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A preclinical evaluation on antioxidant and gastroprotective effect of Dioscorea bulbifera in Wistar rats

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Abstract

The present study was undertaken to evaluate the gastro-protective effect of hydro alcohol extract of *Dioscorea bulbifera* (DB) tubers Linn in indomethacin-induced gastric ulcers in rats at doses of 100, 200 and 400 mg/kg body weight. The gastric lesions were significantly reduced by all doses of DB as compared with the Indomethacin (100 mg/kg body weight) treated group. In the stomach tissues of treated animals, the in-vitro antioxidant levels were evaluated. The administration of indomethacin caused a significant decrease in the levels of peroxidase, catalase (CAT) and an increase in glutathione peroxidase (GPx) and reduced glutathione (GSH), and lipid peroxidation (LPO) level. The administration of all doses of DB reversed the trend, inducing a significant increase of peroxidase, Catalase and a reduction in GPx, GSH and LPO level in tissues. These results suggest that the gastro-protective effect of DB can be attributed to its reducing effect on the oxidative damage in rats.

Key words: : Gastro-protective activity; *Dioscorea bulbifera;* Indomethacin; Antioxidant activity

Introduction

Gastric ulcers are defined as a breach in the mucosa of the alimentary tract, which extends through the muscularis mucosa into the sub mucosa or deeper (Manonmani *et al*., 1995). Stress, smoking, nutritional deficiencies and ingestion of Non-steroidal anti-inflammatory drugs (NSAIDs) augment gastric ulcer incidences (Belaiche *et al*. 2002). NSAIDs are worldwide used for the treatment of pain, rheumatic and cardiovascular diseases, and more recently for the prevention of colon cancer and Alzheimer's disease (To *et al*., 2001). The mechanism by which NSAIDs cause injury to the gastric mucosa is mainly due to the inhibition of cyclo-oxygenase enzyme (COX) and suppression of prostaglandin (PG)-mediated effects on mucosal protection (Wallace *et al*. 2000). Recently, reactive oxygen species (ROS) have also been shown to play a critical role in the development of pathogenesis in acute experimental gastric lesions induced by stress, ethanol and NSAIDs (Banarjee, 1990). NSAID induced ulceration causes accumulation of oxygen free radicals, which play a crucial role in the pathophysiology of gastric ulceration (Szelenyi *et al*., 1988). Oxygen derived free radicals cause lipid peroxidation, which leads to membrane fluidity and increases the influx of Ca^{2+} ions, resulting in reduced membrane integrity of surface epithelial cells, thereby causing gastric ulcers (Bandyopadhyay *et al*., 1999). It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration and scavenging these free radicals can play an appreciable role in healing gastric ulcers (Loguercio *et al*., 1993). Reduced glutathione is found in high concentration in the gastric mucosa of rats and humans (Body *et al*., 1979). Glutathione is important for the maintenance of mucosal integrity, and its depletion in the gastric mucosa induces macroscopic mucosal ulceration (Hoppenkamps *et al*., 1984). The non-availability of GSH decreases the activities of GSH dependent enzymes GPx and rendering these enzymes inactive and/or less active (Fridovich, 1978). Catalase is a common enzyme found in nearly all living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. Peroxidase also reduces hydrogen peroxide to water and oxygen. If CAT and Peroxidase levels have decreased, it led to increase in accumulation of these reactive products and

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thus, has caused increased lipid peroxidation and tissue damage.

Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions (Ariyphisi *et al*., 1986). Plant extracts are some of the most attractive sources for developing new drugs and have been shown to produce promising results in the treatment of gastric ulcers (Lewis and Hanson, 1991). Due to the high cost of orthodox medical care, rural dwellers and low-income earners depend mainly on traditional medicine for health care (WHO, 1991). This is an important reason to investigate antiulcer effect of medicinal plants with traditional use in gastric disease. *Dioscorea bulbifera Linn.* (Dioscoreaceae) is a Twining perennial herb found throughout the India. It has been used in Ayurveda and folk medicine for the treatment of purgative, deflatulent, aphrodisiac, rejuvenating and tonic anthelmintic and is used in hematological disorders, scrofula, hemorrhoids, flatulence, diarrhea, dysentery, worm infestations, general debility, diabetic disorders, polyuric and skin disorders (Sharma, 2005). Despite the popular use of this species as a medicinal plant, there are no data about its antiulcerogenic activity.

