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Research Article

EFFECT OF *PTEROSPERMUM ACERIFOLIUM* BARK EXTRACT ON OXIDATIVE DAMAGES IN THE GASTRIC TISSUE DURING ALCOHOL INDUCED ULCERATION.

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ABSTRACT:

The role of alcoholic fraction of Pterospermum *acerifolium* bark extract on oxidative damages in the gastric tissue during alcohol induced ulceration was investigated. The extract showed significant antiulcer activity against ethanol induced ulceration and as well as significant reduction of tissue lipid peroxidation, catalase, superoxide dismutase and glutathione were observed to occur with the extract.

Key words: Pterospermum acerifolium, Ulcer, Lipid Peroxidation, Gastric Enzyme.

INTRODUCTION

Pterospermum acerifalium wild (Sterculiaceae) commonly known as 'Kanak champa' is a shrubs distributed in tropical Asia. It has been traditionally used for blood troubles, inflammation, ulcer, tumors, leprosy and for small pox eruptions[1]. In an earlier study in our laboratory, the ethanolic extract of *P. acerifolium* were found to possess anti-inflammatory and analgesic activity. The work was aimed at the scientific validation of the ethnopharmacological claim about effect of bark extract on oxidative damages in the gastric tissue during alcohol induced ulceration. As ulcers are essentially due to imbalance between offensive and defensive factor[2,3], the effect of alcoholic extract of *P. acerifolium* on offensive factors like acid-pepsin. Secretion and defensive factors like mucin secretion, cellular mucus, cell shedding and cell proliferation were investigated to as certain the possible mechanism of action, Further the methanolic extract of *P. acerifolium* has been reported to posses significant antioxidant activity. Lipid per oxidation has been postulated to be one of the important factors of ulcerogenesis[4]. Hence gastric antioxidant effect of *P. acerifolium* was investigated as a possible defensive factor. The low molecular weight sterols from P. acerifolium, apart from other, have been proposed to the active constituents from isolation report.

Thus present study was undertaken to evaluate the effect of *Petrospermum acerifolium* on different oxidative damage in the gastric tissue during alcohol induced ulceration.

MATERIAL AND METHOD

Plants materials *P. acerifolium* bark were collected from East Midnapur (West Bengal, India) in July 2007 and authenticated by comparison with a voucher. Specimen in Botanical survey of India, Kolkata.

One kg. of the air dried barks were blended to a fine powder and extracted with Pet. Ether, chloroform and ethanol for 6 days (144 h). The extract was concentrated using a rotavapor. The extract was dissolved in normal saline before orally administrating 150 and 300 mg /kg of the extract to the rats.

Phytochemical screening

The extract and its fraction were tested by the liberman Burchard, Ferric chlorides, Magnesium tracings and Vanilin sulphuric

acid tested to determine the presence of sterols phenolic compound, flavonoids and saponins respectively.

Animals used

Adult charles foster rats (150-200g) housed at normal laboratory condition (24±2^{0}C) for at least 10 days were used for pharmacological experiment .The animals were given standard pellet diet and water ad libitum. All the experiments were performed as per guidelines of institutional animal ethics committee.

Statistical analysis

Results were expressed as mean ± S.E .The statistical evaluation were done by analysis of variance(ANOVA) coupled with dunet's test, $P<0.05$ was considered to be statistically significant.

Ethanol induced gastric ulceration

Groups of albino rats (n = 6) fasted for 24 h. allowing water and ad libitum were used for experiment. *P. acerifolium* bark extract (300 mg/kg) or control vehicle were administered orally. After 30 min following administratrion of test substance and the control vehicle, all the group of animals received ethanol (5 ml/kg, 50% v/v, oral). The animals were sacrificed 1 h after administration of ethanol[9]. The stomachs were removed, opened rinsed with cold saline and the length of lesions (mm) was measured under microscope.

Estimation of oxidative damage in the gastric tissue

After measuring the ulcer index the stomach were washed with 0.9% (w/v) NaCl were cut into small piecs and homogenised with a Potter Eluvehjem glass homogenizer in ice cold 0.15 M KCl solution to produce a 20% (w/v) homogenate. The homogenate was thereafter used for the determination of (i) TBARS as in index of lipid peroxidation estimation[5]. (ii) Estimation of reduced glutathione (GSH) using DTNB solution. (iii) Superoxide dimutase (SOD) activity based on autooxidation of pyrogallol. (iv) Catalase (CAT) activity from the decomposition of H_2O_2 as a marker for oxidative damage.

