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Effect of Ethyl Acetate Extracts from Peel of *Citrus decumana* and *Citrus aurantifolia* on Aspirin Induced Gastric Ulcer in Mice

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Authors' contributions

Author ECE designed the study, statistical analysis and the protocol. Author RUH wrote the first draft of the manuscript. Author AA carried out the laboratory work. All authors have read and approved the final manuscript

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ABSTRACT

Aims: The present study is aimed at investigating the antiulcer activity of ethyl acetate extract of *Citrus decumana* (grapefruit) and *Citrus aurantifolia* (lime) peels.
Study Design: Animals were divided into six groups of six animals each as follows;

- Group EtCD: Ulcer was induced and treated with *Citrus decumana* peel extract.
- Group EtCA: Ulcer was induced and treated with *Citrus aurantifolia.*
- Group EtCD + EtCA: Ulcer was induced and treated with *Citrus decumana* + *Citrus aurantifolia.*
- Group Ranitidine: Ulcer was induced and treated with Ranitidine.

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- Group Ulcer: Ulcer was induced but not treated.
- Group Normal: Ulcer was not induced.

Place and Duration of Study: Department of Biochemistry, Federal University of Technology, P.M.B 65, Minna Niger State, Nigeria.

Methodology: Ethyl acetate extracts of dried peel powder of *Citrus decumana* and *Citrus aurantifolia* were obtained using reflux. Phytochemical screening was done on the extracts to determine their chemical constituents. Ulcer was induced in six groups of mice by oral administration of aspirin. The mice were treated by oral administration of ethyl acetate extract of *Citrus decumana* and *Citrus aurantifolia.* The antiulcerogenic activity was evaluated in aspirin induced ulcerogenic mice models at a dose of 400 mg/kg. Ulcerative indexes as well as oxidative stress markers like thiobarbituric acid reactive species (TBARS), Superoxide dismutase (SOD) and Catalase activity (CAT) in the plasma and gastric tissue were measured in all groups to study the possible involvement of antioxidants with gastro protection.

Results: Pretreatment of animals with ethylacetate extracts of *Citrus decumana* and *Citrus aurantifolia* peel showed a significant decrease in ulcerative index and TBARS Level in both plasma and gastric tissues compared to the control. Although all the treatment groups showed a significant increase in SOD and CAT concentration in plasma and gastric tissue, *Citrus decumana* extract showed a more significant increase in the concentration of SOD when compared to all other groups.

Conclusion: This study indicates that *Citrus decumana* and *Citrus aurantifolia* peel may be used as a natural therapeutic agent in the treatment of ulcers.

Keywords: Citrus decumana; Citrus aurantifolia; antiulcer; aspirin; antioxidant.

1. INTRODUCTION

Ulcer is a common global problem with increasing incidence and prevalence attributed to several factors such as stress, exposure to bacterial infection (*Helicobacter pylori*) and the use of non steroidal anti inflammatory drugs (NSAIDs) as well as decreased the[delete 'the] protective factors such as bicarbonate and mucus.Mucosal damage, an initial step in ulcer development has been known to correlate with oxidative stress by reactive oxygen species generation and hypersecretion of hydrochloric acid through H^+, K^+-ATPase action [1]. Aspirin-induced ulcer is mediated through tissue damaging of free radicals which are produced from the conversion of hydroperoxyl to hydroxy fatty acids, which leads to cell destruction [2]. It is also known that aspirin inhibits cyclooxygenase enzyme that decrease prostaglandin level [3].

It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa [4] and scavenging these free radicals can play an appreciable role in healing the ulcer.

