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ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF RHIZOME OF *PICRORHIZA KURROA* ON INDOMETHACIN INDUCED GASTRIC ULCER DURING HEALING

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RUNNING TITLE : ANTIOXIDANT ACTIVITY OF RHIZOME OF *PICRORHIZA KURROA* ON HEALING

ABSTRACT

Oral administration of ethanol extract of the rhizome of *Pirorhiza kurroa* at a dose of 20mg/kg body weight , for 10 consecutive days , was found to enhance the rate of healing on Indomethacin-induced gastric ulcer in rats . compared to the ulcerated group without treatment. The level of peroxidised lipid ,in terms of thiobarbituric acid reactive species (TBARS), in gastric tissue, was increased in ulcerated rats which was restored to near normalcy on treatment with ethanol extract . The specific activity of *in vivo* antioxidant enzymes , viz SOD and catalase and total tissue sulfhydryl (thiol) group ,which were markedly decreased in ulcerated group ,were found to be significantly elevated ($p< 0.05$) , on treatment with the above extract , at the specified dose ,compared to the indomethacin –induced ulcerated group without any supporting treatment . The present study thus suggests that the ethanol extract of rhizome of *Picrorhiza kurroa* , at the dose of 20mg/kg body weight , accelerated the healing of stomach wall of indomethacin induced gastric ulcerated rats by an *in vivo* free radical scavenging action.

KEY WORDS

Antioxidant , Free radicals, NSAID, *Picrorhiza kurroa*, ulcer healing

INTRODUCTION

It has now been established , that oxygen derived free radicals, primarily superoxide ($O_2^{\cdot}$)anion and hydroxyl radical(OH .), play an important role in the pathogenesis of acute experimental gastric lesions induced by stress, ethanol and non steroidal anti-inflammatory drugs (NSAIDs), viz. Indomethacin, Aspirin etc [1-4]

Helicobacter pylori, although with some controversies, is accepted as a major contributor (70-80%)[5] to the production of peptic ulcer . A possible cause for *H. pylori* infection points towards its profound potentiation of polymorphic nuclear oxidative burst, leading to a considerable production of reactive oxygen species(ROS), the central factor causing irreversible membrane damage, DNA strand breaks , changes in protein secondary and tertiary structures [6-7]

Oxygen handling cells have different systems, e .g. superoxide dismutase (SOD), peroxidase, catalases and tissue thiol group which are able to protect them against the toxic effects of free radicals, one of the most devastating, being, superoxide anion $O_2^{-\cdot}$.

Chander *et al.*1992 have reported that picroliv, picroside –I and kutkoside , obtained from the ethanolic extract of the roots and rhizome of *Picrorhiza kurroa* , are scavengers of superoxide anion ($O_2^{\cdot}$). They have also shown the significant reduction , of increased level of lipid peroxidation in damaged liver tissue, on picroliv administration[8] .

The purpose of the present study was to demonstrate whether or not the antioxidant property of the ethanol extract of rhizome of *P. kurroa* could have any effect on healing of Indomethacin - induced gastric lesions .

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MATERIALS AND METHODS

Extraction of plant materials

Air –dried rhizome of *Picrorhiza kurroa* was supplied by M/s Surendra Nath Das and Co. Kolkata, under the authentic guidance of Plant Chemist from Botanical Survey of India .It was identified from Botanical Survey of India, Indian Botanical Garden, Shibpur, Howrah, West Bengal. An authentic sample of the rhizome of *P. kurroa* has been preserved in our laboratory. The fresh air-dried rhizomes were powdered in a mechanical grinder .Crude powder (250 g) was then soaked in 1 lt of 95% ethanol for 7 days with intermittent shaking .On 8th day , the whole material was filtered through muslin cloth. The filtrate was collected and concentrated under reduced pressure .The residual solvent was removed under vacuum in a rotary evaporator and the solid blackish –brown mass obtained (25.12g) was kept under vacuum, in a vacuum desiccator at 4° C, until further biochemical and chemical analysis, and oral administration.

