ANTIOXIDANT EFFECT OF METHANOLIC EXTRACT OF *SOLANUM NIGRUM* BERRIES ON ASPIRIN INDUCED GASTRIC MUCOSAL INJURY

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ABSTRACT

The antiulcerogenic effects of the methanolic extract of *Solanum nigrum* berries (SBE) on aspirin induced ulceration in rats with respect to antioxidant status in the gastric mucosa have been investigated. Oxygen free radicals are considered to be important factors in the pathogenesis of gastric ulcer. The level of lipid peroxides, which were elevated highly in rats with acute gastric mucosal injury was taken as an index of oxidative stress. The activities of antioxidant defense enzymes were also decreased considerably by oral gastric administration of aspirin. The decreased levels of antioxidant enzymes and increased mucosal injury were altered to near normal status upon pretreatment with (SBE) when compared to the ulcer induced rats. The results indicate that (SBE) may exert its gastroprotective effect by a free radical scavenging action. Our observations suggest that (SBE) may have considerable therapeutic potential in the treatment of gastric diseases.

KEY WORDS

Aspirin, Antioxidant enzymes, *S.nigrum*, ulcer healing

INTRODUCTION

Oxidative stress and free radical mediated processes have been implicated in the pathogenesis of gastrointestinal disorders (1). Nonsteroidal anti-inflammatory drug's (NSAID's) are recognized as the most common etiologic factors associated with gastric ulcer (2). Aspirin injures the gastrointestinal mucosa and because oxygen-derived free radicals mediate injury of this mucosa, oxy-radicals may play a pathogenetic role in the evolution of aspirin – induced erosive gastritis (3). Aspirin induced ulcer has been used as a model for the evaluation of antiulcerogenic agent (4).

Solanum nigrum Linn.(Family solanaceae) is commonly used in the traditional medicine as a remedy for treating various diseases. The berries possesses various medicinal properties such as sedative, diaphoretic, diuretic, hydragogue, expectorant and are useful in the disease of liver, heart and eyes and is also effective against piles, fever and dysentery (5). The leaves are used to heal open wounds and is known to possess hypotensive effect (6). The berries has been used in the treatment of stomach ulcers in the folk medicine in South Africa, European, China and through out India (7). The fruits of *S. nigrum* has been reported to play an adjuvant role in the hepatoprotective property. Inhibition of lipid peroxidation and free radical

scavenging activity has been suggested as a possible mechanism of action (8). Previous studies have demonstrated that aerial parts of *S.nigrum* has the ability to decrease the secretion of gastric acid, pepsin level and stimulate mucus secretion (9).

Therefore, the present study was conducted to evaluate the antiulcerogenic effect *S.nigrum* in terms of its antioxidant status on aspirin induced gastric mucosal damage.

MATERIALS AND METHODS

Plant Material

Berries of *S. nigrum* were purchased from the Native Care and Cure Centre, India. It was authenticated by Prof.P.Brindha, Pharmacology Department, Captain Srinivasamurthy Drug Research Institute, Siddha, Arumbakkam, Chennai-600106. A specimen was submitted to the herbarium of the same institute. The fresh air-dried berries were powdered in an electric grinder. Crushed powder was then soaked in methanol for 5 days with intermittent shaking and the solvent was filtered. The filtrate was collected and then extracted by Soxhlet apparatus (10). The extract was then dried in vacuum drier (yield : 16.7%) and it was stored at -4 °C for further use.

Animals

Adult male albino rats of Wistar strain weighing 150-180 g were purchased from the animal house, Tamil Nadu University of Veterinary and Animal Sciences, Madhavaram, Chennai. They were maintained under

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standard laboratory conditions with standard pelleted diet (M/s. Hindustan Lever Foods, Bangalore, India) and water *ad libitum*. All animal experiments were carried according to the guidelines of Institutional Animal Ethics Committee.

Dosage and Treatment

The doses were selected on the basis of acute toxicity study and LD₅₀ of the extract was found to be 4g/kg body.wt. The (SBE) was employed at oral doses of 250, 500 and 1000 mg/kg body wt. / day as an aqueous suspension using distilled water at different time intervals for 3 and 7 days. After the experimental period, the rats received aspirin orally 400mg/kg.body.wt suspended in (0.5% carboxy methyl cellulose) (11). The rats were sacrificed after 4 hrs of aspirin administration and the stomach was opened along the greater curvature for determination of gastric lesions in the gastric glandular portion under dissecting microscope (12). This study suggested that (SBE) at a dose of 500 mg/kg body.wt given for 7 days appear to be more effective in preventing aspirin induced gastric lesions. Therefore, this dose was chosen as optimum dosage for further studies.