Although there are several orthodox drugs that have been used as antiulcerogenics, most of these drugs produced several side effects including arrhythmias, impotence, gynaecomastia and haematopoeitic changes [5]. In addition, recurrence rates of ulcers are high [6], hence there has been renewed interest in the search for new antiulcer drugs of natural origin capable of combating the side effects of synthetic and orthodox antiulcer drugs. *Citrus decumana* (grape fruit) is a subtropical citrus fruit known for its bitter juice. It originated in Barbados but it is now grown in different subtropical areas of the world including Nigeria. The fruit is green-yellow skinned and largely an oblate spheroid; it ranges in diameter from 10–15 cm. Grapefruit is an excellent source of many nutrients and phytochemicals that contribute to a healthy diet. Grapefruit is a good source of vitamin C[7], and also contains the fibre pectin. *Citrus aurantifolia* is a citrus fruit comm. only known as Key Lime or sour orange. It belongs to the genus of flowering plants in the family Rutaceae (orange family) and a common name for edible fruits of this genus and sometimes related genera. Key Lime is a small shrub-like tree ranging from 3.5 to 9 m in height and 2.5 to 7.5 m in width. The fruit is typically round, green to yellow in color and about 3-6 cm in diameter [8]. It is valued for its unique flavor compared to other limes, with the key lime usually having a more tart and bitter flavor. The name comes from its association with the Florida Keys, where it is best known as the flavoring ingredient in Key lime pie. Key Lime in its natural state is widely used in different parts of Africa particularly in Nigeria. It is used traditionally for different medicinal purposes, for

instance, it is used for the suppression of stomach ache [9]. When added to sugar and palm oil or to honey, the juice has been found to be an excellent cough relieving mixture. The dried peel is burnt in some homes to act as insecticides against mosquitoes [10]. Studies have shown that Key lime juice destroys both human immunodeficiency virus (HIV) and sperm cells [11,12]. The mesocarp is also used as a very good facial scrub and helps in prevention of pimples due to its cleansing action on the skin. The antimicrobial activities of lime in conjunction with other extracts have also been reported [13].

The present study is aimed at investigating the gastroprotective effect of ethyl acetate extract from peel of *Citrus decumana* and *Citrus aurantifolia* on aspirin induced gastric ulcer in mice.

2. MATERIALS AND METHODS

2.1 Collection of Material

The fruits of *Citrus aurantifolia* and *Citrus decumana* were bought from Kure market Minna, Nigeria in the month of June. The peels were removed manually and dried under laboratory condition at room temperature. The dried peels were ground into fine powder and stored in an air tight bottle until use.

2.2 Preparation of Ethyl Acetate Extract

Fifty grams of the dried peel powder of each citrus peel was weighed into two different distillation flasks separately, and 400ml of ethyl acetate solution was added to each flask. The mixture was refluxed for two hours, filtered hot using a muslin cloth and subsequently evaporated using a rotary evaporator at reduced temperature. The semi- dry extracts were weighed, placed in sterile sample bottles and stored in a refrigerator until required.

2.3 Phytochemical Screening

The peel extracts of *Citrus decumana* and *Citrus aurantifolia* were subjected to phytochemical tests according to the methods of Trease and Evans [14]; to determine their chemical constituents.

2.4 Experimental Animals

Albino mice of either sex (mean weight; 20-39 g) were obtained from Biochemistry Department of Ahmadu Bello University, Zaria Kaduna State, Nigeria. They were allowed to acclimatize for two weeks in our own laboratory.

2.5 Experimental Design

Animals were divided into six groups of six animals each as follows:

- Group EtCD: Ulcer was induced and treated with *Citrus decumana* peel extract.
- Group EtCA: Ulcer was induced and treated with *Citrus aurantifolia.*
- Group EtCD + EtCA: Ulcer was induced and treated with *Citrus decumana* + *Citrus aurantifolia.*
- Group Ranitidine*:*Ulcer was induced and treated with Ranitidine.
- Group Ulcer: Ulcer was induced but not treated.
- Group Normal: Ulcer was not induced.

2.6 Aspirin Induced Ulcer in Animal Model

Animals were fasted for 24 hours and ulcer was induced by oral administration of aspirin at dose of 200 mg/ kg [15]. The animals in the treatment groups received extracts in the dose of 400mg/kg body weight for 14 days.

2.7 Administration of Plant Extracts

The extract(s) were weighed and dissolved in 10ml of normal saline and shaken vigorously to dissolve. The mixtures were orally administered to the group A-C mice at a dose of 400mg/kg body weight for 14 days. Ranitidine was used as the standard anti-ulcer agent for comparison (group D).

2.8 Preparation of Tissue and Plasma Samples

Gastric tissue samples were homogenized by using sucrose solution after the measurement of ulcerative index. Centrifugation (3000 rpm) of the homogenized tissues and the collection of supernatants were carried out. These supernatants were stored under refrigeration for further estimation of various biochemical markers. Blood samples were collected in anticoagulant bottles and centrifuged at 3000 rpm. The supernatants were collected and stored under refrigeration for further estimation of various biochemical markers.