Lipid peroxidation

Rats liver homogenate (20%) were prepared in 0.15 M KCl and centrifuged at 800 rpm for 10 minute and the supernatant was used for in vitro lipid peroxidation assays according to underling method- 30 ml of final volume of reaction mixture contain 1 ml of liver homogenate and 2 ml of reaction mixture followed by heating at 100^{o}C for 10 min. Then the reaction mixture was centrifuged at 1000 rpm for 10 min and the absorbance of the supernatant was measured at 532 nm.

Assay of super oxide dismutase (SOD activity)

Superoxide dismutase activity in stomach tissue was determined according to the method followed by Fridovich (1995)[6]. Stomach tissue was scrapped and homogenised in ice cold normal saline medium with the help of homogenizer. Then the tissue homogenate was centrifuged to 10 minutes at 3000 rpm and the supernatant was collected and used for estimation of SOD activity. 10 ml of the solution was taken in a tube and mixed with 0.5 ml of 50 mM phosphate buffer (pH 7.8) and 0.1 mM of EDTA, 0.05 mM Xanthine, oxidase, 0.01 mM cytochrome C and then100 ml of 2.5 ml of xanthine oxidase is added to start the reaction and measure the absorbance at 550 nm.

Assay of catalase activity

Catalase activity in stomach tissue was determined according to the method followed by Leyek (1990)[7] stomach tissue was scrapped and homogenized in ice cold saline medium with the help of homogenizer. The solution was centrifuged for 10 minutes at 3000 rpm and the supernatant was collected

for experiment. 100µl of the supernatant was added to a solution of 3 µl of H_2O_2. Phosphate buffer mixture (50 mM phosphate buffer, pH 7.0 and 30% H_2O_2). The change in optical density at 240 nm per unit time was measured.

The concentration of the mixture was standardized to get the optical density against buffer at 240 nm.

Gluthathione (GSH)

The determination of total tissue sulfyhydryl (thiol) group was carried out according to the method. Gastric tissue was scrapped and homogenized in ice cold phosphate buffer (pH 8.0) medium. The tissue homogenate was centrifuged at 3000 rpm and after centrifugation the supernatant was collected for experiment. The tissue homogenate (supernatant) was taken in 10 mm phosphate buffer (pH 8.0). To this 0.2 ml of 10 mm DTNB (5, 5/-Dithiobis –2 – nitrobenzoic acid) of pH 7.00 prepared in 10 mm. Phosphate buffer was added.

The resulting suspension was mixed thoroughly. The mixture was kept at room temperature for 20 minutes and absorbance was measured in a spectrometer at 412 nm.

Protein Estimation

The method used for protein was according to Lowry et al (1962)[8]. The tissue sample and standards (1 mg/ml BSA in double distilled water) in different tubes were treated with 5 ml of reagent mixture (48% Na, K-tartarate, 2% $CuSO_4$ and 3% Na_2CO_3 in 0.1N NaOH; added in a ratio of 1 : 1 : 48 by volume). Then Folin lowry reagent (half diluted with double distilled water) was added to the reaction mixture with continous shaking. Then the solution was allowed to stands for 30 minutes at room temperature and optical density was measured at 710 nm using water as reagent blank.

RESULT

Alcohol induced gastric ulceration

Pretreatment with *P. acerifolium* bark extract (150mg/kg, 25.82% and 300mg/kg,61.65%) and omeprazole (10 mg/kg, 82.33%) produced significant reduction of alcohol induced ulceration. It was also observed that pretreatment with *P. acerifolium* bark extract (150mg/kg., 300mg/kg) and omeprozole-(10mg/kg, 67.34%) produced significant reduction of tissue lipid peroxidation. It also elevated the GSH content, increase SOD & CAT as compared to control group animal. It was shown in (Table 1, 1A, 1B, 1C, 1D).

Table 1: Effect of *P. acerifolium* bark extract on Alcohol induced gastric ulceration (values are expressed as mean ± S.E.; n = 6) (Dose of *P. acerifolium* bark extract (T_1 – 150 mg/kg and T_2 = 300 mg/kg) standard drug omeprazole = S = 10 mg/kg

Animal No.	*Ulcer Index*			
	Control	**T_1**	**T_2**	***S***
1	8.1	5.1	2.2	1.2
2	8.3	6.8	2.8	1.8
3	8.2	6.1	3.6	1.4
4	7.6	5.6	3.1	1.3
5	7.2	5.4	3.8	1.7
6	8.4	6.2	2.7	1.1
Mean ± S.E.	7.9±0.481	5.86±0.066**	3.03±0.099*	1.41±0.038*
% Inhibition		25.82	61.65`	82.15