The present study has, therefore, been conducted (a) to evaluate the gastroprotective effect of the hydroalcoholic extract (HA) obtained from *Dioscorea bulbifera* tubers on NSAIDAs induced gastric lesions in rats; (b) to determine the enzyme activities, such as CAT, GPx, peroxidase and the levels of LPO and GSH in the stomach tissues of all treated groups.

Materials and Methods

Plant collection and Identification

The *Dioscorea bulbifera* gathered from Thovalai. This plant are authenticated in multi-centres such as Rabinat Herbarium, St. Joseph College - Trichy, St. Xavier's college - Palayamkottai and Botanical survey, CCRAS Unit - Chennai and Govt. Medical college - palayamkottai. The voucher specimens are deposited in the CARISM herbarium and maintained as follows *DB*-0062.

Plant Extract Preparation

The *Dioscorea bulbifera* tubers was shade dried for 15 days and is coarsely powdered using a pulverizer. The dried coarse powder of tubers was subjected to hydro alcoholic extraction in the ratio of water: alcohol as 30:70, adopting cold percolation method. The extracts are then dried in vacuum and stored in a refrigerator

Phytochemical Screening

The extract was subjected to preliminary Phytochemical screening by the method previously described by Sofowora (1994), Trease and Evans (1958) and Kokate (2000).

Animals

Healthy adult Wistar albino rats (150-250g) of either sex were obtained from the animal house at the CARISM, SASTRA University, Thanjavur. Housed individually in plastic cages, maintained under standard conditions (12-h light and 12-h dark cycle; 22 - 24^0 C), the animals were fed with standard rat pellet diet and provided tap water ad libitium. The experimental protocol was approved by the Institutional Animal Ethics Committee of CPCSEA.

Acute oral toxicity study

The procedure was followed as per OECD 425 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of rats and observed for signs of behavioral, neurological toxicity and mortality for 14 days (OECD/OCDE, 2000).

Non-steroidal anti-inflammatory drug (NSAID)-induced ulcer

In this model, after 12 h of fasting, the rats were randomly divided into six groups of six animals each. The first group was given 1ml of vehicle (1% aqueous solution of Tween-80), and the second group was treated with Cimetidine (100 mg/kg). The remaining three groups received 100, 200 and 400 mg/kg of *Dioscorea bulbifera* extract,

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respectively. The last one group is kept as normal control. All the treatments were administered orally for 1 week. One hour after last treatment, all the rats received Indomethacin (100 mg/kg) to induce gastric ulcer. Four hours after treatment with Indomethacin, the animals were sacrificed by cervical dislocation. The stomachs were removed, and opened along the greater curvature. The stomachs were gently rinsed with ice-cold saline. Then ulcer scoring was done. The stomach was also used to the estimation of free radical generation (Nwafor *et al*., 2000; Muriel *et al*. 2008).

Biochemical investigation of Stomach Tissues

After the macroscopic analyses, CAT, GPx, Peroxidase enzyme activities and the GSH and LPO levels in rat stomach tissues were determined. To prepare the tissue homogenates, the fundic part of the stomach was homogenized (5%) with Tris buffer (pH: 7.4) in ice-cold 0.9% saline by using a Potter-Elvehjem glass homogenizer for 30 sec. The homogenate was then centrifuged at 2500 rpm for 15 min and then supernatant collected. The obtained solution was used for determination of enzyme activities.

Catalase activity

The supernatant activity of Catalase activity was determined spectrophotometrically. The assay mixture consisted of 4ml phosphate buffer (0.01 M, pH 7.0), 0.2 ml hydrogen peroxide (0.2 M) and 0.5 ml of supernatant and 1.2 ml distilled water. Decomposition of H_2O_2 in presence of catalase was measured at 630 nm. Results are expressed as micromoles of hydrogen peroxide consumed per min per mg of protein (Aebi, 1974).

