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Research Article

ANTIOXIDANT AND ANTIULCER ACTIVITIES OF PENTATROPIS CAPENSIS (L.F.) BULLOCK

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ABSTRACT

Pentatropis capensis (L.f.) Bullock has been used as traditional medicine in the treatment of ulcer by Siddha medical practioners. The present study was carried out to investigate the antioxidant and antiulcer effects of the ethanol extract from the aerial parts of *Pentatropis capensis* in *in vitro* and *in vivo* models. The *in vitro* antioxidant activity of the extract was measured using DPPH, Superoxide, Hydroxyl and Nitricoxide radicals. The antiulcer activity was evaluated using ethanol-induced ulcer model. The IC_{50} value for the free radical scavenging activity under *in vitro* conditions was found to be moderate when compared with the positive standards namely Butylated Hydroxy Toluene (BHT) and ascorbic acid. The *in vivo* studies exhibited significant antiulcer activity of the extract at the dose of 200 and 400 mg/kg p.o. The antiulcer effect of the higher dose of extract (400 mg/kg) was found to be equally good when compared with the standard drug Omeprazole. The present study proves the scientific validation of the traditional use of this plant against ulcer.

INTRODUCTION

Pentatropis capensis (L.f.) Bullock has been used as traditional medicine in the treatment of ulcer by Siddha medical practioners. The present study was carried out to investigate the antioxidant and antiulcer effects of the ethanol extract from the aerial parts of *Pentatropis capensis* in *in vitro* and *in vivo* models. The *in vitro* antioxidant activity of the extract was measured using DPPH, Superoxide, Hydroxyl and Nitricoxide radicals. The antiulcer activity was evaluated using ethanol-induced ulcer model. The IC_{50} value for the free radical scavenging activity under *in vitro* conditions was found to be moderate when compared with the positive standards namely Butylated Hydroxy Toluene (BHT) and ascorbic acid. The *in vivo* studies exhibited significant antiulcer activity of the extract at the dose of 200 and 400 mg/kg p.o. The antiulcer effect of the higher dose of extract (400 mg/kg) was found to be equally good when compared with the standard drug Omeprazole. The present study proves the scientific validation of the traditional use of this plant against ulcer.

Sample preparation

Coarse powder from the shade dried aerial plant parts of *Pentatropis capensis* (500 g) was exhaustively extracted using Soxhlet apparatus with absolute ethanol (78.5°C). The extract was dried (free of solvent) using a vacuum evaporator for condensation. The extract thus obtained was stored in refrigerator and used for *In vitro* antioxidant activities and pharmacological studies such as toxicity and anti-ulcer activity.

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Estimation of total phenolics, tannins and flavonoids

The total phenolic content and tannins of the extract was determined and calculated as g Tannic acid equivalent from the calibration curve (Siddhuraju and Manian, 2007). The total flavonoid content of sample extract was determined following a colorimetric method and values were expressed as g rutin equivalent (RE)/12q00g of extract (Zhishen *et al*., 1999).

In vitro Antioxidant studies

Free radical scavenging assays include DPPH• (Blios, 1958), Superoxide (Beauchamp and Fridovich, 1971), Hydroxyl (Klein *et al*., 1991), Nitric oxide (Sreejayan and Rao, 1997). Metal chelating activity (Dinis *et al*., 1994), Phosphomolybdenum reduction assay (Prieto *et al.,* 1999) and Ferric reducing antioxidant power (FRAP) assay (Pulide, 2000)

In vivo studies

Male Swiss albino mice, mass ranging from 20-30 g of male breed were used for toxicity studies and Albino rats 150-200 g of male breed were used for antioxidant activity and ethanol induced ulcer. The rats were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. All *in vivo* experiments were carried out as suggested by the Institutional Ethical Committee - CPCSEA (Reg. No. 722 / 02 / a / CPCSEA).

Acute toxicity

Acute oral toxicity studies were performed according to OECD 421 (Organization for Economic Co-operation and Development).

