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EFFECT OF *PTEROSPERMUM ACERIFOLIUM* LEAF 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* leaf 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, catalase, superoxide dismutase and glutathione were observed to occur with the extract.

Key words: Pterospermum acerifolium, Ulcer, superoxide dismutase, Gastric Enzyme.

INTRODUCTION

Pterospermum acerifolium Linn is usually a perennial evergreen plant belonging to family *Sterculiaceae.* It is found in sub Himalayan track and outer Himalayan valleys and hills up to 4000 ft., Bengal, Chittagong, Khasia hills, Manipur, Darjeeling, Jatara Distt-Tikamgarh Madhya Pradesh and extensively planted in the Maharastra state. This plant have plethora of applications in traditional Indian medicine for viz, in ayurvedic anticancer treatment flowers are mixed with sugars and applied locally [(1)] Flowers & bark, charred and mixed with kamala applied for treatment of smallpox. Flowers are made into paste with rice water & applied locally as anti-hemicranias [(2)]Flowers in form of churna are also used in migraine therapy [(3)] Isolation of boscialin glucosides from leaves of *Pterospermum acerifolium* have been reported [(4)] Antidiabetic effect of methanol extract of bark of *Pterospermum acerifolium*

on Type I & Type II diabetic model rats were also reported [5]Chloroform extract of the leaves of *Pterospermum acerifolium* showed significant antihyperglycemic effect on Type I diabetic model rats [3,5] Ethanol and water extract of leaves showed good antimitotic activity against meristmetic cell growth [6] Since available treatments for the diabetes either by the synthetic or from natural sources are limited with certain drawbacks. So the goal of present study is to develop antidiabetic formulation of *Pterospermum acerifolium*.

Resistance of the parasites to existing drugs and their high cost warrants the search for newer anthelmintic molecules. The origin of many effective drugs is found in the traditional medicine practices and in view of this several researchers have undertaken studies to evaluate folklore medicinal plants for their proclaimed anthelmintic efficacy[7]. *Pterospermum acerifolium* (L) Willd (Family: Sterculiaceae) commonly known as “Dinner plate tree”

(English) and “Muchukunda” (Hindi), is widely distributed in North Canada and in many parts India i.e. Himalayan tracts, Dehradun, Bengal, Assam and Manipur[8,9]. The flowers are sharply bitter, laxative, disinfectant, anthelminthic, removes cough (In Ayurveda), useful in leucorrhoea, ulcer, inflammation and leprosy. Leaves are used as haemostatic agent[10]. Barks are used as anthelmintic in treating animals[11]. Flavonoids like keampferol, keampferide, luteolin, steroids and triterpenoids like sitosterol, taraxerol, friedelin, sugars, fatty acids are present in the plant[12,13] .As the people consume this plant to cure helminthic infections as per the literature, we attempted to investigate this medicinal plant for its claimed anthelmintic activity.

MATERIAL AND METHOD

Plants materials *P. acerifolium* leaves were collected from Jatara Distt- Tikamgarh (Madhya Pradesh, India) in July 2011 and authenticated by comparison with a voucher. Specimen in Botanical survey of India, Madhya Pradesh. 1 kg. Of the air dried leaves 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 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±20C) for at least 10 days were used for pharmacological experiment .The animals were given standard pellet diet and water adlibitum. All the experiments were performed as per guidelines of institutional animal ethics committee.

Ethanol induced gastric ulceration

Groups of albino rats (n = 6) fasted for 24 h. allowing water and adlibitum were used for experiment. *P. acerifolium* leaves extract (300 mg/kg) or control vehicle were administered orally. After 30 min following administration 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. 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 estimation5. (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 H2O2 as a marker for oxidative damage.

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.

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.

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.

RESULTS

Alcohol induced gastric ulceration

Pretreatment with *P. acerifolium* leaf 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* leaf 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 (T1 – 150 mg/kg and T2 = 300 mg/kg) standard drug omeprazole = S = 10 mg/kg.