During use, the dried material, after weighing was placed in a mortar –pestle, to which 2% w/v of gum acacia was added and macerated with double distilled water.It was transferred to a small tube and the volume was made up as required for different experiments . The suspensions, thus prepared, fresh before each administration, is referred to as 'drug', in subsequent discussions.

Animals

Charles –Foster strain of rats , weighing between 150-200 g of both sexes were used for the study. The rats were acclimatised to animal house conditions, (light and dark cycle , 12 :12 , temperature ,25 ± 1° C) and fed with pellet food (M/s , Hindustan Lever Limited , Kolkata) and given tap water *ad libitum*. They were housed in standard metal cages.

Animal experiments

Rats were divided into four groups of 6 each , as follows :

Group A	:	Normal Control
Group B	:	Ulcer '0'day
Group C	:	Ulcer , untreated
Group D	:	Experimental

Initially , all the animals of groups B, C, & D were administered orally with indomethacin , suspended in 2% of gum acacia at a dose of 15 mg/kg body weight for 2 consecutive days .3 hours after the last dose of indomethacin, Group B was sacrificed after overnight fasting. From the third day, the animals of group A and C were gavaged with 2% gum acacia solution (vehicle) and those in group D, were administered orally with, ethanol extract of rhizome of *Picrorhiza kurroa* at a dose of 20 mg /kg body weight for 10 days. The animals were sacrificed under ether anaesthesia, 3 hours after the last dose of the 'drug' . The extent of acceleration of healing of the ethanol extract treated group was compared with the normal control and ulcerated, untreated group.[9]

For estimation of biochemical parameters, gastric tissue was taken from the antral portion of the stomach and wet weight was noted.

Assay of malondialdehyde (MDA)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) [10] as standard . Thus, 1 ml of homogenized gastric tissue in 2 ml of normal saline was mixed with 24% TCA and centrifuged at 2,000 rpm for 20 mins. To 2 ml of protein –free supernatant, 1 ml of fresh TBA (0.67%) reagent was added, mixed thoroughly and heated at 95° C for 1 hour, in a water –bath. The suspension was then cooled to room temperature, centrifuged at 2,000 rpm for 10 mins, and the pink coloured supernatent was taken for spectroscopic measurement at 532 nm for the assay of MDA. Lipid peroxide is expressed in terms of nM of MDA /mg of gastric tissue.

Assay of superoxide dismutase (SOD) activity

The method of Mishra and Fridovich[11] was followed involving inhibition of epinephrine autooxidation, in an alkaline medium at 480nm in a UV –vis spectrophotometer.

For the determination of specific activity of SOD in the gastric tissue (scrapped and homogenized), the rate of autooxidation of epinephrine was noted at 30 secs intervals, in the normal control, untreated ulcerated and 'drug' treated group,and the enzyme activity was expressed in arbritary units considering inhibition of autooxidation, as 1 unit of SOD specific activity.

Assay of catalase activity

Catalase activity in the gastric tissue was determined according to the method followed by Lück [12]. The gastric tissue was scrapped off and homogenized in ice- cold normal saline medium. The solution was then centrifuged for 10 mins at 3,000rpm and the supernatant was collected for estimation .100µl of the supernatant was added to a solution containing 3 ml 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 taken as a measure of catalase activity .The concentration of the buffer –H_2O_2 was standardised to get the optical density at 240 nm to 0.500± 0.010 (d=1cm).

Assay of total tissue sulfhydryl (thiol) group (reduced glutathione level)

The determination of total tissue sulfhydryl (thiol) group was carried out according to the method of Ellmann.[13] Gastric tissue was scrapped and homogenized in ice-cold phosphate buffer (pH =8.0) medium. The tissue homogenate was centrifuged at 3,000 rpm for 10 mins and the supernatant was collected for the experiment. The tissue supernatant was then reacted with 10mM DTNB (5,5' –Dithiobis-2-nitrobenzoic acid) of pH 7.0. The resulting suspension was mixed thoroughly and kept at room temperature for 20 mins. The absorbance was measured at 412nm in a UV- visible spectrophotometer, 2mM of reduced glutathione (GSH) being used as standard.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) test, was followed by individual comparison by students' 't' test, for the determination of level of significance among the mean ± SEM. in various groups of animals