Experimental procedure

The rats were divided into four groups of 6 animals each. The animals were kept fasting for 48 hours prior to the experiment but water was permitted *ad libitum*.

Group I	-	Normal Control
Group II	-	Animals received aspirin 400 mg/kg body .wt suspended in (0.5% carboxy methyl cellulose) orally.
Group III	-	Animals received aspirin (400 mg/kg body .wt) + pretreatment with (SBE) 500 mg/kg body.wt/day orally for 7 days.
Group IV	-	Animals received (SBE) 500 mg/kg/body wt/day orally for 7 days.

For biochemical estimation gastric mucosal tissue was taken from the antral portion of the stomach. The gastric mucosa was scrapped with a scraper, weighed and homogenized in icecold phosphate buffer (pH 7.2) to prepare the mucosal homogenate. The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was used for further studies.

Biochemical Estimation

Protein was estimated by the method of Lowry *et al* (13) using Bovine serum albumin as standard. Lipid peroxidation products were estimated by assaying malondialdehyde formation according to the method of Das *et al* (14). The determination of total tissue sulphhydryl (thiol) group (reduced glutathione level) was

carried out according to the method of Ellman (15). The autooxidation of epinephrine was noted by determining the activity of superoxide dismutase (SOD) by the method of Misra (16). Catalase (CAT) activity was measured by following decomposition of H₂O₂ according to the method of Berr's and Sizer (17). Glutathione peroxidase (GSH-Px) was assayed by the method of Rotruck *et al* (18) using H₂O₂ as substrate. Glutathione-S-transferase activity (GST) was measured using 1, chloro 2,4-dinitrobenzene as substrate according to Habig *et al* (19).

Statistical analysis

All the results were expressed as Mean±SEM. Student's 't'-test was used to assess statistical significance in various groups of animals.

RESULTS

(SBE) administered in doses 250,500,1000 mg/ kg.b.wt orally, caused a dose dependent decrease in ulcer index in aspirin induced rats (Table 1). Although a dose of 1000 mg/kg showed maximum ulcer protection (72.70%) ,but revealed toxic symptoms such as diarrhoea and weight loss, where as a dosage of 500 mg/kg.body.wt of (SBE) given for 7 days markedly inhibited the number of lesions and showed ulcer protection (70.12%) without any toxic symptoms, which is safer for further biochemical studies.

Activities of GSH, Lipid peroxides (LPO), SOD, CAT, GSH-Px, GST and protein levels in gastric mucosal tissue are shown in (Table 2). There was a significant increase in lipid peroxidation (p<0.001), decrease in antioxidant enzyme and protein levels in aspirin induced rats when compared with those of control rats (Group I) . (SBE) pretreated rats (Group III) maintained the protein level ,antioxidant enzyme status and LPO levels at near normalcy when compared to ulcerated animals.

DISCUSSION

The oxygen derived free radicals play a key role in the mechanism of aspirin induced acute gastric mucosal lesions (20). Aspirin induces the reactive oxygen metabolites in animal models , which may contribute to mucosal injury (21). The role of these reactive oxygen species and the potential antioxidant protective effect of (SBE) on gastric mucosal tissue are topic of high current interest.

Many reports have demonstrated that most injury of gastric mucosa can be reduced by pretreatment with scavengers of reactive oxygen species (22). In the present study, (SBE) inhibited the increase in area of gastric mucosal lesions in aspirin induced ulceration in rats. These protective effects were observed at oral doses of (SBE) 500 mg/kg body .wt (Group III) and a recovery of 70.12% was observed within 7 days. It offers

Table 1

Effect of *Solanum nigrum* on gastric lesions induced by aspirin in rats.

Values are Mean $\pm$ SEM of 6 animals in each group

Treatment & Dose (mg/kg.b wt)	No. of animals	3 days Lesion score Mean $\pm$ SEM	Percentage of Inhibition	7 days Lesion score Mean $\pm$ SEM	Percentage of Inhibition
Aspirin induced ulcer	6	15.4 $\pm$ 2.3 _a	-	15.4 $\pm$ 2.3 _a	-
Aspirin + pretreated with(SBE)(250mg/kg)	6	12.3 $\pm$ 2.4 _b	20.12	9.1 $\pm$ 1.2 _b **	49.30
Aspirin + pretreated with(SBE)(500mg/kg)	6	8.6 $\pm$ 0.96 _b **	44.15	4.6 $\pm$ 0.60 _b ***	70.12
Aspirin + pretreated with (SBE)(1000mg/kg)	6	7.4 $\pm$ 0.86 _b **	51.94	4.2 $\pm$ 0.54 _b ***	72.70