2.9 Measurement of Ulcerative Index (UI)

Ulcerative index was measured according to the method described by Sood et al. [16], the stomach was opened and washed with running tab water. Then it was placed on a flat glass plate to count the ulcerative area. Standardization was made with a 10x10 cm squared glass plate. The opened stomach was laid on the glass plate and the mucous was exposed, allowing the counting of injuries per square mm. The ulcer index was determined by using the formula:

$$\text{Ulcer index} = 10/X$$

Where:

$$X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

2.10 Estimation of Superoxide Dismutase (SOD) Level in Plasma and Tissue

SOD was measured according to the method of [17].

2.11 Estimation of Lipid Peroxidation in Plasma and Tissue

Lipid peroxide content estimated as thiobarbituric acid reactive species (TBARS) was done according to the method of [18] in the tissue and plasma according to [19].

2.12 Estimation of Catalase Activity (CAT) in Plasma and Tissue

This was determined according to the method of [20].

3. RESULTS

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening (Table 1) revealed the presence of flavonoids, phenols, tannin and steroid in both extracts investigated. Saponin and terpenes were found to be moderately present only in the ethyl acetate extract of *Citrus aurantifolia* while cardiac glycosides, alkaloids and anthracenosides were completely absent in both extract.

3.2 Ulcerative Index

Effects of ethyl acetate peel extracts of both *Citrus decumana* and *Citrus aurantifolia* in aspirin induced ulcer showed a decrease in ulcerative index (Fig. 1). There was a significant increase in the ulcerative index in disease control group whereas all the treated groups at dose of 400 mg/Kg showed a significant reduction in the ulcerative index. However there was no significant difference in the ulcerative index amongst all the extract treated groups.

3.3 Thiobarbituric Acid Reactive Species (TBARS)

Effects of ethyl acetate peel extracts of both *Citrus decumana* and *Citrus aurantifolia* in aspirin induced ulcer had shown a decrease in thiobarbituric acid reactive specie (TBARS) in both plasma and tissue relative to that in the group treated with ranitidine (Fig. 2 and Fig. 3). While thiobarbituric acid reactive species (TBARS) in ulcer untreated remains significantly high. The extracts were able to reduce Thiobarbituric acid reactive species (TBARS) by free radical scavenging mechanism thus reducing the rate of lipid peroxidation. The groups treated with ethylacetate extract of *Citrus decumana* (ETCD) and the Combined extract groups (ETCD +EtCA) as well as the standard drug significantly inhibit lipid peroxidation better than the EtCA treated group alone in the plasma. Also in the gastric tissue of the EtCD treated group showed a more significant reduction in lipid peroxidation compared to the other extracts treated groups.

3.4 Superoxide Dismutase (SOD) Activity

Effects of ethyl acetate peel extracts of both *Citrus decumana* and *Citrus aurantifolia* in aspirin induced ulcer shows an increase in superoxide dismutase in both plasma and tissue relative to that in the group treated with Ranitidine (Fig. 4 and Fig. 5) while superoxide dismutase in ulcer untreated remains significantly low. The extracts in the treated groups were able to boost the inhibition of epinephrine oxidation generally. The EtCD group alone and the combined (EtCA + EtCD) group showed a higher superoxide dismutase activity in the plasma compared to the ETCA group alone, while in the gastric tissue, the EtCA treated group showed the highest superoxide activity compared to the other extracts treated group.

