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Anti-Ulcerogenic Activity of Two Extracts of *Parquetina nigrescens* and their Effects on Mucosal Antioxidants Defence System on Ethanol-Induced Ulcer in Rats

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Absract: The effect of two extracts of *Parquetina nigrescens* on mucosal antioxidants defense system in ethanol-induced ulcer in rats was studied. Activities of superoxide dismutase (SOD), catalase (CAT) and levels of reduced glutathione (GSH) were determined in the gastric mucosa and liver of normal and experimental groups of rats. The rats were pretreated with 500 and 1000 mg kg^{-1} of hexane and chloroform extracts of *P. nigrescens*, respectively dissolved in olive oil for a period of 14 days prior to ethanol induction. It was found that prior to ulcer induction, 14 days pretreatment with hexane and chloroform extract *P. nigrescenes* significantly reduced ethanol-induced gastric damage. The levels of GSH and activities of the antioxidants enzymes (SOD and CAT) were depressed significantly ($p<0.05$) in the ulcerated rats when compared with that of normal control. The activity of SOD was lower significantly ($p<0.05$) in the ulcerated mucosa and liver of the experimental rats when compared to the normal control group. There was a significant increase ($p<0.05$) in the level of CAT in the groups pretreated with the extracts compared to the ethanol group. A similar result was observed for GSH. Pretreatment with hexane extract caused 75.43 and 74.55% elevations in the activities of SOD in the mucosa and liver homogenate, respectively. Similar elevations were observed in the group pretreated with the chloroform extract. The cimetidine group also caused 69.79 and 69.67% elevation in the SOD activity in the mucosa and liver homogenate, respectively. The pretreatment with *P. nigrescens* was found to exact a significant gastro protective and antiulcer effect partly by protecting against the ethanol-induced ulcerogenic effects in experimental rats and probably through the induction of antioxidant enzymes.

Key words: *Parquetina nigrescens*, antioxidant enzymes, mucosa, antiulcer activity, free radicals

INTRODUCTION

There is evidence concerning the participation of reactive oxygen species in the etiology and physiopathology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such

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as gastrointestinal inflammation and gastric ulcer. The role of these reactive oxygen species in several diseases and the potential antioxidant protective effect of natural compounds on affected tissues are topics of current interest (Repetto and Llesuy, 2002). Free radicals are constantly being generated in the body as a result of normal metabolic processes; damaged due to free radicals is countered by antioxidants. Sometimes excessive free radical formation occurs in the body and the oxidant systems in the body cannot cope with the situation. This situation is known as oxidative stress. This oxidative stress is a general term used to describe a state of potential oxidative damage caused by the free radicals (Priscilla and Heather, 2000). Lutomski *et al.* (1991) have shown that oxygen derived free radicals are directly implicated in ulcerogenesis and use of antioxidants may lead to gastro protective effects.

Peptic ulceration is one of the common diseases affecting millions of people. It is now considered to be one of the modern age epidemics affecting nearly 10% of world population. Research advances during last decade have offered new insights in the therapy and prevention of peptic ulceration. Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess antiulcer properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved antiulcer drugs (Shah *et al*., 2006).

Parquetina nigrescens (Periplocaceae) a shrub found in equatorial West Africa (Irvine, 1961; Mabberly, 1987) has been in traditional medicine practice for centuries. The leaves, roots and the latex are the parts of the plants used for traditional medicine (Gill, 1992). In Oyo state Nigeria, the leaves have been reputed for the treatment of helminotocasis (intestinal worm) while the roots are reputed for use as antirheaumatic purpose (Adeyemi, 1994). Over the years, *Parquetina nigrescens* has been used as an ingredient in the medications for in-sanity (Iwu, 1993) and as aphrodisiac in East Africa (Kokwaro, 1976). Decoction of the bark is given as a cardioctonic, while the whole plant is used to stupefy fish in Ghana and Liberia, the leaves and latex are used for treatment of rickets, diarrhea, skin lesions and tropical skin diseases (Gill, 1992; Oliver, 1960). The leaves of *P. nigrescens* have been used for the treatment of wound in Africa (Irvine, 1961; Mabberly, 1987) and have sympathomimetic effect (Datte *et al*., 1999). This study is designed to investigate the gastroprotective and antiulcer property of the Hexane and chloroform extracts of *Parquetina nigrscens* in ethanol-induced ulcer in experimental animals.