'P' values vs. control (by student 't' test)* p <0.01),** p <0.05

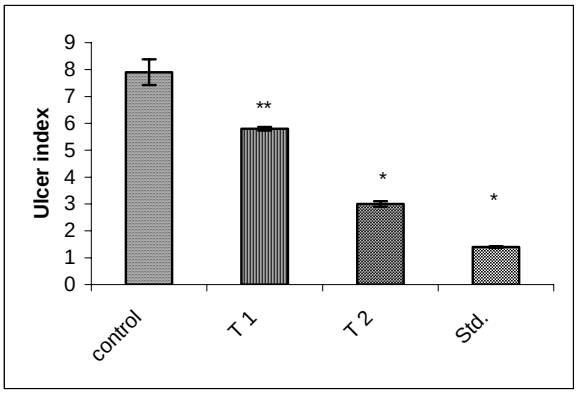

Fig. 1: Effect of *P. acerifolium* bark extract on Alcohol induced gastric ulceration (values are expressed as mean ± S.E.; n = 6) (Dose of *P. acerifolium* bark extract (T_1 – 150 mg/kg and T_2 = 300 mg/kg) standard drug omeprazole = S = 10 mg/kg
'P' values vs. control (by student 't' test)* p <0.01), p<0.05**

Table 1A: Effect of *P. acerifolium* bark extract on lipid peroxidation (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Animal No.	Lipid Peroxidation (n mol of TBARS/kg of protein				
	Normal	Control	T_1	T_2	S
1	5.01	10.39	6.82	4.95	3.92
2	8.63	11.89	5.95	4.99	3.21
3	8.01	11.62	5.28	4.79	3.46
4	8.31	11.46	6.64	4.07	3.84
5	8.47	12.08	6.28	5.12	3.96
6	9.15	11.98	5.33	5.33	3.53
Mean ± S.E.	7.43±0.52	11.57±0.25	6.04±0.271*	4.87±183***	**3.65±0.246
% change		55.72	47.79	57.9	68.45

'P' values vs. control (by student 't' test)p<0.01 ,**p<0.001**

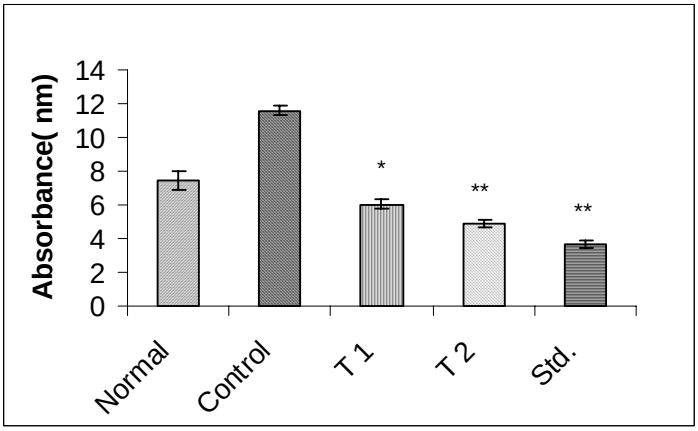

Fig. 1A: Effect of *P. acerifolium* bark extract on lipid peroxidation (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Table 1B: Effect of *P. acerifolium* bark extract on reduced Glutathione (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Animal No.	*GSH (µg/ml of gastric tissue)*				
	Normal	**Control**	**T_1**	**T_2**	**S**
1	11.02	2.04	3.64	8.41	9.02
2	12.24	2.98	3.46	8.86	9.16
3	11.46	3.19	4.02	9.16	9.88
4	12.41	2.89	4.16	8.56	9.01
5	10.25	2.24	3.81	8.43	9.06
6	11.85	3.62	4.17	9.41	9.98
Mean ± S.E.	11.53±	2.82±	3.87±	8.80±	9.54±
	0.33	0.197	0.118*	0.155*	0.185*
% change		75.54	37.10	212.05	231.21