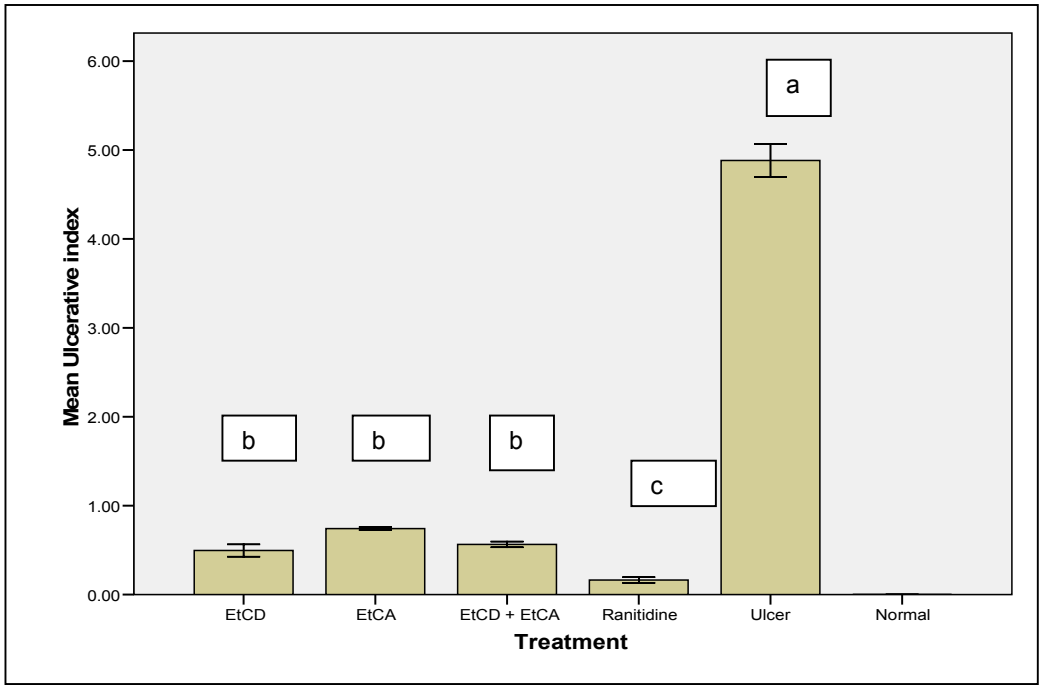

Fig. 1. Effect of EtCD and ETCA on ulcerative index in aspirin induced peptic ulcer. Values are Mean±SEM six animals. Values with different alphabet are significantly different from each other (p≤0.05) but those with the same alphabet are not (p>0.05)

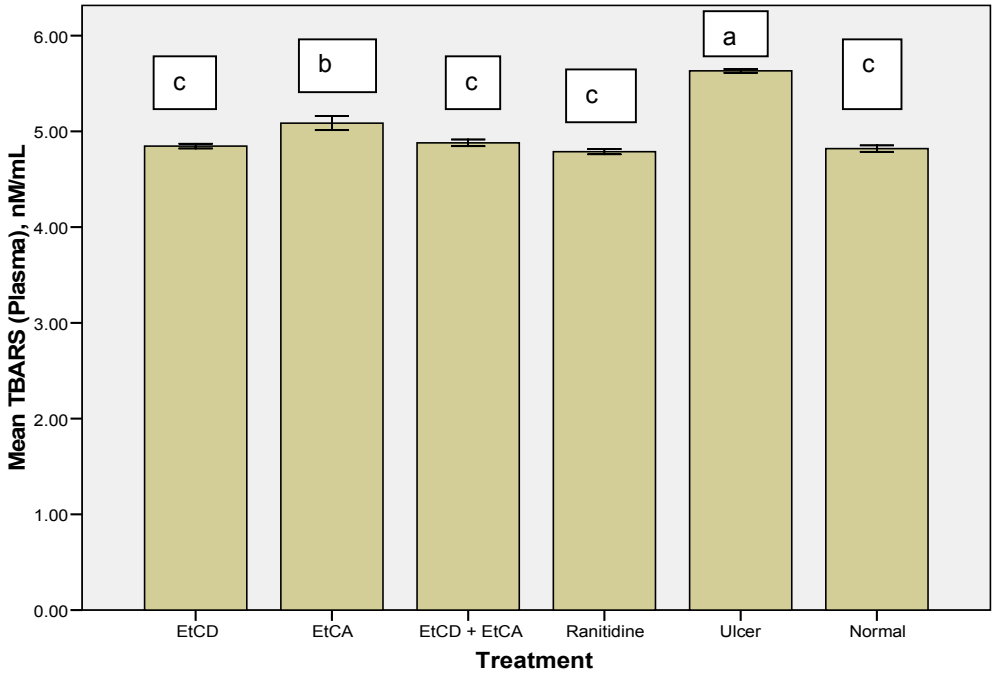

Fig. 2. Effect of EtCD and ETCA on Plasma Thiobarbituric acid reactive species in aspirin induced peptic ulcer. Values are Mean±SEM six of animals. Values with different alphabet are significantly different from each other (p≤0.05) but those with the same alphabet are not (p>0.05)