Animal No	Ulcer Index			
	Control	T1	T2	S
1	8	4.9	2	1.2
2	8.2	5.9	2.5	1.9
3	8.3	6	3.5	1.5
4	7	5.5	2.8	1.3
5	7.5	5.2	3.5	1.6
6	8.6	6	2.5	1
Mean±S.E.	7.93	5.58	13.51	1.41
% Inhibition		25.02	60.65	80.23

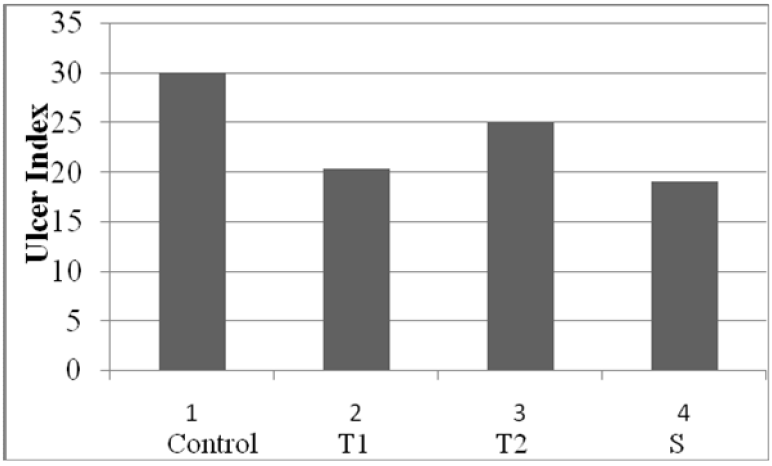

Fig. 1: Effect of *P. Acerifolium leaf* extract on Alcohol induced gastric ulceration (values are expressed as mean + S.E.; n = 6) (Dose of *P. acerifolium* leaf extract (T1 – 150 mg/kg and T2 = 300 mg/kg) standard drug omeprazole = S = 10 mg/kg.

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

Table 1A: Effect of *P. acerifolium* leaf extract on reduced Glutathione (Dose of *P.acerifolium* bark extract T1 – 150 mg/kg and T2 = 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	T1	T2	S
1	11.00	2.00	3.5	8.4	8.88
2	12.22	2.75	3.41	8.76	9.15
3	11.44	3.09	3.99	9	9.87
4	12.20	2.80	4.1	8.55	8.79
5	10.02	2.20	3.8	8.49	9
6	11.75	3.56	4.1	9.56	9.89
Mean±S.E.	11.44±	2.73±	3.82±	8.79±	9.26±
%Change		75.00	36.86	210.10	233.22

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

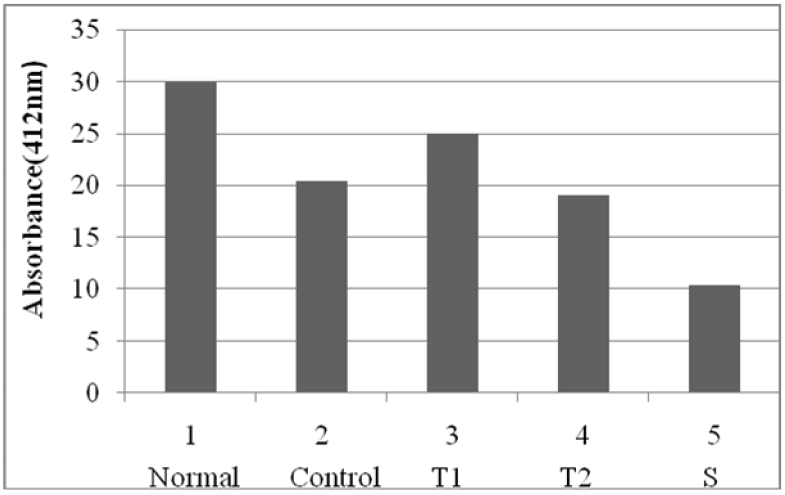

Fig. 1A: Effect of *P. acerifolium* leaf extract on reduced Glutathione (Dose of *P. acerifolium* bark extract T1 – 150 mg/kg and T2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean + SE; n = 6].

