International Journal of Tropical Medicine 6 (2): 25-29, 2011
ISSN: 1816-3319
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Antioxidant Properties of Methanol Extract of Parquetina Nigrescens in Ulcerated Rats

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Abstract: *Parquetina nigrescens* is a plant used in folk medicine in treating gastric ulcer. This study investigated the antioxidant properties of *P. nigrescens* in ethanol ulcerated rats. Rats were pretreated for 7 or 14 days with graded doses (500 and 1000 mg kg^{-1}) of methanol extract of *P. nigrescens* before ulcer was induced on 8th or 15th day with absolute ethanol (1 mL/rat). The antioxidant properties of *P. nigrescens* were assessed using standard procedures. Intoxication with ethanol caused increased lipid peroxidation, reduced activities of Super Oxide Dismutase (SOD) and Catalase (CAT) and reduced protein concentration. Pretreatment with *P. nigrescens*, however, lowered lipid peroxidation, caused increases in activities of SOD, CAT and protein levels. This study therefore, shows that *P. nigrescens* possesses antioxidant properties that can protect against free radical induced ulcer.

Key words: *Parquetina nigrescens*, SOD (Superoxide Dismutase), CAT (Catalase), ethanol, ulcer, Nigeria

INTRODUCTION

Ulcer is circumscribed erosion of mucosal tissue that occurs at various sites in the gastrointestinal tract which are exposed to gastric juice. Excessive ethanol ingestion, administration of Non Steroidal Anti-Inflammatory Drugs (NSAIDS), colonization of the stomach with Helicobacter pylori (Carter, 1980) are etiological factors that give rise to gastritis characterized by mucosal edema, sub-epithelial hemorrhage, cellular exfoliation and inflammatory cell infiltration (Kvietys and Beveleign, 1990). These are pertinent to the disruption of the vascular endothelium, resulting in increased vascular permeability and epithelial lifting.

Cytochrome P_{450E1} is involved in the metabolism of ethanol. During alcohol metabolism free radicals and its intermediate by-products are generated. The increase in the concentration of reactive oxygen species tends to disturb the antioxidant/oxidants equilibrium and hence contributes significantly to the pathogenesis of peptic ulcer (Pihan *et al*., 1987; Banerjee *et al*., 1994). Polyphenolic compounds are used against various diseases that are related to inflammatory reactions generated by free radicals. This, however is significant in stemming reactive oxygen species production in ulcer induced by alcohol. Therefore, raising antioxidant status in the biological system would probably be prophylactic or therapeutic in medical intervention of free radical induced ulcer.

Parquetina nigrescens is a plant used in folk medicine in many countries (Gill, 1992; Iwu, 1993; Sofowora, 1993a; Burkill, 1997). Some scientific findings have confirmed the efficacy of this plant in the treatment of some diseases such as anemia (Agbor and Odetola, 2001). It is one of the herbs commonly used in the South Western part of Nigeria for the treatment of gastrointestinal disorders (Odetola *et al*., 2006). Different part (leaves, bark, latex and roots) are also used as constituents of medications used for treatment of diverse diseases such as rickets, diarrhea, skin lesions, menstrual disorders and gonorrhea (Sofowora, 1993a, b). *Parquetina nigrescens* has been used for wounds in Africa (Irvine, 1961; Mabberly, 1987) and has sympathomimetic effects (Datte *et al*., 1999). This study investigates the antioxidant properties of the methanol extract of *Parquetina nigrescens* in the prevention of gastric ulcers.

MATERIALS AND METHODS

Plants: The dried leaves of *Parquetina nigrescens* were screened for phytochemicals. The method of Miller and Tainter (1944) was used to test the toxicity of *P. nigrescens* in mice.

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Preparation of extracts

Methanol extract: About 200 g of powdered shade dried leaves of *Parquetina nigrescens* was soxhlet extracted for 16 h and evaporated in vacuum at 40°C. The crude methanol extract (141 g) was stored at 4°C throughout the experiment.