Antiulcer activity

Ethanol-induced gastric ulcer (Morimoto *et al.,* 1991)

Six groups of male Wistar rats (n = 6) were fasted over night prior to the start of the experiment but have free access to water *ad-libitum*. The first and the second groups received distilled water (10 ml/kg /day p.o.), while the third group was treated with Omeprazole (10 mg/kg/day p.o.) whereas fourth and fifth groups were administered with the ethanol extract of *Pentatropis capensis* (200 and 400 mg/kg /day p.o.). On day third ethanol (1ml/kg) was given as a single oral dose to the groups II - V to induce gastric ulcers, after 60 min of Omeprazole and *Pentatropis capensis* extracts treatments. After 1 h the animals were sacrificed with over dose of diethyl ether and each stomach was examined for ulcer index.

Ulcer index was determined by following the scoring method of Suzuki *et al*. (1998).

Score 1: maximal diameter of 1mm
Score 2: maximal diameter of 1-2 mm
Score 3: maximal diameter of 2-3mm
Score 4: maximal diameter of 3-4 mm
Score 5: maximal diameter of 4-5mm
Score 10: maximal diameter of 5 mm and above
Score 25: a perforated ulcer.
The sum of the length (mm) of all lesions for each stomach was used as the ulcer
index (UI), and the protection percentage was calculated from the following formula:
[(UI control - UI treated)/UI control] X100.

Biochemical estimations

Superoxide dismutase was determined by the method of (Das *et al*., 2000). Catalase was estimated by the method given by (Sinha, 1972). Glutathione peroxidase was determined by the method of Ellman, 1959. Glutathione S Transferase was estimated by the method of Habig *et al*., 1974. Lipid peroxidation was estimated by the method of Ohkawa *et al* (1979).

Statistical analysis

For *in vitro* antioxidant assays, the values are expressed as means of triplicate analysis of the samples (n=6) ± standard deviation (SD). For *in vivo* ulcer the values are expressed as mean (n=6) ± standard deviation (SD). Statistical significance of difference between groups was determined by one way analysis of variance (ANOVA) P values of <0.05 are considered significantly different.

RESULTS

The quantitative estimations of total phenolics, tannins and flavonoids from ethanol extract of *Pentatropis capensis* revealed that the extract has 32.44±2.49, 26.20±1.44and 11.28±0.05g/100g extract of total phenolics, tannins and flavonoids respectively (Table-1).

The ethanolic extract of *Pentatropis capensis* exhibited low free radical scavenging activity for DPPH (IC501562.5 mg/ml), superoxide (IC501250 mg/ml) nitric oxide (IC501063.83 mg/ml) and hydroxyl (IC50 943.39 mg/ml) radicals. Metal chelating activity (3.48±0.10 mg EDTA equivalent / extract) of ethanolic extract of *Pentatropis capensis* was moderate and it also recorded moderate values for phosphomolybedenum (189.87±3.75 mg ascorbic acid eq/gram extract) and FRAP (64.07±7.02 Mmol) assay (Table-2-6).

Table 1 Estimation of Total Phenolics, Tannins and Flavanoids content of Ethanolic extract of *Pentatropis capensis*

Sample	Total Phenolics mg TAE/g extract	Tannins mg TAE/g extract	Flavanoids mg RE/g extract
Ethanol extract	32.44±2.49	26.20±1.44	11.28±0.05

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

TAE -Tannic acid equivalent, RE - Rutin equivalent

Table 2 DPPH radical scavenging activities of *Pentatropis capensis*

Sample	Concentration(µg)	Percentage activity	IC_{50}
Ethanol extract	200	7.08±0.26	1562.5
	400	14.46±0.09	
	600	20.89±0.24	
	800	25.91±0.22	
	1000	31.79±0.29	
BHT	10	36.19±0.011	48.99
	50	50.12±0.02	
	100	69.01±0.01	
	150	82.52±0.05	
	200	96.63±0.02	

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Table 3 Superoxide radical scavenging activities of *Pentatropis capensis*

