ORIGINAL ARTICLES

Protective Effect of *Carica papaya* L Leaf Extract against Alcohol Induced Acute Gastric Damage and Blood Oxidative Stress in Rats

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ABSTRACT

The effects of Carica papaya leaf (CPL) aqueous extract on alcohol induced acute gastric damage and the immediate blood oxidative stress level were studied in rats. The results showed that gastric ulcer index was significantly reduced in rats pretreated with CPL extract as compared with alcohol treated controls. The in vitro studies using 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) assay showed strong antioxidant nature of CPL extract. Biochemical analysis indicated that the acute alcohol induced damage is reflected in the alterations of blood oxidative indices and CPL extract offered some protection with reduction in plasma lipid peroxidation level and increased erythrocyte glutathione peroxidase activity. Carica papaya *leaf may potentially serve as a good therapeutic agent for protection against gastric ulcer and oxidative stress.*

Efecto Protectivo del Extracto de la Hoja de *Carica papaya L* Contra el Daño Gástrico Agudo Inducido por Alcohol y el Estrés Oxidativo en Ratas

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RESUMEN

Los efectos de; extracto acuoso de la hoja de Carica papaya *(CPL) en el daño gástrico agudo inducido por alcohol y el nivel de estrés oxidativo inmediato en la sangre, fueron estudiados en ratas. Los resultados mostraron que el índice de úlcera gástrica se reducía significativamente en ratas pretratadas con extracto de CPL, en comparación con los controles tratados con alcohol. Los estudios in vitro mediante el ensayo con 2,2-difenil-1-picrihidrazilo) mostraron la fuerte naturaleza antioxidante de extracto de CPL. El análisis bioquímico indicó que el daño agudo inducido por alcohol se refleja en las alteraciones de los índices oxidativos de la sangre y el extracto de CPL ofreció cierta protección con la reducción del nivel de peroxidación lipídica del plasma y el aumento de la actividad de la glutatión peroxidasa de los eritrocitos. La hoja de la* Carica papaya *puede servir potencialmente como un buen agente terapéutico para la protección contra la úlcera gástrica y el estrés oxidativo.*

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INTRODUCTION

The papaya, *Carica papaya* L, is a member of the small family Caricaceae allied to the Passifloraceae. As a dual or multipurpose, early bearing, space conserving, herbaceous crop, it is widely acclaimed, despite its susceptibility to natural enemies (1). In some islands of the West Indies, it is known as pawpaw (2). 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* leave (CPL) is used as food or as medication in folk medicine. It is consumed as a vegetable by the Malay community in Malaysia and by the natives in the East Indies. Traditionally, the leaf extract was used as a tonic for the heart, analgesia and treatment for stomach ache (3). The extract is also known to have antioxidant properties (4) but there are no scientific data reported on the protective effect of this extract on alcohol

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induced acute gastric damage. 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 (5). 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. Therefore, in this study, we aimed to investigate whether the treatment with a single dose of CPL extract (500 mg/kg) might reduce acute gastric ulceration induced by absolute ethanol and if it is so, to determine the immediate oxidative stress level in blood.

METHODS

The CPLs were collected from Puchong, Selangor, Malaysia and was identified as *C papaya* by one of the authors (Kuppusamy). A voucher specimen (code: CPL2) was deposited at the Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Malaysia. The fresh leaves were cut into small pieces and homogenized in cold distilled water to obtain the juice which was filtered and subjected to lypholization in a freeze drier. The percentage weight of the freeze dried plant material was 7.14%. The antioxidant activity of the crude aqueous extract was measured using the DPPH assay (6). Male adult *Sprague-Dawley* rats (body weight 180–220 g) bred and reared in The University of Malaya animal unit were used for the experiment. All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (7). A total of 24 rats were divided into four groups (6 rats per group). The first group was a control group treated with 5 ml kg^{-1} distilled water and the second group was administered with 5 ml of CPL aqueous extract (500 mg/kg body weight) orally. The third group was administered a single oral dose of absolute ethanol (1 ml/animal). The last group was administered with 5 ml (500 mg/kg) CPL aqueous extract 30 minutes before being given a single oral dose of absolute alcohol. The rats were sacrificed 20 minutes later and their stomachs were rapidly removed and fixed in 10% buffered formalin. The gastro-haemorrhagic lesion index was measured using a microscope with a square grid eyescope and was expressed as ulcer index (mm^2). The serum was collected in plain and EDTA tubes for various oxidative marker measurement namely xanthine oxidase (XO) (EC 1.2.3.2), malondialdehyde (MDA), glutathione peroxidase (GPx; EC 1.11.1.19), catalase (EC 1.11.1.6) and ferric reducing antioxidant potential (FRAP) based on known established methods (8). All reagents used for the determination of oxidative indices were purchased from Sigma chemicals (St Louis, Mo, USA). Other reagents of analytical grade were obtained from normal commercial sources. The data for various parameters were analyzed using Duncan multiple range test and $p < 0.05$ was regarded as significant.