RESULTS

Effect of ethanol extract on healing of gastric ulceration induced by indomethacin

Ulcer , induced by Indomethacin (NSAID) , healed in a progressive manner by oral administration of the ethanol extract of the rhizome of *P. kurroa* at a dose of 20mg/kg body weight daily, for 10 days, compared to the untreated ulcerated group (Table I). The ethanol extract treatment , showed significant reduction in the ulcer index (4.8 ± 1.10) and also healed at a faster rate, (80.3%) within 10 days whereas, the untreated ulcerated group, showed only 57.4% recovery.

Effect of ethanol extract on lipid peroxidation

The level of lipid peroxide (MDA) was reduced significantly (Table 2) in the gastric tissue, on oral administration of the ethanol extract, at a dose of 20mg/kg body weight for 10 days to the ulcerated group of rats compared to the Indomethacin – induced ulcerated control.

Effect of ethanol extract on SOD activity

SOD activity in gastric tissue was reduced significantly in the indomethacin –induced ulcerated group. However , oral administration with the ethanol extract for 10 days at the dose of 20 mg/kg body weight ,to the experimentally ulcerated group, increased the SOD activity (382.26 ± 11.05 units/g tissue)(Table 2)

Effect of ethanol extract on catalase activity

Table 2 shows that oral administration of ethanol extract of the drug for 10 days (20 mg/kg body weight) to the experimental group of rats significantly increased the catalase activity (8.35 ± 0.085 units /mg of protein) compared to the untreated ulcerated group.

Effect of ethanol extract on total tissue sulfhydryl group

A significant ($p<0.05$) increase in level of total tissue thiol group (Reduced glutathione level) in gastric tissue was observd (Table 2) in the group of rats treated with the ethanol extract (20 mg/kg body weight) for 10 days compared to the untreated ulcerated group.

DISCUSSION

In our experimental therapeutic study, the morphological findings (Table I) clearly indicate that oral administration of ethanol extract of the rhizome of *Picrorhiza kurroa* to the ulcerated rats recover 80.3% within 10 days from gastric mucosal lesions caused by indomethacin,whereas untreated ulcerated rats recovered only 57.4%.

Ulcer formation induced by Indomethacin, a non – steroidal anti –inflammatory agent, is known to be

corelated with inhibition of cyclooxygenase, that prevents prostaglandin biosynthesis [14,15], which in turn inhibits the release of mucus[16], a defensive factor against gastrointestinal damage. Ethanol extract of rhizome of *Picrorhiza kurroa*, stimulate prostaglandin synthesis /release.[17]

Recently, much attention has been focused on oxygen derived free radicals which play an important role in the pathogenesis of peptic ulcer apart from the interactive processes like many other tissue degeneration situations.[18] Oxygen derived free radicals cause tissue injury through lipid peroxidation. Oxygen handling cells have different systems, e.g. superoxide dismutase (SOD), peroxidases and catalases which are able to protect them against the toxic effects of oxygen derived free radicals.

Chander et al have reported [7] that picroliv , picroside –I and kutkoside from *P kurroa* are scavengers of superoxide anions . They have also shown the reduction of increased level of lipid peroxidation in damaged liver tissue on picroliv administration. The antioxidant property of picroliv , picroside –I and kutkoside depends on preventing the formation of free radicals or by scavenging superoxide anions, possibly acting like superoxide dismutase, xanthine oxidase inhibitors and metal ion chelators.

Our results (Table 2) show that treatment with ethanol extract at the dose of 20mg/kg body weight significantly decreased the level of lipid peroxidation product (MDA) in gastric tissue compared to ulcerated rats . The activities of both SOD and catalase were decreased in ulcerated groups and maintained to near normalcy in drug treated group.