Sample	Concentration(µg)	Percentage activity	IC $_{50}$
Ethanol extract	200	8.39±0.28	1250
	400	16.05±0.47	
	600	24.01±0.47	
	800	33.03±0.35	
	1000	40.66±0.56	
Ascorbic acid	10	41.66±0.01	33.10
	50	59.14±0.06	
	100	65.07±0.05	
	150	82.93±0.02	
	200	96.02±0.02	

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Table 4 Nitric oxide radical scavenging activities of *Pentatropis capensis*

Sample	Concentration(µg)	Percentage activity	IC $_{50}$
Ethanol extract	200	10.06±0.23	1063.83
	400	20.20±0.20	
	600	28.70±0.62	
	800	37.82±0.51	
	1000	45.71±1.13	
BHT	10	34.85±0.01	63.39
	50	46.47±0.01	
	100	57.04±0.01	
	150	70.42±0.01	
	200	88.73±0.02	

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Table 5 Hydroxyl radical scavenging activities of *Pentatropis capensis*

Sample	Concentration(µg)	Percentage activity	IC $_{50}$
Ethanol extract	200	15.10±0.60	943.39
	400	25.24±1.18	
	600	33.04±0.77	
	800	42.20±0.44	
	1000	51.55±0.60	
Ascorbic acid	10	29.13±0.01	72.64
	50	44.09±0.04	
	100	61.81±0.02	
	150	74.01±0.01	
	200	83.07±0.01	

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Table 6 Metal chelating, Phosphomolybdenum and FRAP assay of ethanolic extract of *Pentatropis capensis*

Sample	Metal chelating mg EDTA/g extract	Phosphomolybdenum mg ascorbic acid eq/g extract	FRAP mmol (Fe(II)/mg extract
Ethanol extract	3.48±0.10	186.87±3.75	64.07±7.02

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Acute toxicity study

The acute toxicity study of the ethanolic extract of *Pentatropis capensis* was carried out in mice model. The oral administration of the ethanolic extract did not produce mortality in mice up to 10,000 mg/kg. A summary of the gross behavioral symptoms of toxicity observed before and after the acute oral administration of the ethanolic extract are presented in Table -7. All the animals were alert with normal grooming, touch response and there was no sign of passivity, stereotype and vocalization. Their motor activity and excretary signs were also normal. All the animals appeared uniformly healthy till the end of the study.

Table 7 Toxicological evaluation of ethanolic extract of Pentatropis capensis in Swiss albino mice

S.No.	Response	Animals Before treatment	After treatment
1.	Alertness	Normal	Normal
2.	Grooming	Absent	Absent
3.	Restlessness	Absent	Absent
4.	Touch response	Normal	Normal
5.	Torch response	Normal	Normal
6.	Pain response	Normal	Normal
7.	Tremors	Absent	Absent
8.	Convulsion	Absent	Absent
9.	Righting reflex	Normal	Normal
10.	Gripping strength	Normal	Normal
11.	Pinna reflux	Present	Present
12.	Corneal reflux	Present	Present
13.	Writhing	Absent	Absent
14.	Pupils	Normal	Normal
15.	Urination	Normal	Normal
16.	Salivation	Normal	Normal
17.	Skin color	Normal	Normal
18.	Lacrimation	Normal	Normal

Effect of Pentatropis capensis on the severity of gastric lesion in ulcer models

Gastric lesions were induced in rats by oral administration of ethanol (1ml/kg). Oral administration of ethanolic extract of *Pentatropis capensis* registered a significant dose-dependent decrease in the extent of gastric mucosal damage in ethanol induced ulcer models. In ethanol induced ulcer model, the protective effect of the extract at a dose level of 400 mg/kg (ulcer index 14.02 ± 0.03; 67.03% protection) was slightly lower than that of the standard drug Omeprazole (ulcer index 10.78 ± 10.78; 74.64% protection). Ethanol induced depletion of gastric wall mucus has been significantly (P<0.01) reduced by ethanol extract of *Pentatropis capensis* at the dose levels of 200 and 400 mg/kg body weight showing ulcer index 39.62% and 67.03% respectively (Table 8).