MATERIALS AND METHODS

Animals and Treatment

Adult male albino Wister strain rats (150-250 g) were used for the study. They were obtained from the animal house, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were bred and housed in the Primate Colony Department of Biochemistry, University of Ibadan, Nigeria. They were kept in wire meshed cages and fed with commercial rat chow (Ladokun Feeds Nigeria Limited) and liberally supplied with clean water.

Thirty-five rats were divided into seven groups of five animals each. Groups A, B C and D were pretreated with 500 mg kg^{-1} hexane, 1000 mg kg^{-1} hexane, 500 mg kg^{-1} chloroform and 1000 mg kg^{-1} chloroform extracts of parquetina nigrescens, respectively. Groups E and F were normal and ethanol groups received physiological saline. Group G was given a standard ulcer drug cimetidine (50 mg kg^{-1}). All treatments were administered orally for 14 consecutive days (once daily). The animals were sacrificed 24 h after the last treatment. They

were deprived of food but allowed free access to water until 1 h before they were challenged with ethanol administration (Rodriguez *et al*., 2007).

Plant Material

Fresh leaves of *P. nigrescens* were collected within the premises of University of Ibadan. They were identified and authenticated in the Herbarium at the Department of Botany, University of Ibadan.

Preparation of Extract

The leaves were air dried in the laboratory so as to avoid the loss of bioactive constituents which may be destroyed by irradiation. After drying, the sample was ground into powder. The powdery form was soxhlet extracted with hexane and chloroform to give 8.15 and 2.58% (w/w) yield, respectively.

Acute Toxicity

The acute toxicity of *P. nigrescens* was evaluated and LD_{50} value was calculated according to the method of modified by Sheth *et al.* (1972). The male mice were observed 4 h after oral administration and twice daily for 7 days for signs of behavioral changes and mortality.

Ulceration Index

One hour after absolute ethanol challenge to the animals, they were sacrificed by cervical dislocation. The livers were removed for subsequent biochemical assays. The stomachs were also removed and opened along the greater curvature and their lesions were examined and scored (mm^2) macroscopically, using a hand lens (Nwafor *et al*., 2000). The gastric mucosa was scrapped with glass slides and stored at 4°C for subsequent biochemical assays. Ulcer Index (UI) and percentage inhibition were calculated thus:

$$UI = \frac{\text{Total ulcer score}}{\text{No. of animals ulcerated}}$$

$$\text{Percentage inhibition} = \frac{\text{UI of ethanol treated group - UI of pretreated groups}}{\text{UI of ethanol treated group}} \times 100$$

Preparation of Tissue Homogenate

The liver and gastric mucosa scrapings were weighed and homogenized in 4 volume of ice cold isotonic phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min at 4°C using cold centrifuge. The resultant supernatants were used for subsequent biochemical assays (Agnihotri *et al*., 2007).

Protein Determination

Protein content of all fractions was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

Determination of Antioxidant Enzyme Activities

Catalase (CAT) activity of the liver and the gastric mucosa homogenate were determined according to the procedure of Clairborne (1989) by following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 250°C.

The procedure of Misra and Fridovich (1972) as described by Magwere *et al.* (1997) was used for the determination of superoxide dismutase (SOD) activity by measuring the inhibition of autooxidation of epinephrine at pH 10.2 at 30°C.

Determination of Reduced Glutathione (GSH)

The content of reduced glutathione (GSH) was estimated according to Jollow *et al.* (1974).

Statistical Analysis

All data are expressed as Mean±SD. Statistical analysis was performed using the student t-test. Values of $p<0.05$ were considered significant (Bamgboye, 2002).

RESULTS

Table 1 shows the ulcer scores obtained from rats induced experimentally with absolute ethanol after 14 days pretreatment with the hexane and chloroform extracts of *P. nigrescens* and cimetidine. Both the extracts and cimetidine showed a significant reduction ($p<0.05$) in the severity of the ulcer damage caused by ethanol induction. The 1000 mg kg^{-1} chloroform extract reduced the ulcer index from (23.0 ±5.57) mm^2 in the ethanol group to (11.0±2.83) mm^2. While the 1000 mg kg^{-1} chloroform extract reduced the ulcer index from (23.0±5.57) to (6.7±3.51) mm^2. The cimetidine group also effected reduction from (23.0±5.57) mm^2 to (8.5±1.73) mm^2. The results obtained from the pretreatment with the extracts are comparable to that of the cimetidine. The 1000 mg kg^{-1} chloroform extract tend to show a greater significant decrease ($p<0.05$) than cimetidine.

