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Ameliorative Effect of Ethanolic Extract of *Laportea aestuans* (L.) Chew on Gastric Oxidative Damage in Aspirin - Induced Gastric Ulcer in Male Rats

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are connected with the generation of free radicals and one of its adverse effects is gastric ulceration. Ulceration was induced orally using aspirin. Twenty-four male Wistar rats were used for this study (120-150g). Rats were divided into 6 groups with each group containing 4 rats. Rats were pre-treated orally with cimetidine, a reference drug. Group 1 rats orally received 1% gum acacia solution as the control group, Group 2 rats orally administered 25 mg/kg aspirin and served as the ulcerated, untreated group, rats in groups 3 and 4 were pre-treated orally with 200 mg/kg and 400 mg/kg respectively for 3 days while rats in groups 5 and 6 were pre-treated orally with 50 mg/kg cimetidine and 50 mg/kg catechin respectively for 3 days. The result of this study shows that the ulcerated, untreated rats showed increased concentrations of gastric volume with a concomitant decrease in protein concentration compared to the control group, cimetidine and catechin reversed these observations. Activities of glutathione, catalase were decreased in the ulcerated, untreated group but pre-treatment with different doses, cimetidine and catechin reversed these observations. In conclusion, the ethanoic extract of *L. aestuans* can be said to be used as an anti-ulcerogenic agent against aspirin-induced gastric ulcer which is due to the presence of phytochemicals in the plant.

Keywords: Gastric Ulcer, Oxidative Stress, Cimetidine, Phytochemicals, Aspirin, *Laportea aestuans*

1. INTRODUCTION

Laportea aestuans (L.) Chew on (*Urticaceae*) is widely distributed in tropical rainforest generally called stinging nettle¹⁸. Aspirin-acetylsalicylic acid is an effectual non-steroidal anti-

inflammatory drug (NSAID) that is importantly used for inflammation, fever, and body pains¹⁹. Aspirin is widely used for the management of rheumatoid arthritis and also for cardiovascular thrombosis prevention¹⁷. Bleeding, perforation, ulceration, and gastric mucosal erosions have been related to the prolonged use of NSAIDs¹⁹. Dyspepsia occurrence in adults is about 30-40%³. A situation where the stomach generates excess gastric acid is dyspepsia in which its symptoms are nausea, vomiting, heartburn, reflux, ulcer, and restlessness^{2,5}. Peptic ulcer disease, duodenitis, gastro-oesophageal reflux disease, gastritis, and functional dyspepsia are the main causes of dyspepsia^{2,5}.

The oil extracts of *L. aestuans* contain methyl salicylate which has antimicrobial and antioxidant properties¹. The production of endogenous prostaglandins is being impeded in gastric injury due to the actions of NSAIDs. The gastric mucosa is being shielded against a wide range of causative agents due to the build-up of prostaglandins from arachidonic acid from the two isoforms of cyclooxygenase¹². COX-1 is generally evidenced in the stomach and is responsible for the generation of prostaglandins involved in mucosal defense. COX-2 is the isoform responsible for inflammation and responsible for the display of prostaglandins involved in the healing of gastric ulceration¹².

Gastric ulcer is controlled by the production of gastric acid secretion using chemical drugs, neutralizing the acid by antacid drugs, or delay in cellular apoptosis by cytoprotective drugs²⁰⁻²¹. Similarly, these drugs have side effects such as impotence, gynecomastia, altered heartbeat, and joint pains²⁰⁻²¹. The present study is aimed at finding the natural antioxidants from plant materials, thus providing a natural remedy against aspirin-induced gastric ulcer to replace synthetic drugs for the effective treatment of therapeutic drug toxicity and also to evaluate the antioxidative properties of *L. aestuans* to cure gastric ulcer, thereby encouraging the use of medicinal plants against certain illnesses.

2. MATERIALS AND METHODS

1% Bovine Serum Albumin, 0.01M Hydrochloric Acid, 0.35M Trichloroacetic acid, 0.1N sodium hydroxide, 1N Folin- Ciocalteu reagent, Alkaline copper reagent, 4% Sodium potassium tartrate, 30% Trichloroacetic acid, 0.75% Thiobarbituric acid, 0.15M Tris-KCl buffer (pH7.4), 5% Dichromate solution, 0.2M Hydrogen Peroxide, Dichromate/acetic acid, 0.05M Carbonate buffer, 0.3 mM Adrenaline, GSH working standard, 0.1M phosphate buffer (pH 7.4), potassium chloride, dipotassium hydrogen orthophosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), tris base, ethanol, Distilled water. All chemicals used in this study were gotten from Sigma Aldrich, USA. These chemicals and all others used in this study were of standard analytical grade. The drugs used are aspirin (Bayer Pharmaceuticals, Germany) and cimetidine (Cimebios 200, Jiangsu Pharmaceutical Ltd. Jiangsu Province, China)²⁵⁻²⁸.

2. 1. Collection of plant material

L. aestuans were collected within Bowen University, Iwo, Osun State in January 2015. For botanical identification and voucher specimen referencing was done at FRIN, Ibadan, Oyo State, Nigeria (Voucher specimen number: FHI 110899). Plant authentication was done by Dr Ayanbamiji in the Microbiology and Botany Department, Bowen University, Iwo, Osun State.

2. 2. Animals

120-150g adult male wistar rats were obtained from the Veterinary Anatomy Department of University of Ibadan, kept at the animal house. They were fed *ad libitum* with standard rat chow throughout the period of the experiments with free access to water and were housed in polyethylene walled cages. The animals were kept on a 12 hour light: 12 hour dark regime at 28 °C. The study was by following the ethical directives (2010/63/EU) for animal handling proposed by the European parliament and was approved by the Department of Biochemistry, Animal Ethical Committee, Bowen University, Iwo, Osun State.