Table 1B: Effect of *P. acerifolium* leaf extract on superoxide dismutase status [Dose of *P. acerifolium* leaf extract T1 = 150 mg/kg, T2 = 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	T1	T2	S
1	6.4	3.02	3.99	2.11	4.14
2	5.02	2.9	3.65	1.85	4.33
3	6	3.1	3	2.01	4.93
4	5.85	2.95	3.35	1.22	4.55
5	5.55	3.1	3.45	1.59	4.77
6	5.15	2.9	3.54	1.31	3.33
Mean±S.E.	5.66	3.00	3.50	1.68	4.34
%Change		60.00	15.15	42.95	44.47

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

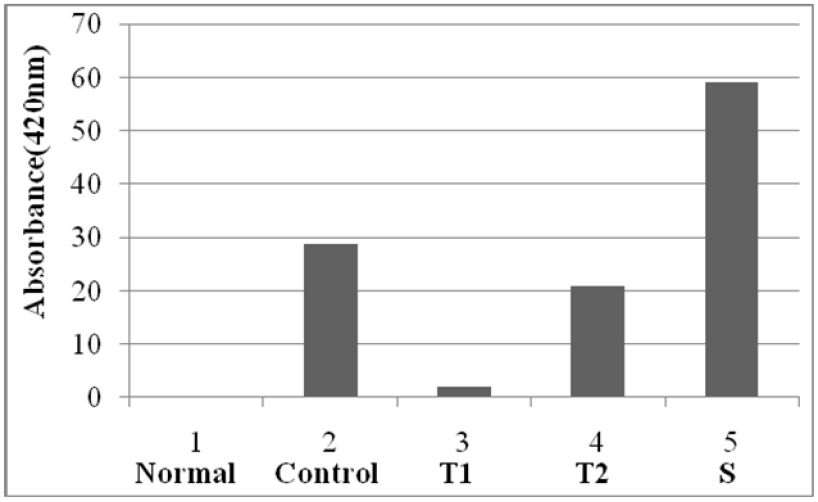

Fig. 1B: Effect of *P. acerifolium* leaf extract on Superoxide dismutase. (Dose of *P. acerifolium* leaf extract T1 – 150 mg/kg and T2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean + SE; n = 6]

Table 1C: Effect of *P. acerifolium* leaf extract on Catalase status during Alcohol (5ml/kg v/v p.o.) induced gastric ulceration.) (Dose of *P. acerifolium* bark extract T1 – 150 mg/kg and T2 = 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	T1	T2	S
1	5.89	3.33	4.1	3	1.85
2	5.55	3.45	3.99	3.12	1.06
3	4	3.45	3.85	3.16	2.21
4	4.98	2.95	4.15	2.98	1.77
5	5.01	3.56	4.75	3.17	1.47
6	4.56	3.65	4.1	3.56	1.97
Mean±S.E	5.00	3.40	4.16	3.17	1.72
%Change		28.85	1.98	20.89	58.98

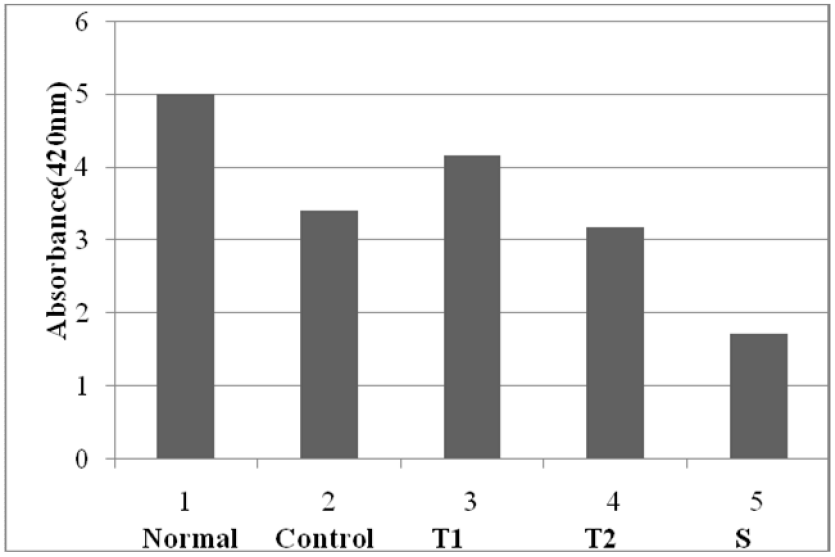

Fig. 1D: Effect of *P. acerifolium* leaf extract on Catalase status during Alcohol (5ml/kg v/v p.o.) induced gastric ulceration.) (Dose of *P. acerifolium* leaf extract T1 – 150 mg/kg and T2 = 300 mg/kg, oral) standard drug omeprazole S = 10 mg/kg) (Results are expressed as mean + SE; n = 6]

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-410. This finding suggests a possible role for the lipooxygenase.

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 H2O2 , 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 Changes in antioxidative molecules levels may be an important factor in ulcer generation [17,18]. 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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