'P' values vs. control (by student 't' test)p<0.001*

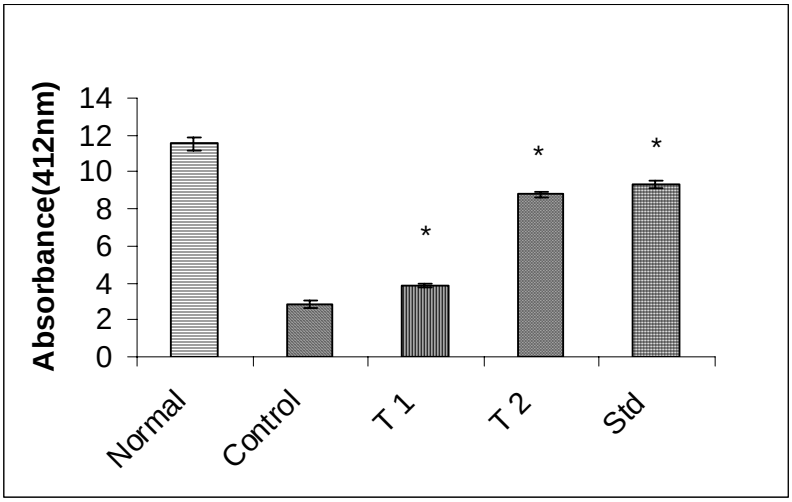

Fig. 1B: Effect of *P. acerifolium* bark extract on reduced Glutathione (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Table 1C: Effect of *P. acerifolium* bark extract on superoxide dismutase status [Dose of *P. acerifolium* bark extract T_1 = 150 mg/kg, T_2 = 300 mg/kg, orals) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Animal No.	Superoxide dismutase status expressed as units/mg of protein				
	Normal	Control	T_1	T_2	S
1	6.47	3.02	3.97	2.08	4.14
2	5.11	2.88	3.62	1.83	4.43
3	6.02	3.15	3.04	2.14	4.93
4	5.89	2.91	3.37	1.26	4.52
5	5.52	3.12	3.41	1.57	4.78
6	5.19	2.99	3.54	1.31	3.39
Mean ±S.E.	5.7±0.214	3.11±0.044	3.49±0.146*	1.69±0.155**	**4.36±0.225
% change		60.70	15.95	43.85	44.85

'P' values vs. control (by student 't' test)* p <0.001,**p<0.01,***p<0.025

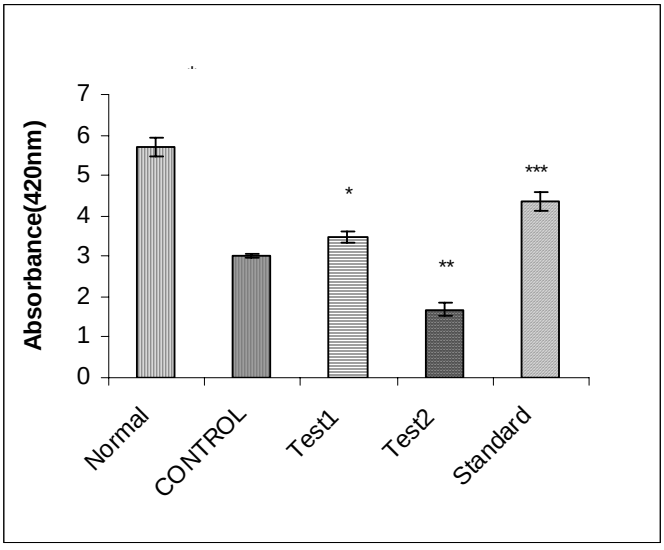

Fig. 1C: Effect of *P. acerifolium* bark extract on Superoxide dismutase. (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Table 1D: Effect of *P. acerifolium* bark extract on Catalase status during Alcohol (5ml/kg v/v p.o.) induced gastric ulceration.) (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

Animal No.	Catalase expressed as unit/min./μg of enzyme				
	Normal	Control	T_1	T_2	S
1	5.93	3.32	4.12	3.61	1.82
2	5.42	3.43	4.04	3.02	1.02
3	4.08	3.41	3.92	3.17	2.04
4	5.02	2.98	4.19	2.98	1.79
5	5.11	3.87	4.78	3.15	1.47
6	4.93	3.67	4.13	3.46	1.96
Mean ± S.E.	5.86±0.1018	4.11±0.1146	4.19,*** ±0.123	3.23±0.2924**	1.68±0.056*
% change		29.86	1.94	21.41	59.12