Experimental design

Animal experiment: About 50 male albino rats were used for this study. A two by five factorial design was employed in grouping the animals. This composed of two large groups A and B, each with five sub-groups. Group A was pretreated for 7 days while group B was pretreated for 14 days. The five sub-groups are described as follows according to the treatment they received:

- Group 1: Control group: Normal diet
- Group 2: Ulcer group: Normal diet+1 mL ethanol 1 h before sacrifice
- Group 3: Cimetidine group: Normal diet+15 mg kg^{-1} cimetidine 1 h before giving ethanol+1 mL ethanol 1 h before sacrifice
- Group 4: *P. nigrescens* (1000 mg kg^{-1}) group: Normal diet+daily pretreatment with 500 mg kg^{-1} plant extract+1 mL ethanol 1 h before sacrifice
- Group 5: *P. nigrescens* (500 mg kg^{-1}) group : Normal diet+daily pretreatment with 1000 mg kg^{-1} plant extract+1 mL ethanol 1 h before sacrifice

Assessment of gastric ulceration by means of scoring technique: The animals were fasted overnight and gastric ulcer was induced by oral administration of 1 mL ethanol per rat. The animals were left for 1 h before they were sacrificed. The scoring of the gastric ulceration was done according to the method described by Alphin and Ward (1967) and used by Elegbe (1978) (Table 1).

Preparation of gastric juice and gastric mucosal: The animals were anesthetized by intraperitoneal injection of 0.6 mL of Urethane per 100 g of body weight. A lateral dissection of the abdominal region was carried out with the animal placed on dissecting board and the stomach removed. The stomachs were dissected from the pylori region along the greater curvature and the gastric content was emptied into a beaker. This was centrifuged at 200 rpm for 15 min and the supernatant decanted as gastric juice. The surface mucosal of the stomachs was scrapped using a blunt knife into a beaker and made into solution using phosphate buffer this was centrifuged and supernatant decanted for assays.

Table 1: Scoring of gastric ulceration according to method described by Alphin and Ward (1967)

Ulcer score	Criteria
0	Normal stomach
0.5	Punctuate hemorrhage or pin-point ulcer
1	Two or more hemorrhagic ulcers
2	Ulcers >3 mm in diameter

Biochemical assays: Phytochemical screening for alkaloids, saponins, flavonoids, tannins, anthraquinones and phylobatanin were carried out according to the methods of Sofowora (1993a, b), Harborne (1993) and Trease and Evans (2002). Lipid peroxidation was assayed using the procedure of Rice-Evans *et al.* (1986). Protein concentration was determined using Biuret reaction as described by Gornall *et al.* (1949). Catalase was estimated by the method of Sinha (1972). The level of Superoxide Dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972).

RESULTS AND DISCUSSION

Phytochemical studies revealed that *Parquetina nigrescens* contains bioactive ingredients such as flavonoids, anthraquinones, alkaloids, tannins, saponin and phylobatanin as shown in Table 2. Figure 1 shows the result of the acute toxicity test. *Parquetina nigrescens* has LD50 of 4.5 g kg^{-1}. Table 3 shows the mean ulcer score in the different animal groups. Mean ulcer score was significantly reduced with 1000 or 500 mg kg^{-1} extract pretreated (3.5±0.004 and 5.0±0.006) animals compared with the ethanol ulcerated rats (9.4±0.009). The ulcer score in animals pretreated for 14 days are not significantly different from that in 7 days before induction of ulcer. Table 4 shows the effect of the pretreatment with *P. nigrescens* for 7 days before ethanol intoxication on lipid peroxidation, protein concentration and activities of antioxidant enzymes (SOD and CAT) in the gastric juice of ethanol intoxicated rats. Ethanol intoxication caused