Table 8 Effect of ethanolic extract of *Pentatropis capensis* on ethanol induced ulcer in experimental rats

Groups	Ulcer spots	Percentage protection
Control	---	---
Induced	42.54 ± 3.91	---
Standard	10.78 ± 4.15	74.64
Low dose	25.68 ± 5.11	39.62
High dose	14.02 ± 0.03	67.03

Standard–Omeprazole (10mg/kg body weight)
Low Dose – 200mg/kg body weight
High Dose – 400mg/kg body weight

Effect of ethanolic extract of Pentatropis capensis on certain antioxidant markers in ethanol induced ulcer model

In order to determine the effect of the ethanolic extract on oxidative stress induced in ethanol induced ulcer model, the levels of lipid peroxidation and activities of antioxidant enzymes such as SOD, CAT, GPx and GST were measured in

gastric tissue. Ulcer induction in ethanol induced ulcer model was associated with marked reduction in the activities of SOD, CAT, GPx and GST and increase in the level of LPO. Treatment with the ethanolic extract of *Pentatropis capensis* (200 and 400mg/kg p.o.) produced a significant increase in the activities of antioxidant marker enzymes and concomitantly decreased the level of LPO (Table 9) (Plate-2). The recovery of all the above said biochemical parameters by treatment of the experimental animals with the ethanolic extract of *Pentatropis capensis* at the dose of 400 mg/kg was almost comparable to the activity shown by the standard drug Omeprazole (10 mg/kg).

Table 9 Effect of ethanolic extract of *Pentatropis capensis* on antioxidant markers (From stomach tissue homogenate in ethanol induced ulcerogenic rats)

Groups	SOD	CAT	GPx	GST	LPO
Control	2.16 ± 0.03	21.93 ± 1.08	31.00 ± 2.00	10.32 ± 0.17	1.45 ± 0.09
Induced	1.12 ± 0.14a**	10.86 ± 2.022a**	17.36 ± 1.67a**	5.03 ± 1.04a**	6.23 ± 0.90a**
Standard	2.07 ± 0.13b**	21.13 ± 2.17b**	29.10 ± 1.58b**	10.10 ± 0.58b**	1.70 ± 0.17b**
Low dose	1.72 ± 0.09b**	13.85 ± 0.44b**	22.11 ± 0.59b**	6.87 ± 0.58b**	3.07 ± 0.21b**
High dose	2.00 ± 0.05b**	20.78 ± 0.58b**	28.11 ± 1.14b**	9.67 ± 0.43b**	2.03 ± 0.14b**

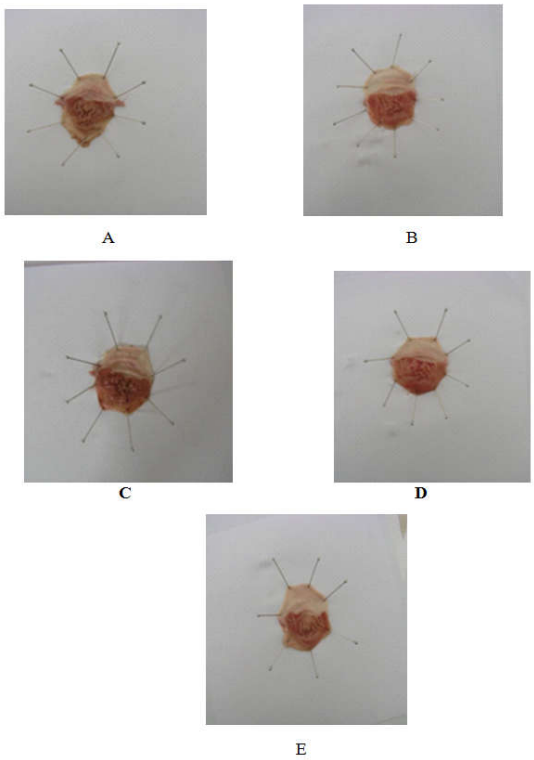
Values are mean ± SD of six samples in each group
Group Comparison : a - G1 vs G2; b - G2 vs G3, G4, G5
* - Significant at p<0.05 ** - Significant at p<0.01 ns – Not significant