'P' values vs. control (by student 't' test)* p <0.01,**p<0.02,***p<.0.05

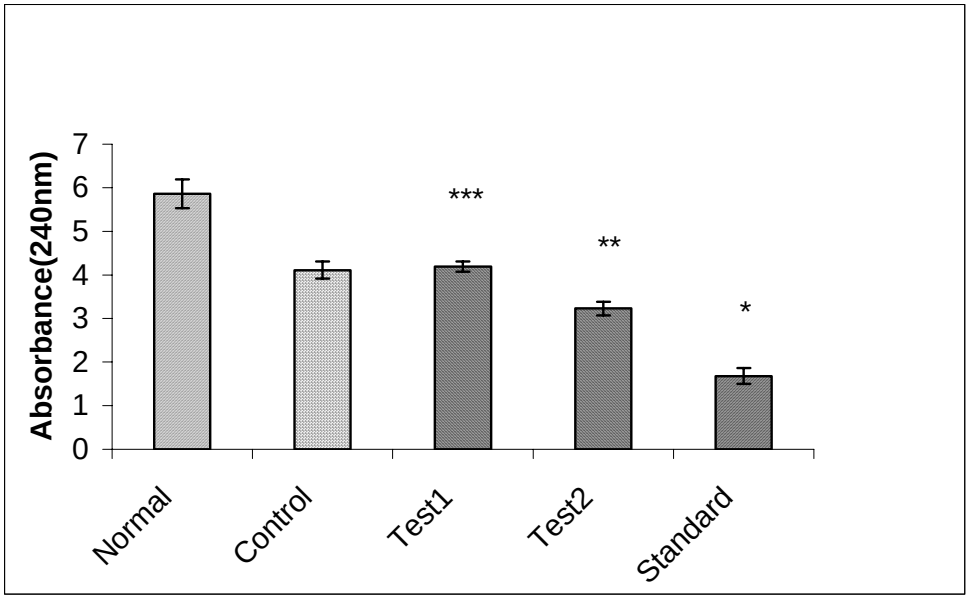

Fig. 1D: Effect of *P. acerifolium* bark extract on Catalase status during Alcohol (5ml/kg v/v p.o.) induced gastric ulceration.) (Dose of *P. acerifolium* bark extract T_1 – 150 mg/kg and T_2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean ± SE; n = 6]

DISCUSSION

Oral administration of absolute alcohol causes severe gastric damage to rats, prostaglandins effectively protect the gastric mucosa, against the haemorrhagic and necrotic effect of ethanol (Robert, 1979, Lay, 1986). Therefore treatment with analogous prostaglandins could be effective in reducing this type of damage. Alcohol also causes ulceration due to excess production of gastric mucosal LTC-4 and LTD-4[10]. This finding suggests a possible role for the lipo-oxygenase product (Leukotriene) in the pathogenesis of such lesions[11,12]. In our early studies protection has been observed following pretreatment with bark extract of *P. acerifolium* in ethanol induced ulcer. Lipid per oxidation propagates through membrane, the protein molecules present in the membranes are also affected, radical like peroxyl and alkoxyl, aldehyde and other product generated within membranes can also inflict severe damage to the protein present[15,17]. Antioxidant significantly delays or prevents reduction in antioxidant enzymes reserve in-vivo. Super oxide dismutase is one of the important enzyme due to the high reaction, specificity of SOD. It is also used as probe for investigating involves of super oxide radical in biological system similar logic, appears to the involvement of other enzymes such as catalase(CAT) which is one of the two enzymes, they can directly catalase decomposition of H_2O_2 , during oxidative stress, SOD converts the reactive O_2 to H_2O_2 which is not scavenged by CAT, can initiate lipid per oxidation. Glutathione peroxidase is perhaps another enzymes, which is known to play an important role towards antioxidant defense, glutathione catalyses removal of H_2O_2, following oxidation of reduced glutathione. It reacts with different free radical species, generating thiol radical which in turn generate super oxide. The body has developed several endogenous antioxidant system to deal with the production of reactive oxygen species. These system can be divided into enzyme and nonenzymatic groups (Das et al; 1997). The enzymatic antioxidant super oxide dismutase (SOD) which catalyses the conversion of O_2 to H_2O_2 . These enzymes also requires trace metal co-factors for maximum efficacy, including selenium for glutathione peroxidase, copper, zinc or manganese for SOD and iron for catalase. The non-enzymatic antioxidants include in lipid soluble vitamins and GSH. Glutathione, which is synthesized intracelllary from cysteine. The majority of GSH is synthesized in liver. Its biological role is believed to be a defence against lipid peroxidation. Changes in antioxidative molecules levels may be an important factor in ulcer generation[17-19]. Ethanol induced lesion formation is due to different factor like stasis of gastric blood flow contributing significantly to the development of haemorrhagic as well as necrotic aspects of tissue injury. The products of 5-Lipoxygenase pathway may also play a key role in the development of such ulcer[11]. Protection rendered by bark extract of *P. acerifolium* in alcohol induced ulcer is probably due to restoration of superoxide dismutase (SOD) and catalase (CAT) enzyme or due to 5-lipoxygenase antagonism.

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