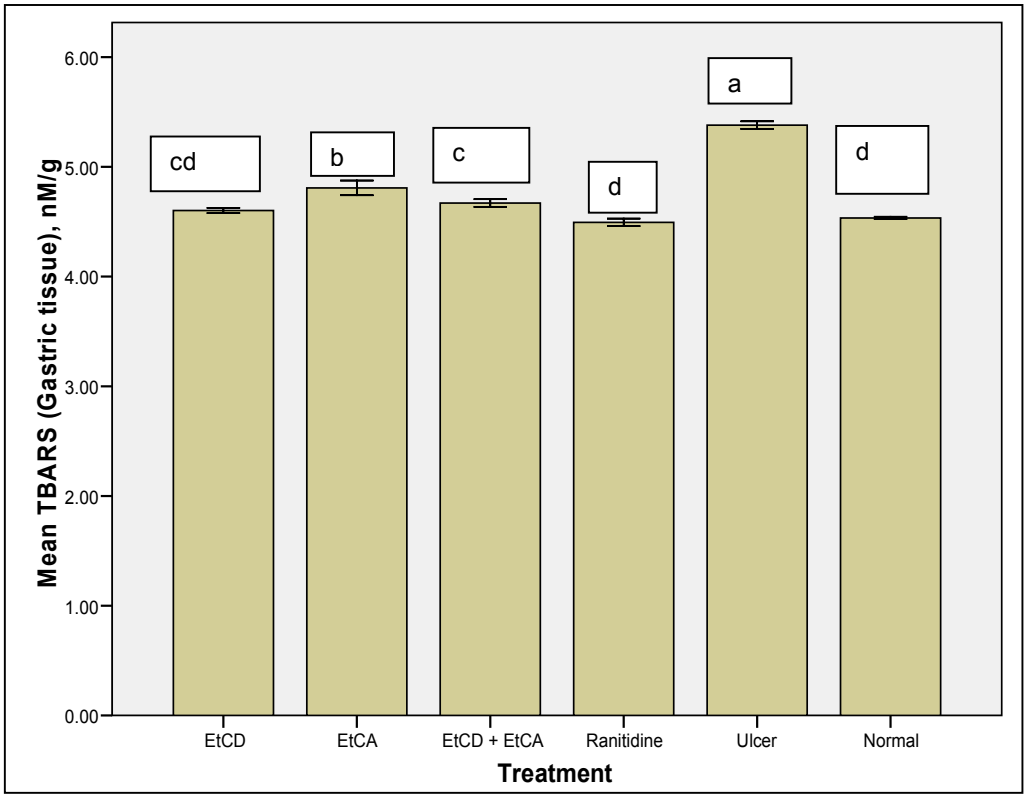

Fig. 3. Effect of EtCD and ETCA on Gastric Tissue Thiobarbituric acid reactive species in aspirin induced peptic ulcer. Values are Mean±SEM six animals. Values with different alphabet are significantly different from each other (p≤0.05)

3.5 Catalase (CAT) Activity

Effects of ethyl acetate peel extracts of both *Citrus decumana* and *Citrus aurantifolia* in aspirin induced ulcer showed an increase in catalase level in both plasma and tissue relative to that in the group treated with Ranitidine (Fig. 6 and Fig. 7). While catalase level in ulcer untreated remains significantly lower. This increase in the level of catalase defends the membrane against oxidative stress and effects of high Hydrogen peroxide (H_2O_2) concentration by catalyzing its decomposition into molecular oxygen and water without the production of free radicals.

3.6 Statistical Analysis

All values were expressed as mean ± SE. Statistical analysis was performed using one-way analysis of variance (ANOVA) and individual comparisons of the group mean values was done using Duncan test.

The phytochemical compounds present in the peels *of Citrus decumana* and *Citrus aurantifolia* are shown in Table 1.