Glutathione peroxidase (GPx) activity

The reaction mixture consisted of 0.2 ml phosphate buffer (0.4 M, pH 7.0), 0.2 ml of EDTA (0.8 mM), 0.2 ml of sodium azide (10 mM), 0.2 ml of GSH (4 mM), 0.2 ml of H_2O_2 (20 mM) 0.5 ml dithio bis nitro benzoic acid (1 M) and 0.2 ml Supernatant. The absorbance was measured at 420 nm. The activity of GPx was determined by measuring the amount of oxidized GSH as nmol per min per mg protein. Results were expressed as nmol/min/mg protein (Wendel, 1981).

Estimation of Reduced Glutathione (GSH)

0.2 ml sample was mixed with 1.8 ml of precipitation reagent and it was centrifuged at 2500 rpm for 10 min. 1.0 ml supernatant was collected; to this add 4.0 ml 0.3 M phosphate buffer and 0.5 ml 0.01M DTNM reagent. The absorbance was read at 405 nm and the amount of glutathione is expressed as nmol GSH/g mucosal tissue (Ellman, 1959).

Estimation of Lipid peroxidation

Lipid peroxide content in gastric mucosal tissues was determined by Thio-barbituric acid reaction. The reaction mixer consisted of 0.2 ml of homogenate with 3.0 ml reagent, placed in serological water bath for 20 minutes at boiling temperature and then centrifuged at 3000 rpm for 7 minutes. Collect the supernatant and taken OD at 532 nm in UV-visible spectroscopy. The lipid peroxide concentration was expressed as MDA nmol/mg protein (Ohkawa *et al*. 1979).

Estimation of protein

The reaction mixer consisted of 0.01 ml of homogenate, 5 ml of distilled water and 2ml of Lowry's reagent. The tubes were allowed to stand for 1 hour and 0.2 ml of folin phenol reagent was added. The colour developed was read at 620 nm after 20 minutes. The values are expressed in mg/g of tissue (Lowry *et al*., 1951).

Results and Discussion

Phytochemical screening

Dioscorea bulbifera showed the presence of various Phytochemical constituents such as alkaloids, carbohydrate, protein, glycosides. The presence of alkaloids, carbohydrates and protein in *Dioscorea bulbifera* indicates the antioxidant activity of that plant.

Acute oral toxicity studies

An herbal extract of DB is used for the acute oral toxicological study for appreciating the

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usefulness of the chosen plant DB and in understanding the effects of the same in relation to the ulcer disease. The group of animals is administered by a single dose of the drug orally. They are found to be safe without mortality even up to 2000 mg/kg body weight. It reflects that LD_{50} determination of DB extract is

>1000 mg/kg body weight. The acute oral toxicological study does not show any deviation from the normal behavior of the albino wistar rats during the entire study period. Therefore, there are no acute toxicological changes for the herbal drug DB up to 2000 mg/kg body weight of the animal.

Gastroprotective effect of *Dioscorea bulbifera* tubers on Indomethacin - induced gastric damage in rats

Table 1. Gastro-protective effect of *Dioscorea bulbifera* tubers at 100, 200 and 400 mg/kg doses on Indomethacin

S. No	Treatment	N	Dose mg/kg body wt	Ulcer Index (mm^2)
1	Indomethacin	6	100	1.4
2	*Dioscorea bulbifera*	6	100	1.0
3	*Dioscorea bulbifera*	6	200	1.2
4	*Dioscorea bulbifera*	6	400	1.2
5	Cimetidine	6	100	0.75

The gastroprotective effect of *Dioscorea bulbifera* tubers at 100, 200 and 400 mg/kg doses on indomethacin induced gastric damage was macroscopically determined in rats (Table 1). The ulcer indexes in rats receiving *Dioscorea bulbifera* at doses of 100, 200 and 400 mg/kg were 1, 1.2 and 1.2 respectively. It indicates that *Dioscorea bulbifera* showed potent gastroprotective effect against NSAIDs induced gastric damage at all doses.

