

Antioxidant Effect of *Carica papaya* on Ethanol Induced Gastric Lesion in Adult Male Wistar Rats

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Abstract: This study was performed to elucidate the role of some important constituents of antioxidant defence such as Glutathione Peroxidase (GPx), Thiobarbituric Acid Reaction (TBAR), the activity of the enzyme Glucose-6-Phosphate Dehydrogenase (G-6-PDH) of *Carica papaya* on ethanol induced gastric lesion in adult male wistar rats. Twenty-four male adult Wistar rats weighing 180- 220 g were used in this study. Animals were divided into three groups (n = 8 per group). The control group A received phosphate buffered saline orally, with the aid of a cannula. 1 mL of 50% ethanol was administered orally, by a cannula, to produce the gastric lesion in group B (n = 8). And in the group C, 500 mg/kg body weight of paw paw leaf extract was administered orally, with a cannula, twelve hours after ethanol administration to the rats. This experiment lasted for twenty one consecutive days. The result showed that TBARS in gastric mucosa as an index for oxidative stress level was significantly increased after ethanol administration. CPL did not reduce significantly the levels of TBARS in the gastric mucosa. G-6-PDH activity was significantly increased in gastric mucosa after ethanol administration, but in rats treated with CPL, a reverse of G-6-PDH activity was observed. Ethanol induced a remarkable and significant decrease of GPx activity in gastric mucosa, whereas CPL induced a significant reversion of ethanol's effect on the enzyme. The results therefore demonstrate that CPL treatment exerts antioxidant effects on ethanol-induced gastric lesions in wistar rats.

Keywords: *Carica papaya* (CPL), enzyme, glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GPx), stomach, thiobarbituric acid test (TBARS)

INTRODUCTION

Carica Papaya (CP) is a member of the family *Caricaceae*, the third most important horticultural crop after citrus and plantain in Nigeria (Oluma and Amuta, 1999). CP is a dual or multi-purpose, early bearing, space conserving, herbaceous crop, is widely acclaimed, despite its susceptibility to natural enemies (Hernandez *et al.*, 2007). In some islands of the West Indies, it is known as pawpaw (Fermin *et al.*, 2005). Originally from Southern Mexico, Central America and Northern South America, the papaya is now cultivated in most countries with tropical climate like Malaysia and the West Indies. *Carica Papaya* Leaf (CPL) is used for food or as medication in folk medicine. It is consumed as a vegetable by the Malay community in Malaysia and by the natives of the East Indies. Traditionally, the leaf extract was used as a tonic for the heart, an analgesic and in the treatment of stomach

ache (Giose Nakazawa, 1996). The extract is also known to have antioxidant properties (Rahmat *et al.*, 2004), but there are no scientific reports on the protective effect of this extract on alcohol induced acute gastric damage. Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barrier. There are many products used for the treatment of gastric ulcers, such as antacids, proton pump inhibitors or antihistaminic agents, but most of these drugs produce several adverse reactions. Thus, there is a need for more effective and less toxic anti-ulcer (Rahmat *et al.*, 2004). The manifestation of oxidative stress through generation of free radicals is one of the numerous mechanisms involved in the gastro-toxic effect of ethanol. Free radicals play an important role in tissue injury by altering the oxidant-antioxidant equilibrium (Afzal and Armstrong, 2007). The altered balance is a risk for the development of various disorders

of the digestive tract. An efficient therapy to control redox status balance in gastric ulcer is important in order to minimize the damage associated with oxidative stress (Afzal and Armstrong, 2007). The objective of this study was to determine the antioxidant activity of Peroxidase (GPx), Thiobarbituric Acid Reaction (TBAR) and the activity of the enzyme Glucose-6-Phosphate Dehydrogenase (G-6-PDH) of *Carica papaya* on ethanol induced gastric lesion in adult male wistar rats.

MATERIALS AND METHODS

Animals: 24 adult male Wistar rats weighing 190- 210g, which were purchased from Animal house University of Ilorin were used in this project, study. They were each weighed and randomly assigned to three groups (A, B and C). They were kept and maintained under standard laboratory conditions of temperature, humidity, and light. They were allowed free access to standard laboratory diet pellets and water liberally. All animals were fasted 24 h before the commencement of the experiment. This study was carried out in the month of March 2010 at Anatomy Department University of Ilorin kwara State Nigeria.

