and haemorrhages. Administration of *D.Virginiana* berry extracts in graded doses significantly decreased gastric secretions, total acidity, free acidity and increased PH of gastric juice when compared to control and exhibited dose dependent action. Results shown by Chloroform extract at a dose of 200mg/kg b.w is almost significant with that of standard drug and it was found that 70% protection against gastric ulcers. Moderate significant action was shown by ethanolic extract where as minimum action or least

action was shown by petroleum ether extract. Whereas no or less significant action was shown by petroleum ether extract.

CONCLUSION

From this work we conclude Berries of *D.Viriginiana* were found to be good gastro protective agents as well as nutrient supplements. Chloroform extract at a dose of 200mg/kg b.w has shown best protective action against ulcer induced by pyloric ligation it could be due to presence of flavonoids and alkaloids.[14] Hence folklore usage has been validated for D.Virginiana as antiulcer agent.

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