Comparison of enzyme activities in rat stomach tissues

In order to explore the effects of antioxidant defenses on the ulceration process, in all stomach tissues, the antioxidant levels (CAT, GPx, GSH and LPO) were evaluated. The results are presented in Table 2. This table shows that GPx, and GSH levels in indomethacin-administrated groups were lower as compared with that of healthy rat group. However, as compared with GPx and GSH levels, the opposite results were found for the levels of CAT and LPO in indomethacin administrated tissues. In contrast to indomethacin, all doses of DB and Cimetidine showed the increasing effect on the levels of GPx and GSH. Likewise, the activities of CAT enzymes, increased by indomethacin, were reduced by administration of all doses of DB and Cimetidine. lipid peroxidation (LPO) in rat stomach ($p < 0.05$). Table 2 also shows that, all doses of DB and Cimetidine had a significant decreasing effect on rats. Values are expressed as mean ± S.D. for six animals in each group. NS: Non-significant. LPO, nmol of MDA/ mg of protein; GSH, nmol GSH/mg of protein; GPx, change in OD/min/mg of protein; Catalase, change in OD/min/mg of protein; Cimetidine: 100 mg/kg of body weight.

Conclusion

In these present studies, reveals the hydro alcohol extract *Dioscorea bulbifera* tubers contains carbohydrates, glycoside, alkaloids and proteins and its physicochemical analysis (ash values and extractive values) shows the presence of foreign particles are within the limits as mentioned in pharmacopoeia. The acute toxicity study indicates that there is no observed adverse effect up to the dose of 2000 mg / kg body weight.

Table 2. Doses of DB and Cimetidine on rats

Parameters	Normal	Disease control	Low dose	Medium dose	High dose	Cimetidine
LPO	292.7±63.8	870.4±162.7*	739.4±129.3 NS	673.4±99.9**	653.9±111.4**	637.7±115.9**
GSH	275.0±113.4	266.9±98.08**	281.2±646.0*	282.6±306.4 NS	344.7±101.81*	277.9±34.24 NS
GPx	47.5±2.7	30.3±10.3 **	38.2±3.1**	38.6±3.3*	40.6±3.1*	38.8±4.0**
Catalase	0.01±0.04	0.05±0.003***	0.04±0.02*	0.03±0.01*	0.01±0.02***	0.02±0.03***
*-p<0.05, **p<0.1,***p<0.001						

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As shown in Table 1, the gastric lesions were significantly reduced by the administration of DB at 100, 200 and 400 mg/kg compared with the indomethacin-treated group. All doses of DB also showed a greater gastroprotective effect in comparison to Cimetidine, which is used as a H_2-receptor blocker. The gastric ulcer production by Indomethacin is due to the fact that this compound inhibits the synthesis of cytoprotective prostaglandins, synthesized by COX-1 and COX-2 in the stomach tissue (De Souza *et al*., 2002). Recently, it has been also shown that ROS possess an important role in the pathogenesis of mucosal damages caused by Indomethacin, ethanol and other agents besides the inhibition of COX enzymes (Das *et al*., 1997; Elliot and Wallace, 1998). Superoxides produced by peroxidases in the tissues might damage the membranes and stomach tissues by increasing the lipid peroxidation (Takeuchi *et al*., 1991; Miura *et al*., 2002). Similarly, our results showed that there was a significant increase in the LPO level in rat stomach tissues-administrated with Indomethacin. However, significant decreases in the LPO level were observed by the administration of all doses of DB and Cimetidine (100 mg/kg body weight). On the other hand, organisms have enzymatic and non-enzymatic defenses, including GSH, CAT, and GPx against to the lipid peroxidation in tissues, caused by ROS (Mates *et al*., 1999). Previously, it has been shown that the administration of NSAIDs and ethanol decreased the levels of GSH, and GPx in tissues (El-Missiry *et al*., 2001; Bafna and Balaraman, 2004). Similarly, in the present study, the levels of GPx and GSH in rat stomach tissues were significantly reduced by administration of Indomethacin (Table 2). Contrarily, the administration of DB at 100, 200 and 400 mg/kg and ranitidine (100 mg/kg) resulted in a significant increase in the GPx and GSH levels. However, according to present results, the level of CAT was found to be increased in Indomethacin-treated rat tissues as compared with tissues of healthy rat (Table 2). This increase may be due to an increase in the mucosal H_2O_2 and OH^- level, occurred by inhibition of peroxidases (Banarjee, 1990). The activities of these enzymes were lowered by the administration of all doses of DB, which can be attributed to the decrease in the tissue H_2O_2 and OH levels. In view of the present results, it can be concluded that DB and Cimetidine possess a reducing effects against the oxidative damage in rat stomach tissues.

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