Table 1. Phytochemical Compounds present in peels of *Citrus decumana* and *Citrus aurantifolia*

Phytochemicals	*Citrus decumana*	*Citrus aurantifolia*
Cardiac glycoside	-	-
Saponin	-	++
Steroid	+++	+++
Tannin	+	+
Alkaloid	-	-
Terpene	-	++
Flavonoid	++	+
Anthracenosides	-	-
Phenol	+++	+++

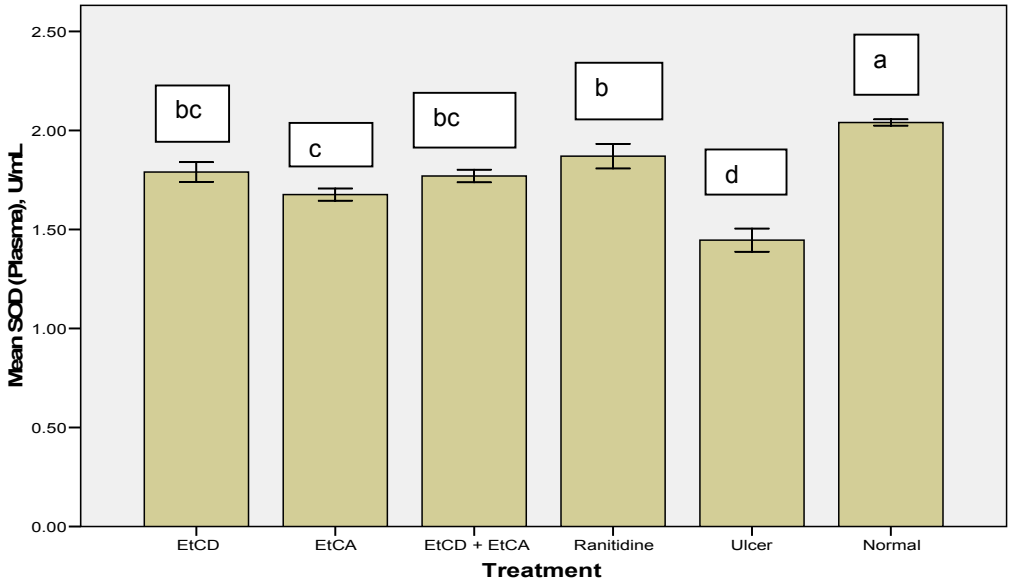

Fig. 4. Effect of EtCD and ETCA on Plasma Superoxide dismutase activity in aspirin induced peptic ulcer. Values are Mean±SEM six animals. Values with different alphabet are significantly different from each other (p≤0.05)

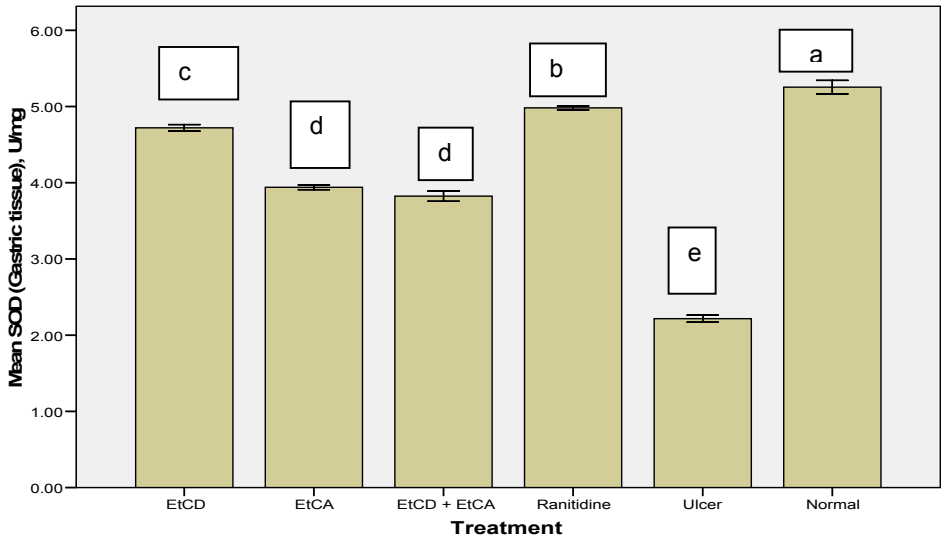

Fig. 5. Effect of EtCD and ETCA on gastric tissue superoxide dismutase activity in aspirin induced ulcer. Values are Mean±SEM six animals. Values with different alphabet are significantly different from each other (p≤0.05) but those with the same alphabet are not