Plant material and extract preparation: Leaves of *Carica papaya* leaves were collected within the premises of College of Medicine University of Ilorin Kwara State Nigeria. Identification of plant was carried out in the department of Botany University of Ilorin. A large quantity of leaves were collected, washed clean with water, air dried and reduced to powder using a mortar and pestle. Powdered leaves of plant weighing 100 g was mixed with 1litre of 70% ethanol and the mixture was left for 24 h, mixture was filtered and the filtrate was concentrated in a water bath (maintained at $60\pm 0.2^{\circ}\text{C}$) to yield 15 g of dark green solid extract. The solid extract was stored in a refrigerator.

Treatment: Group A received oral phosphate buffered saline (PBS) and served as control. Group B (Ethanol) received 1.0mL of 50% ethanol orally every 12 h (Ofusori *et al.*, 2008). Group C (Ethanol + CPL) received papaw leaf extract (500 mg/kg) orally 12 h after 1.0 mL 50% ethanol for 21 consecutive days. 24 h after the last administration the animals were sacrificed following cervical dislocation and the stomach was excised. The samples needed for biochemical estimation were immediately blotted dry, weighed, homogenized with mortar and pestle in cold media. The following biochemical tests were carried out, Thiobarbituric Acid Reaction test TBARS, Glutathione Peroxidase (GPx) and the activity Glucose-6-phosphate Dehydrogenase G-6-PDH was also evaluated.

Biochemical activities: Effects of both ethanol and CPL on gastric lesion in gastric tissue were evaluated by the

estimation of oxidative stress markers (Glutathione Peroxidase GSH-Px and Thiobarbituric Acid Test TBAR) and the action of an enzyme (Glucose- 6- Phosphate Dehydrogenase). Statistical analysis was done with SPSS software.

RESULTS AND DISCUSSION

Fig. 1 shows TBARS in gastric mucosa as an index for the oxidative stress level was significantly increased after ethanol administration. CPL did not reduce significantly the levels of TBARS in the gastric mucosa.

Fig. 2 G-6-PDH activity was significantly increased in gastric mucosa after ethanol administration, but in rat treated with CPL, a reverse of G-6-PDH activity was observed.

Fig. 3 shows that ethanol induced a remarkable and significant decrease of GSH-Px activity in gastric mucosa, whereas CPL induced a significant reversion of ethanol effect on the enzyme.

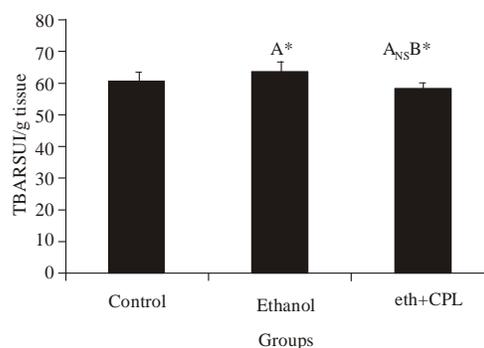


Fig. 1: Thiobarbituric acid reaction test activity in gastric lesion induced by ethanol. The results are mean values in (UI/g tissue). Values are expressed as mean \pm S.E.M of four rats in each group; A: compared with group PBS; B: compared with group ethanol; *: $p < 0.05$; NS: not significant

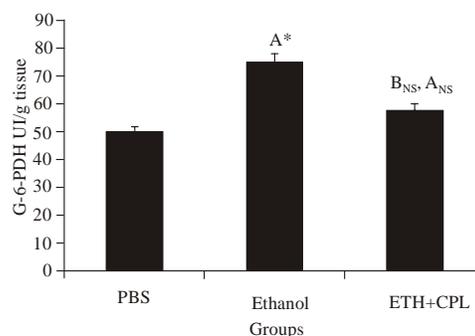


Fig. 2: Glucose-6-phosphate dehydrogenase activity in gastric lesion induced by ethanol. The results are mean values (UI/g tissue). Values are expressed as mean \pm S.E.M of four rats in each group; A: compared with group PBS; B: compared with group ethanol; *: $p < 0.05$; NS: not significant