Superoxide dismutase (SOD) content in gastric tissue is a point of controversy [19,20]. The decrease in the level of lipid peroxidation and increase in the activities of free radical scavenging enzymes in the drug treated gastric mucosa compared to ulcerated group, suggest the drug's ability to protect the gastric mucosa against free radical mediated tissue injury. The protective action may be ,via the increase and maintenance of near normalcy in the activity of SOD, which is said to prevent neutrophil induced damage[21,22]

Glutathione, a major non –protein thiol in living organisms, plays a central role in co-ordinating the body's antioxidant defense processes. Reduced thiols have long been reported to be essential for recycling of antioxidants like vitamin E and vitamin C[23]. Administration of thiol compounds such as glutathione, cysteine and methionine have been shown to protect against oxidative stress in humans and animals. Treatment with test 'drug' resulted in increased level of total tissue sulfhydryl (thiol) group compared to the untreated ulcerated rats .(Table 2) Yoshikawa *et al* [24] have shown that indomethacin induced gastric mucosal injury decreased the glutathione peroxidase activity and aggravated the injury due to accelerated accumulation of H_2O_2 and lipid peroxidation. Furthermore, excessive peroxidation causes increased glutathione consumption [6].Indomethacin induced depletion of gastric glutathione is counteracted by maintenance of normal glutathione tissue concentration after treatment with the 'drug' which inhibits gastric mucosal injury possibly through scavenging indomethacin generated metabolites.[25]

Thus , it is evident that the test drug prepared from the rhizome of *P. kurroa* exerts therapeutic action on gastric ulcer predominantly by its antioxidant property.

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TABLE I

Effect of ethanol extracts of rhizome of *Picrorhiza kurroa* (drug) on healing of gastric ulceration induced by indomethacin . (Values are mean ± SEM of 6 rats in each group)

EXPERIMENTAL GROUPS	DOSE (mg/kg body weight	0 Day		7th Day		10th Day
		ULCER INDEX(mm^2)	HEALING PROMOTION(%)	ULCER INDEX (mm^2)	HEALING PROMOTION	ULCER INDEX(mm^2)
ULCER '0' DAY	-	24.40± 1.01	-	-	-	-
ULCER UNTREATED, VEHICLE ONLY	-	-	-	15.20 ±1.21	37.70	10.40±1.31
ULCER, ETHANOL, EXTRACT TREATED	20	-	-	8.80** ±1.22	63.90	4.8*±1.10

(Values are mean ± SEM, FOR 6 RATS IN EACH GROUP)

* Significant , compared to untreated ulcerated group, $p< 0.005$ (10th day)

**Significant , compared to untreated ulcerated group, $p< 0.01$(7th day)

Table 2

Effect of ethanol extract of rhizome of *Picrorhiza kurroa* (drug) on lipid peroxidation (MDA), superoxide dismutase (SOD), catalase and total tissue thiol level during the process of healing of gastric tissue induced by indomethacin (Values are mean ± SEM of 6 rats in each group)

TREATMENT GROUPS	PEROXIDISED LIPID [MDA] (nM /mg gastric tissue)	SOD ACTIVITY Units /mg of protein	CATALASE ACTIVITY Units /mg of protein	TOTAL THIOL (-SH) GROUP nM/mg protein
NORMAL CONTROL	9.21 ± 0.17	364.57 ± 9.79	7.87 ± 0.11	75.17± 1.05
ULCER '0'DAY INDOMETHACIN INDUCED	14.70 ± 0.22[a]	255.86± 8.24[a]	6.84 ± 0.08[b]	55.60± 1.52[a]
ULCER , '10' DAYS , UNTREATED	12.15 ± 0.13 **	305.46 ± 12.25	4.68 ± 0.13	61.27 ± 2.39
ULCER , '10' DAYS , DRUG TREATED	8.2 ± 0.08 *	386.26 ± 11.05**	8.35 ±0.09**	73.96± 2.75**

(Values are mean ± SEM, FOR 6 RATS IN EACH GROUP)

* Significant , compared to untreated ulcerated control, $p<0.01$

**Significant , compared to untreated ulcerated control, $p< 0.05$

[a] Significant , compared to control group, $p< 0.005$

[b] Significant , compared to control group, $p<0.05$