The effects of the pretreatment of rats with the extracts on gastric mucosal superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) levels are shown in (Table 2). Significant reductions ($p<0.05$) were observed in the activities of the SOD

Table 1: ULCER SCORES $(mm)^2$ of rat induced experimentally with absolute ethanol

Experimental groups	Ulcer index $(mm)^2$	Inhibition (%)
Normal Control	0.0±0.00	_
Ethanol (1 mL/200 g)	23.0±5.57	_
Cimetidine (50 mg kg^{-1}) + Ethanol	8.5±1.73	63.0
HE_1 (500 mg kg^{-1}) + Ethanol	8.7±2.08	62.2
HE_2 (1000 mg kg^{-1}) + Ethanol	11.0±2.83	52.2
CE_1 (500 mg kg^{-1}) + Ethanol	15.7±6.35	31.7
CE_2 (1000 mg kg^{-1}) + Ethanol	6.7±3.51	70.9

Table 2: Effects of *P. nigrescens* extracts on gastric mucosal CAT, SOD and GSH

Experimental groups	Gastric Mucosal CAT activity (nmol of H_2O_2 decomposed/min/mg protein)	Gastric Mucosal SOD activity (U/min/mg protein)	Gastric Mucosal GSH (mg mg^{-1} protein)
Normal Control	7.25±0.28	13.75±1.48	0.96±0.018
Ethanol (1 mL/200 g)	5.45±0.32	6.15±0.74	0.55±0.011
Cimetidine (50 mg kg^{-1}) + Ethanol	7.09±0.31^{ab}	20.36±2.67^{ab}	1.17±0.013^{ab}
HE_1 (500 mg kg^{-1}) + Ethanol	9.04±0.26^{abc}	25.12±2.15^{abc}	2.12±0.015^{abc}
HE_2 (1000 mg kg^{-1}) + Ethanol	11.43±0.39^{abc}	20.00±1.16^{ab}	2.00±0.010^{abc}
CE_1 (500 mg kg^{-1}) + Ethanol	8.46±0.20^{ab}	21.40±2.18^{ab}	1.15±0.013^{ab}
CE_2 (1000 mg kg^{-1}) + Ethanol	8.86±0.25^{ab}	23.86±2.14^{abc}	1.78±0.013^{abc}

Student t-test was used in the analysis of this data. The results are expressed as Mean±SD (n = 5); $^a p<0.05$ significantly different from the control group; $^b p<0.05$ significantly different from the ethanol group; $^c p<0.05$ significantly different from the cimetidine group

Table 3: Effects of *P. nigrescens* extracts on Liver CAT, SOD and GSH

Experimental groups	Liver CAT activity (nmol of H_2O_2 decomposed/min/mg protein)	Liver SOD activity (U/min/mg protein)	Liver GSH (mg mg^{-1} protein)
Normal control	20.12±0.42	12.86±2.30	0.52±0.019
Ethanol (1 mL/200 g)	11.36±0.37	6.21±1.37	0.21±0.011
Cimetidine (50 mg kg^{-1}) + Ethanol	37.26±0.96^{ab}	20.47±2.68^{ab}	0.67±0.026^{ab}
HE_1 (500 mg kg^{-1}) + Ethanol	48.53±0.43^{abc}	24.40±2.22^{abc}	0.90±0.015^{abc}
HE_2 (1000 mg kg^{-1}) + Ethanol	56.26±0.59^{abc}	20.20±2.16^{ab}	0.75±0.019^{ab}
CE_1 (500 mg kg^{-1}) + Ethanol	30.66±0.90^{ab}	20.96±2.12^{ab}	1.00±0.017^{abc}
CE_2 (1000 mg kg^{-1}) + Ethanol	32.54±0.72^{ab}	21.00±1.96^{ab}	0.95±0.016^{abc}

Student t-test was used in the analysis of this data. The results are expressed as Mean±SD (n = 5); $^{a}p<0.05$ significantly different from the control group; $^{b}p<0.05$ significantly different from the ethanol group; $^{c}p<0.05$ significantly different from the cimetidine group

and CAT and GSH levels in the ethanol group when compared to the normal control group. Pretreatment with *P. nigrescens* hexane extract (500 mg kg^{-1}) and Cimetidine (50 mg kg^{-1}) caused an elevation by 308 and 231% for SOD, 65.87 and 30.09% for CAT activities respectively and 285 and 113% for GSH levels when compared with the ethanol group. The chloroform extracts (500 mg kg^{-1}) and (1000 mg kg^{-1}) also caused an increased in activities ($p<0.05$), 248 and 288% for SOD, 55.23 and 62.57% for CAT and 109 and 224% for GSH levels when compared to the ethanol group.