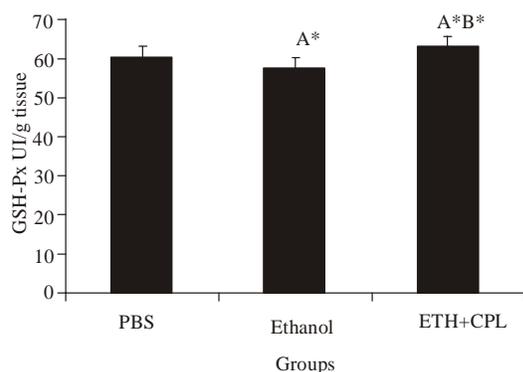


Fig. 3: Glutathione peroxidase activity in gastric lesion induced by ethanol. The results are mean value (UI/g tissue). Values are expressed as mean±S.E.M of four rats in each group; A: compared with group PBS; B: compared with group ethanol; *: p<0.05; NS: not significant

DISCUSSION

In this study the effect of CPL was also evaluated on some anti oxidant parameters in gastric tissue of the rats. Oral administration of ethanol in rat is noxious for the stomach, affecting the gastric mucosa by disrupting its barrier and provoking pronounced micro/macrovascular changes a few minutes after its administration (Gonthier *et al.*, 2004). In addition, erythrocytes are highly sensitive to peroxidative damage probably due to the high content of unsaturated fatty acid in their membrane (Kaliman and Pavychenko, 2005). Malondialdehyde, an end product of lipid peroxidation, is widely used as a marker of lipid peroxidation. Glutathione peroxidase is an important enzyme which plays a key role in the elimination of hydrogen peroxide and lipid hydro peroxide in gastric mucosa cells (Kaliman and Pavychenko, 2005). Ferric reducing antioxidant potential assay actually detects the level of non-enzymatic plasma antioxidants (Khosla *et al.*, 2004). It is tempting therefore to speculate the existence of synergism between enzymatic and non-enzymatic antioxidants in preventing oxidative stress (Khosla *et al.*, 2004). During the experimental period, it was noted that after each ethanol administration the physical activity of the rats increased immediately, and after some time they became slow in action. Both the ethanol and CPL treated groups did not consume their feeds as much as the PBS control group and this must be responsible for the loss in weight of both groups. After 21 days of administration, the weight of the rats were noted and measured in percentage weight change. The weight of the PBS control group increased by 0.38%, that of ethanol group was reduced by 4.83% and the CPL treated group was reduced by 2.33%. According to various studies, alcohol induced damage may result from disturbance of pro-oxidant and anti-oxidant balance that is found in cells (Gonthier *et al.*,

2004). Currently, there is a consensus that former deleterious effects of alcohol on gastric mucosa are the consequences of enhanced lipid peroxidation and decreased GPx level or *vice versa* (khosla *et al.*, 2004). In this study TBARS in gastric mucosa was used as an index for the oxidative stress level, and was significantly increased after ethanol administration in the ethanol group. And was reduced after CPL administration, but reduction in levels of TBARS in the gastric mucosa of CPL treated was not statistically significant. The evaluation of G-6-PDH activity was significantly increased in gastric mucosa after ethanol administration, but in animals treated with CPL, a reverse of G-6-PDH activity was observed. This means that the activity of G-6-PDH was not statistically reduced after CPL administration. The ethanol induced a remarkable and significant decrease of GPx activity in gastric mucosa of rats in the ethanol group, whereas CPL induced a significant increase in activity of GPx on the gastric mucosa of CPL treated group. The results therefore demonstrate that CPL treatment exerts antioxidant effects on ethanol-induced gastric lesions in wistar rats.

CONCLUSION

The present study showed that *Carica Papaya* Leaves (CPL) treatment exerts antioxidant effects on ethanol-induced gastric lesions in wistar rats. Extract of CPL possessed antioxidant properties in ethanol-induced gastric lesions in Wistar rats, which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are currently under way to isolate and characterized the active components of the crude extract of this plant.

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