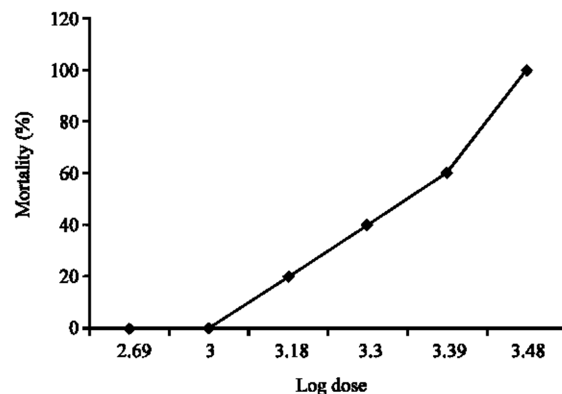

Fig. 1: The LD50 of *P. nigrescens*

significant increases in lipid peroxidation, i.e., from 0.065±0.001 in the normal group to 0.192±0.068 MDA^{-1} tissue in the ethanol intoxicated group. It also depressed significantly ($p<0.05$) the concentration of protein in normal group (16.8±0.90 mg mL^{-1} tissue) compared to ulcer group (12.7±0.82 mg mL^{-1} tissue) and activities of antioxidant enzymes; SOD (1.11±0.017-0.764±0.001 min^{-1} mg^{-1} protein) and CAT (0.061±0.022-0.035±0.011 min^{-1} mg^{-1} protein).

Pretreatment with *P. nigrescens* reversed these alterations to values that were not significantly different ($p<0.05$) from normal values. For instance, lipid peroxidation in the 500 and 1000 mg kg^{-1} exract pretreated groups was reduced to 0.068±0.004 and 0.069±0.004 MDA^{-1} tissue, respectively. The action of *P. nigrescens* was not dose dependent as values were not significantly different in the 500 mg kg^{-1} extract and 1000 mg kg^{-1} extract pretreated groups e.g. Catalase activity in gastric juice of the 1000 mg kg^{-1} pretreated group was 0.089±0.038 while it was 0.087±0.03 min^{-1} mg^{-1} protein in the 500 mg kg^{-1} pretreated group.

Table 5 shows the effect of the pretreatment with *P. nigrescens* for 7 days on lipid peroxidation, protein concentration and activities of antioxidant enzymes (SOD and CAT) in the gastric mucosa homogenate. Intoxication with ethanol induced similar effects as observed in the gastric juice. The effect of extract on parameters studied was similar in the gastric mucosa homogenate and gastric juice.

Table 6 and 7 shows the effects of the pretreatment with *P. nigrescens* for 14 days on lipid peroxidation, protein concentration and activities of antioxidant enzymes (SOD and CAT) in the gastric juice and gastric mucosa homogenate of ethanol intoxicated, respectively.

The anormaly caused by alcohol intoxication was corrected as a result of pretreatment with *P. nigrescens*. However, the values were also reduced compared with pretreatment for 7 days indicative of possible toxic effects resulting from prolonged treatments.

The present study evaluates the effect of methanolic extract of *Parquetina nigrescens* on gastric cyto-protection and antioxidant properties. It has been suggested that oxygen derived radicals, mast cell excretory products, breakdown of the mucosa lead to lipid peroxidation in the mucosa lining (Terano *et al*., 1986). This is consistent with the finding of this study as administration of ethanol increased the level of lipid peroxidation product (MDA) in the gastric juice and mucosal homogenate. Pretreatment with extract of *P. nigrescens* reduced the lipid peroxidation product (MDA) formed showing that the methanol extract of *P. nigrescens* is cytoprotective. The standard drug,

Table 2: Phytochemical screening of the leaves of *P. nigrescens*

Test	Result	Percentage
Tannin	+	1.27
Phylobatanin	+	1.58
Saponin	+	0.14
Anthraquinone	+	0.09
Flavonoids	+	0.13
Alkaloids	+	1.72

+Positive test (present)