Units

SOD-Superoxide dismutase (Units/min/mg protein)
CAT-Catalase (µ moles of H_2O_2 consumed/min/mg protein)
GPx-Glutathione peroxidase (µ moles of GSH oxidized/min/mg protein)
GST-Glutathione-S-transferase (µ moles of CDNB conjugation formed/min/mg protein)
LPO-µmoles/ mg protein **Standard**–Omeprazole (10mg/kg body weight)
Low Dose –200mg/kg body weight. **HighDose-**400mg/kgbodyweight.

Plate-2

Effects of Ethanolic Extract of *Pentatropis capensis* on Ethanol Induced Ulcer in Experimental rats



DISCUSSION

Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances. A fairly high percentage of useful plant derived drugs were discovered as a result of scientific follow-up of well known plants used in traditional medicine and it can be assumed that this is a good approach for discovering useful drugs from plants.

Pentatropis capensis have been used traditionally in the treatment of various diseases, most common usage is for the treatment of gastrointestinal disorders. For this reason, the antiulcer activity of the plant extract was evaluated using ethanol induced ulcer models.

Several hypotheses have been put forward to explain the mechanisms of ethanol induced ulcer. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and narcotic aspects of tissue injury (Nordin *et al.*, 2014). Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of sodium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Surendra., 1999; Shawon and Gautam, 2012). Ethanol produces severe gastric hemorrhagic lesions by increasing super oxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa. These and other reactive metabolites react with most of the cell components, changing their structures and functions, or contributing to other mechanisms that finally promote enhanced oxidative damage.

The phytoconstituents like flavonoids, tannins, terpenoids, and saponin have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants (Nwafor, 1996; Nwafor, 2000; Al-Rehailey *et al*., 2002; Berenguer, 2005 and Al-Batron *et al.*, 2013) Alkaloids and terpenoids prevent ulcers induced by stress. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models (Di carlo *et al*., 1999 and Zayachkivska *et al.,* 2005) by increasing the amount of neutral glycoproteins (Di carlo *et al*., 1999) and the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion
(Borrelli and Izzo, 2000).

Saponins, especially triterpenes have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect from acid effects by selectively inhibiting PGF2α (Agwu and Okunji, 1986 and Abdulla *et al*., 2010).

Phenols have a dual effect on prostaglandin biosynthesis, with low concentrations stimulating and high concentrations inhibiting PGHS (Alanko *et al*., 1999 and Roy *et al.,* 2013).

As cited above *Pentatropis capensis* also contains flavonoids, tannins, phenols and any of these phytochemicals could have imparted the antiulcer activity to *Pentatropis capensis*. The *in vitro* studies revealed that *Pentatropis capensis* is not an efficient antioxidant but *in vivo* studies have proved it to be an efficient anti-ulcer drug. Thus our studies did not show correlation between *in vitro* and *in vivo* studies. Similar results were observed by (Burguete, 2007)

This may be due to the fact that, ethanolic extract of *Pentatropis capensis* as such is not an efficient free radical scavenger under *in vitro* conditions, but after entering in to the complex biological system showed high antiulcerogenic efficacy by increasing the levels and subsequently the activities of the antioxidant enzymes such as SOD, CAT, GPx and GST which are involved the free radicals scavenging activity. Thus indirectly *Pentatropis capensis* becomes an efficient free radical scavenger in *In vivo* environment and thus act as an efficient anti-ulcer drug.

CONCLUSION

It was found that *Pentatropis* contains flavonoids, tannins and phenols and any of these phytochemical could have imparted the antiulcer activity to *Pentatropis capensis*. As for as antioxidant studies are concerned we found no correlation between *In vitro* and *In vivo* studies. *In vivo* studies have proved high anti-ulcerogenic efficacy by increasing the activity of antioxidant enzymes and thus involved in free radicals scavenging activity. Due to the remarkable non-toxic antiulcer activity of *Pentatropis capensis* further studies are needed.

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