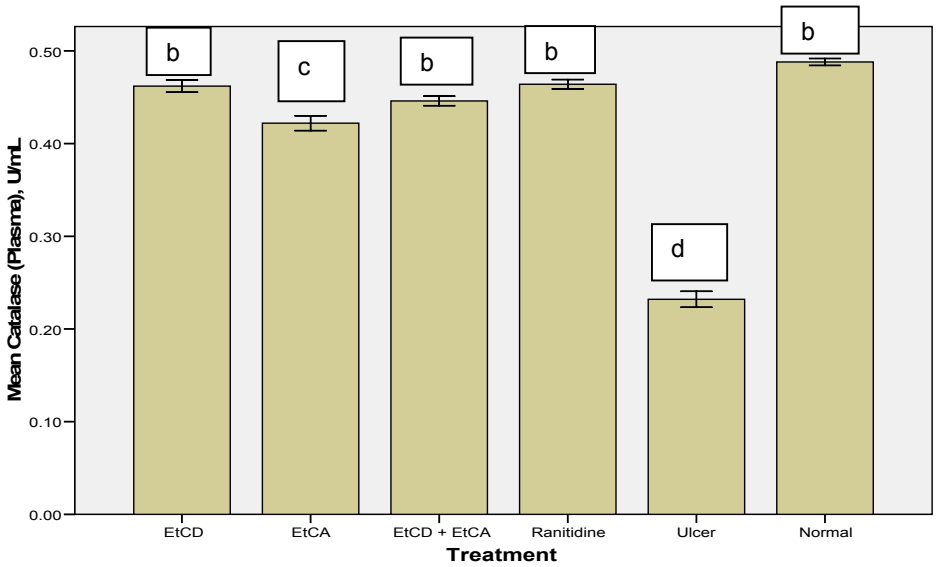

Fig. 6. Effect of EtCD and ETCA on Catalase (in plasma) activity in aspirin induced peptic ulcer. Values are Mean±SEM of six animals. Values with different alphabet are significantly different from each other (p≤0.05)

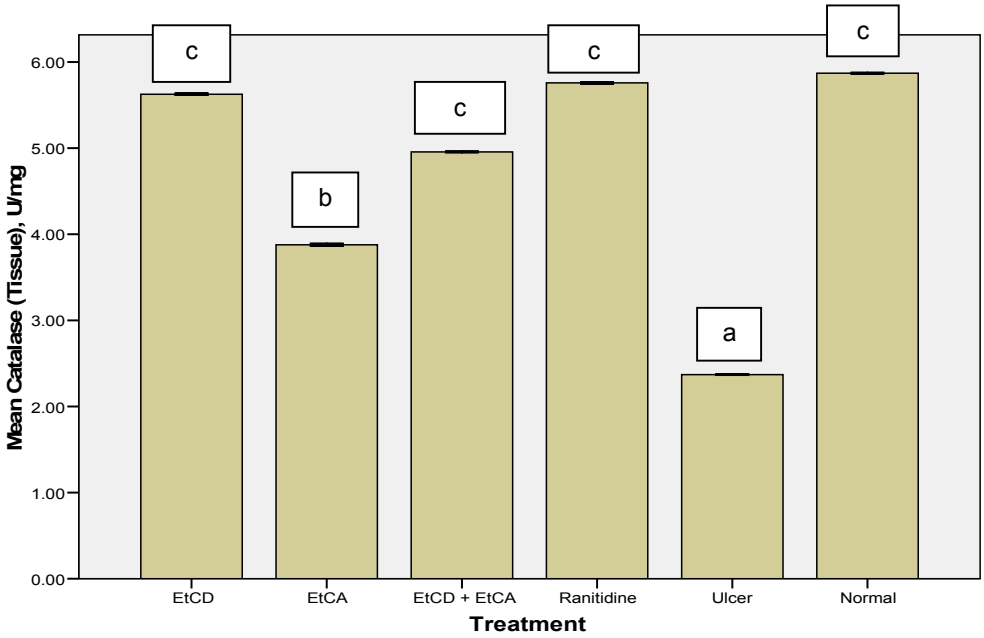

Fig. 7. Effect of EtCD and ETCA on Catalase (in gastric tissue) activity in aspirin induced peptic ulcer. Values are Mean±SEM of six animals. Values with different alphabet are significantly different from each other (p≤0.05)

4. DISCUSSION

Oral aspirin administration to rats is known to cause high ulcerative index in rats' gastric tissues [21]. Aspirin causes mucosal damage by hindering prostaglandin synthesis, increasing acid secretion and back diffusion of H ions [22]. In the present study the high Ulcerative Index in Ulcer group (Untreated) was significantly brought down in all the treated groups generally. Within

the treatment groups, there was no significant difference in the reduction in the ulcerative index however, the standard drug ranitidine had a slightly significant decrease in the ulcerative index as compared to other groups. This result is in close agreement with the result of a recent study [23] which reported that A*egle marmelos* fruit was able to reverse the Ulcerative Index induced in aspirin in ulcerogenic rats. The observed increase in the ulcer untreated group is an indication that aspirin treatment lead to disrupted mucosal barrier which may have arisen from failure in gastroprotection and repair mechanism.