Table 3: Ulcer scoring in the different animals groups

Groups	No. of animals	Ulcer score in 7 day pretreated rats Mean±SD	Ulcer score in 14 day pretreated rats Mean±SD
Control	5	0.0	0.0
Ulcer	5	7.5±0.004	9.4±0.009
Cimetidine	5	2.1±0.003*	2.2±0.001*
1000 mg kg^{-1}extract	5	3.1±0.002*	3.5±0.004*
500 mg kg^{-1} extract	5	4.8±0.003*	5.0±0.006*

*Significantly lower than ulcer group at $p<0.05$; SD: Standard Deviation

Table 4: Effect of 7 days pretreatment with methalonic extract of *P. nigrescens* on lipid peroxidation, protein concentration and antioxidant enzymes in gastric juice of ulcerated rats

Groups	Lipid peroxidation (MDA^{-1} tissue)	Protein concentration (mg mL^{-1} tissue)	Catalase activity (Units mg^{-1} protein)	SOD activity (Units mg^{-1} protein)
Control	0.065±0.001	16.8±0.90	0.061±0.022	1.11±0.017
Ulcer	0.192±0.068**	12.7±0.82	0.035±0.011**	0.76±0.001**
Cimetidine	0.074±0.003	18.8±1.02	0.059±0.019	1.86±0.011
1000 mg kg^{-1} extract	0.069±0.040*	28.5±1.38*	0.089±0.038*	2.04±0.018*
500 mg kg^{-1} extract	0.068±0.004*	32.0±1.19*	0.087±0.030*	2.22±0.001*

Values are Mean±SD of 5 rats. SD = Standard Deviation. SOD: Superoxide Dismutase; **Significantly different from normal control at $p<0.05$; *Significantly different from ulcer group at $p<0.05$

Table 5: Effect of 7 days pretreatment with methalonic extract of *P. nigrescens* on lipid peroxidation, protein concentration and antioxidant enzymes in gastric mucosa homogenate of ulcerated rats

Groups	Lipid peroxidation (MDA^{-1} tissue)	Protein concentration (mg mL^{-1} tissue)	Catalase activity (Units mg^{-1} protein)	SOD activity (Units mg^{-1} protein)
Control	0.070±0.003	16.8±1.05	0.059±0.017	1.054±0.120
Ulcer	0.201±0.071**	11.0±0.81**	0.031±0.024**	0.810±0.011
Cimetidine	0.078±0.005	19.0±1.33	0.063±0.074	1.105±0.099
1000 mg kg^{-1} extract	0.080±0.006*	32.0±1.72*	0.091±0.047*	2.112±0.015*
500 mg kg^{-1} extract	0.096±0.005*	28.5±1.28*	0.089±0.025*	2.091±0.109*

Values are Mean±SD of 5 rats. SD = Standard Deviation. SOD: Superoxide Dismutase; **Significantly different from normal control at $p<0.05$; *Significantly different from ulcer group at $p<0.05$

Table 6: Effect of 14 days pretreatment with methalonic extract of *P. nigrescens* on lipid peroxidation, protein concentration and antioxidant enzymes in gastric juice of ulcerated rats

Groups	Lipid peroxidation (MDA $^{-1}$ tissue)	Protein concentration (mg mL^{-1} tissue)	Catalase activity (Units mg^{-1} protein)	SOD activity (Units mg^{-1} protein)
Control	0.073±0.002	16.8±1.20	0.041±0.010	1.028±0.011
Ulcer	0.201±0.079**	10.4±0.89**	0.022±0.004**	0.065±0.070**
Cimetidine	0.058±0.003	25.0±1.40	0.050±0.019	0.042±0.160
1000 mg kg^{-1} extract	0.085±0.006*	27.0±0.98*	0.074±0.016*	2.194±0.210*
500 mg kg^{-1} extract	0.092±0.005*	27.5±1.12*	0.060±0.008*	2.100±0.210*

Values are Mean±SD of 5 rats. SD: Standard Deviation, SOD: Superoxide Dismutase; **Significantly different from normal control at $p<0.05$; *Significantly different from ulcer group at $p<0.05$