In the liver homogenate, similar results were observed as there was a significant depletion ($p<0.05$) in the activities of SOD and CAT and GSH levels in the ethanol group as shown in (Table 3). This depletion was reversed with pretreatment with P. nigrescens extracts and the results comparable with cimetidine. The chloroform extract (1000 mg kg^{-1}) and cimetidine (50 mg kg^{-1}) caused an increase by 238 and 230% for SOD, 186 and 228% for CAT activities, 352 and 219% for GSH levels, respectively when compared to the ethanol group. The Hexane extracts (500 mg kg^{-1}) and (1000 mg kg^{-1}) caused an elevation by 293 and 225% for SOD, 327 and 395% for CAT activities 329 and 257% for GSH levels when compared to the ethanol group.

DISCUSSION

The hexane extract of *P. nigrescens* was well tolerated by mice (used for the acute toxicity test) through the oral route of administration. The LD_{50} of *P. nigrescens extract* was obtained to be 7.5 g kg^{-1} b.wt. The considerable high LD_{50} of 7.5 g kg^{-1} b.wt. obtained for *P. nigrescens* hexane extract suggest that administration by oral route is relatively safe. Hayes (1987) and Brock *et al.* (1995) stated that doses for LD_{50} higher than 5 g kg^{-1} b.wt. are generally not considered as dose related toxicity.

In this study, absolute ethanol (1 mL/ 200 g b.wt.) induced gastric ulcer in all treated animals and this agrees with reports in other previous studies (Lee *et al.*, 2005; Shah *et al.*, 2006). The significant percentage inhibition of ulceration shown by the groups pretreated with cimetidine and *P. nigrescens* extracts suggest that these treatment agents have gastroprotective properties.

Phytochemical studies of *P. nigrescens* have revealed the presence of bioactive substances such as Alkaloids, Tannins, Saponins, Flavonoids and Glycosides etc (Kokwaro, 1993). Research has also shown that these constituents of plants can function as natural antioxidants (Free radical scavengers) and thereby preventing diseases (Halliwell *et al.*, 1999). *Parquetina nigrescens* contains active constituents which are natural antioxidants, especially flavonoids which exhibits a wide range of biological effects.

The significantly decreased ($p<0.05$) activities of the ROS scavenging enzymes, viz CAT and SOD observed in rats by acute exposure to ethanol, conform to previous work of Coskun *et al.* (2004). These decreased activities may be due to continuous usage of the enzymes, or could be due to a direct effect of aldehyde formed from oxidation of ethanol on these enzymes (Sandhir and Gill, 1999). Present results revealed that *P. nigrescens* extracts could induce the activities of these antioxidant enzymes and also reduce the free radicals generation and gastric mucosa damage. This induction will enhance the free radical scavenging property of the mucosa. The liver homogenate showed increased activities of CAT and SOD in the groups pretreated with *P. nigrescens* extracts and cimetidine. This suggests that, as *P. nigrescens* extracts potentiate gastroprotective and antioxidant status of the mucosa, they simultaneously improve the antioxidant status of the liver, likely by the induction of these enzymes.

The ingestion of alcohol is known to increase lipid peroxidation and lead to membrane damage. In defense against this type of membrane damage several systems have evolved including glutathione (GSH) system. Glutathione is involved in three types of mechanisms: catabolism of the hydroperoxide formed, conjugation with reactive xenobiotics and as a direct free radical scavenger. Glutathione protects the cell from oxidative damage. We observed a significant ($p<0.05$) increase in the level of reduced glutathione in the pretreated groups when compared to the ethanol group which suggests that the extracts and cimetidine also protect from oxidative damage through the induction of glutathione. There seems to be a correlation between the degree of ulcer inhibition by the extracts of *P. nigrescens* and the elicited increases in the antioxidant enzymes. Therefore, we conclude that the results of our findings has enabled us to achieve the aim of this work which was to investigate the gastroprotective and anti-ulcer property of the hexane and chloroform extracts of our plant *Parquetina nigrescens*.

In summary, our data suggest that the leaf extract of *P. nigrescens* appears to be effective against gastric ulcer induced by ethanol. We speculate that the gastro protective and antiulcer effect could be attributed to free radical scavenging property of the plant extract, inhibition of gastric acidity and strengthening of the gastric mucosal barrier through antioxidant enzyme induction.

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