Oxidative stress plays vital roles in the pathogenesis of ulcers. This may be due to radicals capable of promoting mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism [24]. The damage to the membrane proteins decreases membrane permeability, activities of enzyme and receptors and activation of cells. Evident from this study is the significant decrease in lipid peroxidation in the entire extract treated groups in both plasma and gastric tissue as seen in the TBARS reactive species level (Figs. 2 and 3). The groups treated with ethylacetate extract of *Citrus decumana* (ETCD) and the combined extract groups (ETCD +EtCA) as well as the standard drug significantly inhibit lipid peroxidation better than the EtCA treated group alone in the plasma. Also, in the gastric tissue, the EtCD treated group showed a more significant reduction in lipid peroxidation compared to the other extracts treated groups. Lipid peroxidation is usually used as index for measuring damage that occurs in membrane tissue as a result of free radical generation [25,26]. Significant increase in Lipid peroxidation as seen in the aspirin treated group is possibly due to the generation of free radicals via autooxidation or superoxide catalyzed oxidation process while the decrease in lipid peroxidation by extracts may be due to the presence of flavonoids and phenols in the extract. Many studies show that flavonoids present in the citrus peel possess strong antioxidant, anti-atherogenic, antiviral, antiaggregatory, antimutagenic, antiulcer and antitumor effects [27,28,29]. Phenolic compounds have also been reported to have a beneficial role in gastric ulcers, as it has been suggested that phenols stimulate PGE_2 formation [30]. Polyphenols have been shown to clearly improve the status of different oxidative stress biomarkers [31]. The biological mechanisms of these possible effects have been attributed to their antioxidant properties through several possible mechanisms, such as their ability to scavenge free radicals, break radical chain reactions, directly reducing peroxides, and stimulating the activities of antioxidant defense enzymes [32]. Therefore the presence of flavonoids and phenols in both extract may be responsible for their protective effect.

Superoxide dismutase (SOD) and catalase (CAT) are defensive antioxidant enzymes capable of reversing oxidative stress arising from harmful free radicals and reactive oxygen species. In the diseased control group (Ulcer), superoxide dismutase (SOD) and catalase activity in gastric tissue and plasma decreased probably due to increase in free radicals generation caused by Aspirin. Administration of the extracts significantly increased the SOD and CAT activities in both plasma and gastric tissue. However, EtCD treated group and the combined ETCD and EtCA treated group increased the activities of these antioxidant enzymes more significantly in a similar manner like ranitidine (standard drug) in both plasma and gastric tissue. This result is in concurrence with previous work which illustrated that peel of *Citrus decumana* had a positive effect on Glutathione level, SOD and CAT activities on stress induced peptic ulcer [33]. These extracts can be said to exert its antioxidant defense mechanism probably by metabolising lipid peroxides and scavenging endogenous superoxide radicals and H_2O_2. CAT traps the harmful hydrogen peroxide and converts into water and oxygen. SOD is known to scavenge superoxide radicals by speeding up their dismutation, while CAT removes H_2O_2 by converting it to water and oxygen. Thus detoxification of the superoxide anion is not a terminating step in free radical scavenging since dismutation results in the production of H_2O_2 which ultimately accumulates in the mitochondria and cytosol. Flavonoids, phenols majorly and other phytochemicals inherent in the ethylacetate of Citrus and Citrus may be responsible for their protective role against aspirin induced ulceration.

5. CONCLUSION

Our results showed that *Citrus decumana* and *Citrus aurantifolia* peel extracts possess

gastroprotective effect at a dose level of 400 mg kg^{-1} in Aspirin induced groups. Thus, the extracts may be a potent natural therapeutic agent for the treatment of ulcerogenic disorders.

CONSENT

Not applicable.

ETHICAL APPROVAL

Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guideline and Protocol Review (CCAC, 19997).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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