Table 7: Effect of 14 days pretreatment with methalonic extract of *P. nigrescens* on lipid peroxidation, protein concentration and antioxidant enzymes in gastric mucosa homogenate of ulcerated rats

Groups	Lipid peroxidation (MDA^{-1} tissue)	Protein concentration (mg mL^{-1} tissue)	Catalase activity (Units mg^{-1} protein)	SOD activity (Units mg^{-1} protein)
Control	0.072±0.040	16.8±1.10	0.052±0.001	1.067±0.103
Ulcer	0.203±0.014**	11.5±0.81**	0.030±0.010**	0.672±0.015**
Cimetidine	0.065±0.070	23.0±1.34	0.055±0.018	1.24±0.0100
1000 mg kg^{-1} extract	0.095±0.006*	25.0±1.02*	0.068±0.019*	1.997±0.097*
500 mg kg^{-1} extract	0.096±0.006*	26.5±1.22*	0.058±0.070*	2.003±0.020*

Values are Mean±SD of 5 rats. SD: Standard Deviation. SOD: Superoxide Dismutase; **Significantly different from normal control at $p<0.05$; *Significantly different from Ulcer group at $p<0.05$

Cimetidine reduced the level of MDA more than the extract but the reduction brought about by the extract showed that it is equally effective in reducing lipid peroxidation. However, the observed slight increase in the level of lipid peroxidation in 14 days pretreatment group compared to 7 day pretreatment group could be as a result of the prolonged use of extract. Thus a shorter period of exposure could be safer.

Treatment with absolute ethanol has been shown to damage epithelial cells which leads to reduction in protein concentration (Kaunitz, 1999). The mucous membrane which acts as the first layer of defense of stomach tissue was also eroded by ethanol. The gastric mucosa prevents contact between the digestive enzymes such as pepsin and the stomach wall (Kaunitz, 1999). Similar observation was made in this study as the administration of ethanol reduced the protein concentration by 38.09%. Pretreatment with the extract enhanced the generation of epithelial cells that gave rise to significant increase in protein concentration (40%) in the gastric secretion of the pretreated group (1000 mg kg^{-1}).

It is now currently held that superoxide anion (O_2) is involved in ulcer formation (Smith and Kvietys, 1988). The decrease in SOD activity in rats exposed to reactive oxygen metabolite has been associated with a number of diseases such as ischemia reperfusion injury and inflammation in some organs (Halliwell, 1990; Parks *et al.*, 1983).

Superoxide and hydroxyl radicals are important mediators of oxidative stress that play vital role in some clinical disorders. Any compound, natural or synthetic with antioxidant activities might contribute towards the total/partial alleviation of such damage. Therefore, removing superoxide and hydroxyl radical could contribute to defense of a living body against disease (Jung *et al.*, 1999; Cao *et al.*, 2000). Superoxide Dismutase (SOD) converts superoxide to hydrogen peroxide and subsequently, catalase converts hydrogen peroxide to water. Reduced activities of SOD and CAT were observed in the gastric secretion and gastric mucosa homogenate of ulcerated rats. This might have resulted from their utilization for the decomposition of superoxide anion generated by lipid peroxidation. Lowered activities of these enzymes may result in a number of deleterious effects. Pretreatment with the methanol extract of *Parquetina nigrescens* increased the activities of SOD and CAT which may be the mechanism of action of the observed reduction in lipid peroxidation. This also justifies the use of *P. nigrescens* in folk medicine for the prevention/treatment of ulcer.

CONCLUSION

As shown from the results of this study, intoxication with ethanol increased the mean ulcer score and lipid peroxidation with a concurrent decrease in the protein concentration and the activities of antioxidant enzymes (SOD and CAT). Pretreatment with methanolic extract of *P. nigrescens* reversed all these alterations which suggests that the methanolic extract of *P. nigrescens* has protective property against ethanol induced ulceration.

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