P values: * <0.05 , ** <0.01 *** <0.001

(a) aspirin induced Vs (b) SBE pretreated +aspirin

Table 2

Effect of *Solanum nigrum* on number of lesions, lipid peroxidation, antioxidant enzymes and protein levels of gastric mucosa in rats

Values are Mean $\pm$ SEM of 6 animals in each group

Parameters	Group I	Group II	Group III	Group IV
Number of lesions	-	15.4 $\pm$ 2.3***	4.6 $\pm$ 0.60***	-
Protein (mg/100 mg tissue)	16.3 $\pm$ 1.6	7.9 $\pm$ 0.68***	14.8 $\pm$ 1.5***	16.1 $\pm$ 1.4 ^{NS}
Lipid peroxide (nmoles of MDA/mg protein)	3.8 $\pm$ 0.31	9.7 $\pm$ 0.74***	4.2 $\pm$ 0.29***	3.9 $\pm$ 0.22 ^{NS}
Superoxide dismutase (units/mg protein)	5.3 $\pm$ 0.32	3.4 $\pm$ 0.13***	4.9 $\pm$ 0.21***	5.5 $\pm$ 0.35 ^{NS}
Catalase activity (nm of H ₂ O ₂ decomposed /min/mg protein)	6.8 $\pm$ 0.42	3.2 $\pm$ 0.28***	5.9 $\pm$ 0.45***	6.9 $\pm$ 0.44 ^{NS}
Glutathione peroxidase (μ g GSH utilised / minute / mg protein)	158.2 $\pm$ 10.2	60.1 $\pm$ 5.8***	142 $\pm$ 9.2***	165.4 $\pm$ 10.9 ^{NS}
Reduced sulphhydryl group (nm/g protein)	4.8 $\pm$ 0.14	2.0 $\pm$ 0.11***	3.9 $\pm$ 0.14***	4.7 $\pm$ 0.15 ^{NS}
Glutathione-S-transferase (nmoles of CDNB conjugated/min./mg protein)	4.6 $\pm$ 0.32	2.8 $\pm$ 0.19***	3.9 $\pm$ 0.29***	4.4 $\pm$ 0.31 ^{NS}

P values: *** <0.001 ; NS - Non-significant

Group II and Group IV was statistically compared with Group I

Group III was statistically compared with Group II.

gastroprotection against aspirin induced ulcer by significantly blocking lipid peroxidation. Prashanth kumar *et al* have reported that *S.nigrum* also exhibited significant hydroxyl radical scavenging potential, thus suggesting its propable mechanism of cytoprotection (23). Scavenging activity of this extract also reduces lipid peroxidation. In the present study aspirin pretreatment significantly reduced the protein concentration of the stomach . It may be due to accumulation of toxic free radicals in the mucosal cells (24). (SBE) pretreated rats provided protection against the action of aspirin by increase in protein content of the gastric mucosal tissue. Oxygen handling cells have antioxidant enzymes such as CAT, SOD, GST, GPX and GSH which are the first line of cellular defense against oxidative injury, decomposing O_2 & H_2O_2 before they interact to form more reactive $(OH^\cdot)$ radicals, SOD mainly act by quenching of superoxide $(O^\cdot)$ and active oxygen free radical, produced in different aerobic metabolism (25). SOD and CAT enzymes are highly specific in their catalytic mode of actions and it decreases the gastric mucosal damaging effect of aspirin (26). Although the increase in catalase is necessary for effective antioxidant activity, the changes in CAT levels were significant (27). Hence the antioxidant activity of (SBE) may be one of the important defensive factors involved in its ulceroprotective effect.

The role of GSH as an endogenous gastric antioxidant in mucosal protection, however, remains controversial since recent evidence has indicated an inverse correlation between gastric mucosal GSH levels and mucosal protection (28). GST and GSH-Px are essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell .The extract maintained the activity of GSH-Px almost at near normalcy by its ability to increase the level of reduced GSH, and to decrease lipid peroxidation. Previous studies have shown that the increase in GST activity when rats were fed on *Solanum nigrum* diet (29). The enhancement in GST activity was observed in (SBE) pretreated gastric mucosal tissues compared to ulcerated tissues. Many research have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer (30). The observed cytoprotective and antioxidative activity of the extract is attributed to the presence of biologically active phytoconstituents with having antioxidative nature .

In conclusion it can be said that (SBE) exhibit a protective effect through free radical scavenging properties and reduces oxidative damage caused by aspirin. These results provide additional support for the popular use of this plant as an antiulcer remedy in the Indian traditional medicine. A further detailed study on various other parameters of mucosal defensive factors could elucidate their exact mechanism of actions and their usefulness in the treatment of ulcer.

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