RESULTS

The CPL showed a DPPH activity with an IC_{50} of 60.2 µg/ml. Oral administration of CPL extract before administration of ethanol led to significant protection of the stomach compared to the control group. There were severe haemorrhagic lesions visible in the dissected stomach of rats treated with alcohol alone but in CPL pretreated rats, only mild lesions were visible (Figs. 1, 2). The gross examination of the stomach showed that the extract alone did not cause any changes but in the presence of alcohol, thick mucous formation and increase in pH of the stomach secretion were observed. The crude aqueous extract showed a pH of 9.2. The Table shows the ulcer index and levels of blood oxidant-antioxidant markers in the various treatment groups. Rats treated with CPL had significantly lower MDA levels as compared to the alcohol treated group. Glutathione peroxi-

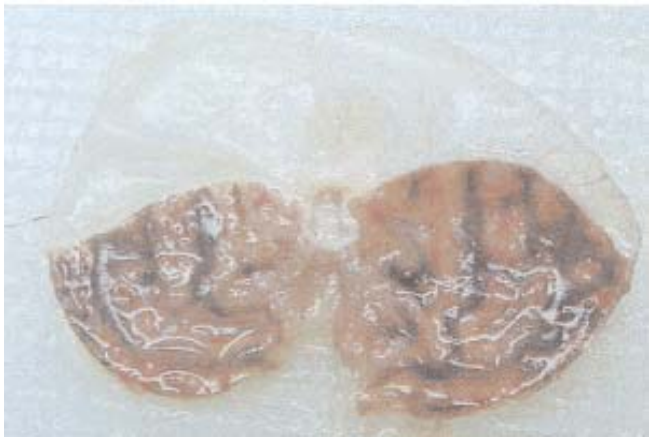

Fig. 1: Necrosis of gasric mucosa (gross). Gastric mucosal damage caused by absolute ethanol. Absolute ethanol produced extensive visible haemorrhagic necrosis of gastric mucosa.

Fig. 2: Cytoprotection of aqueous extract 500 mg kg^{-1} against absolute ethanol. Aqueous extract was able to prevent or reduce the formation of gastric lesions by absolute ethanol.

Table: Ulcer index and levels of blood oxidant-antioxidant markers in the various treatment groups

	Control	*C papaya* leaf *extract*	Alcohol	*C papaya* leaf extract^{+} Alcohol
Lipid Peroxidation (MDA/µmol/L)	0.099 ± 0.018	0.084 ± 0.025	0.133 ± 0.019[a]	0.102 ± 0.020[a,b]
FRAP (µmol/L) Xanthine	210.56 ± 27.188	211.67 ± 30.180	248.06 ± 30.77[a]	243.61 ± 28.92[a]
Oxidase (U/L)	0.054 ± 0.045	0.061 ± 0.029	0.070 ± 0.022	0.068 ± 0.033
GPx (U/mg protein)	2.694 ± 0.310	2.720 ± 0.365	2.229 ± 0.281[a]	2.475 ± 0.214[a,b]
Catalase (U/mg protein)	0.078 ± 0.013	0.080 ± 0.015	0.068 ± 0.037	0.067 ± 0.029
Mean Ulcer Index (mm^2)	0	0	6235.2 ± 386.33[a]	993.6 ± 141.384[a,b]

Data are expressed as mean ± standard deviation. Statistical evaluation of data was performed using SPSS version 14.0 and the level of significance was evaluated by Duncan's multiple range test.

[a] $p < 0.05$ compared to control and *C papaya* leaf extract treated groups

[b] $p < 0.05$ compared to alcohol treated group

dase activity was significantly decreased in blood erythrocytes after ethanol treatment, but in rats pretreated with CPL, a significant increase was observed. In contrast, neither catalase nor XO showed significant changes in all the treatment groups. In addition, FRAP level was significantly higher in the alcohol treated group. The ulcer index was significantly lower ($p < 0.05$) in the CPL-treated group.

DISCUSSION

The present result demonstrates that a single dose (500 mg/kg) of CPL aqueous extract is able to protect the rat gastric mucosa against haemorrhagic lesions produced by alcohol. This dose was chosen after preliminary assessment using a wide dose range of this extract (result not shown). The DPPH method showed strong radical scavenging activity (antioxidant) and this property is most likely contributed by polyphenols present in this extract. 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. According to various studies, alcohol induced damage may result from disturbance of pro-oxidant and antioxidant balance that is found in cells (9). In this study, the measurement of oxidant-antioxidant parameters was done in blood because it is a better indicator of changes in metabolite and energy metabolism related enzyme activity. In addition, erythrocytes are highly sensitive to peroxidative damage probably due to the high content of unsaturated fatty acid in their membrane (10). 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 hydroperoxide in gastric mucosa cells. Currently, there is a consensus that former deleterious effects of alcohol on gastric mucosa are the consequence of enhanced lipid peroxidation and decreased GPx level or *vice versa* (11). The present study confirmed that both the levels of MDA and GPx were reversed by CPL treatment. The result also revealed an increase in the mean FRAP value for alcohol and CPL treated groups. Ferric reducing antioxidant potential assay actually detects the level of non-enzymatic plasma antioxidants It is tempting therefore to speculate the existence of synergism between enzymatic and non-enzymatic antioxidants in preventing oxidative stress. In contrast, no changes were observed in blood catalase and XO activity. These results could be due to the short duration of treatment. Moreover, the results of the study is in concordance with other studies (12, 13) which reported that GPx is the main anti-oxidant involved in the removal of hydrogen peroxide whereas catalase shows a lower affinity for that reactive oxygen species. The absence of increased XO activity suggests against the involvement of this superoxide generating enzyme in the development of acute gastric ulcers. The gross observation of the increasing pH of the stomach secretion and the alkaline content of CPL extract were interesting findings. It is possible that the extract induced both mucous and $HC0_3^-$ secretion to protect the stomach lining against alcohol assault apart from directly neutralizing the stomach acidity. Drugs used to treat ulcer such as omeprazole, lansoprazole and famotidine are also known to act *via* the same mechanism (14).

CONCLUSION

In conclusion, the CPL aqueous extract offered some protection against alcohol induced oxidative damage to the gastric mucosa. The antioxidant system present in CPL might play a protective role against the production of reactive oxygen species and lipid peroxidation by-products. Work is

in progress to study the molecular mechanism behind the efficacy of this plant and also to isolate the active components of the plant. The present study revealed that CPL extract is a promising candidate for the development of phytomedicine against gastric ulcer, and further studies are needed in this direction.

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