2. 3. Preparation of the leave extract of *L. aestuans*

The leaves of *L. aestuans* were air-dried at room temperature for a period of 15 days and pulverized with electric blender (model MS-223; Blender/Miller III, Taiwan, China), and the 500g of the powdered was extracted with 2. 5 liters of ethanol by maceration for 48 hours with intermittent shaking. The extract obtained was strained, filtered, evaporated to dryness on a rotatory evaporator (Model 349/2 Corning/England) at 40 °C.

2. 4. Experimental Design and Grouping of Animals

Twenty four Wistar rats (120-150g) were used in this study. Rats were grouped into 6 groups with 4 rats in each group. Feed was withdrawn from the rat 16-18 hours before the start of the experiment but the rats were allowed access to water. Rats in Group 1 served as the control group (1% gum acacia), Group 2 received 25 mg/kg aspirin alone (ulcerated, untreated group). Rats in Groups 3 and 4 were pre-treated for 3 days with different doses of the ethanol extract of *L. aestuans*, rats in Group 5 were pre-treated for 3 days with 50 mg/kg of cimetidine, rats in Group 6 were pre-treated for 3 days with 50 mg/kg of catechin. Pre-treatment was done for 3 days. On the third day, one hour after the last dose of the ethanol extract, cimetidine and catechin, 25 mg/kg of aspirin was administered orally to rats in Groups 3, 4, 5 and 6.

Four hours after the administration of the ulcerogenic agent (aspirin), all the animals were sacrificed by cervical dislocation, their stomachs were excised and opened along the great curvature and the gastric contents were collected with the washings. Both the washings and gastric contents were centrifuged together at 4000 rpm for 10 minutes, stored in test tubes and kept in a freezer at -4 °C, total protein concentration ⁴, glutathione level ⁶, catalase ⁷.

2. 5. Statistical Analysis

The SPSS was used and a value of $P < 0.05$ was statistically significant. The data were analyzed using one way analysis of variance and mean values were compared using Duncan test. Results were expressed as mean $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

In Figure 1 above, oral administration of 25 mg/kg ASP alone showed an increased gastric juice which was also statistically significant when compared to the ulcerated, untreated group. Pre-treatment with different doses of the treatment decreased gastric juice compared to that of the ulcerated, untreated group which had increased gastric juice concentration.

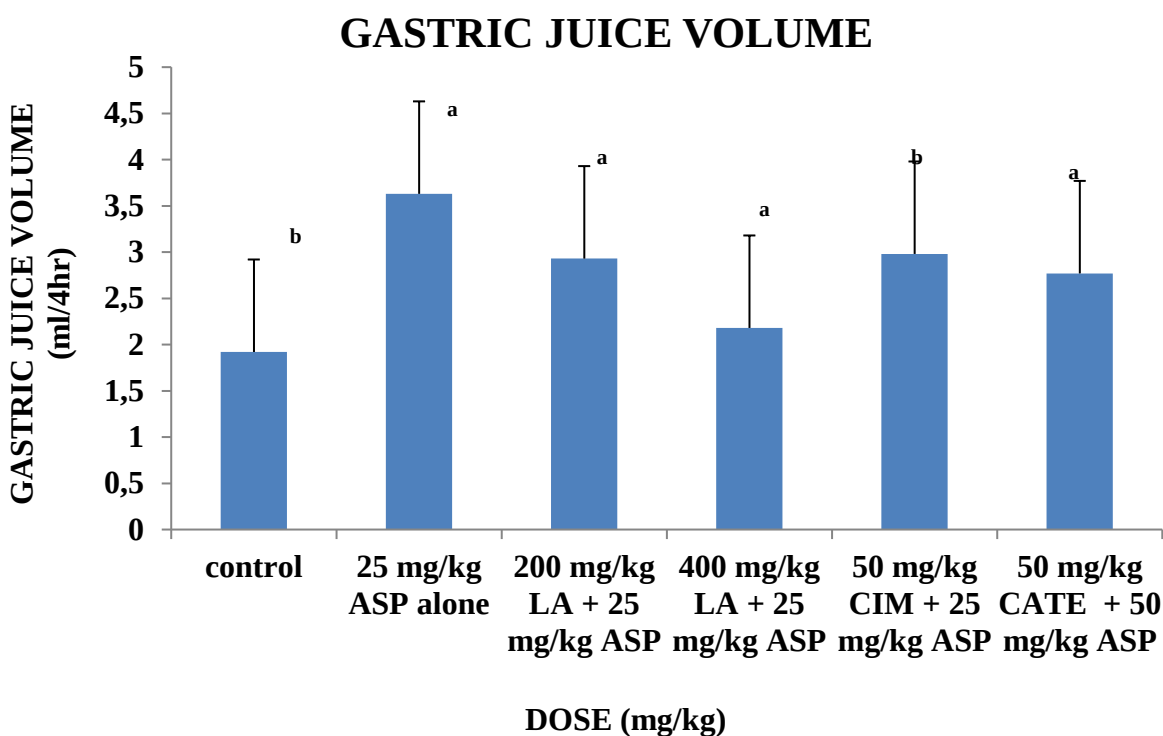


Figure 1. Shows the effect of different treatment on gastric juice volume in aspirin-induced gastric ulcer in male Wistar rats. Each bar represents Mean $\pm$ SD of 4 rats. ^a value differ from normal control group, ^b values differ from ulcerated untreated group (p<0.05).

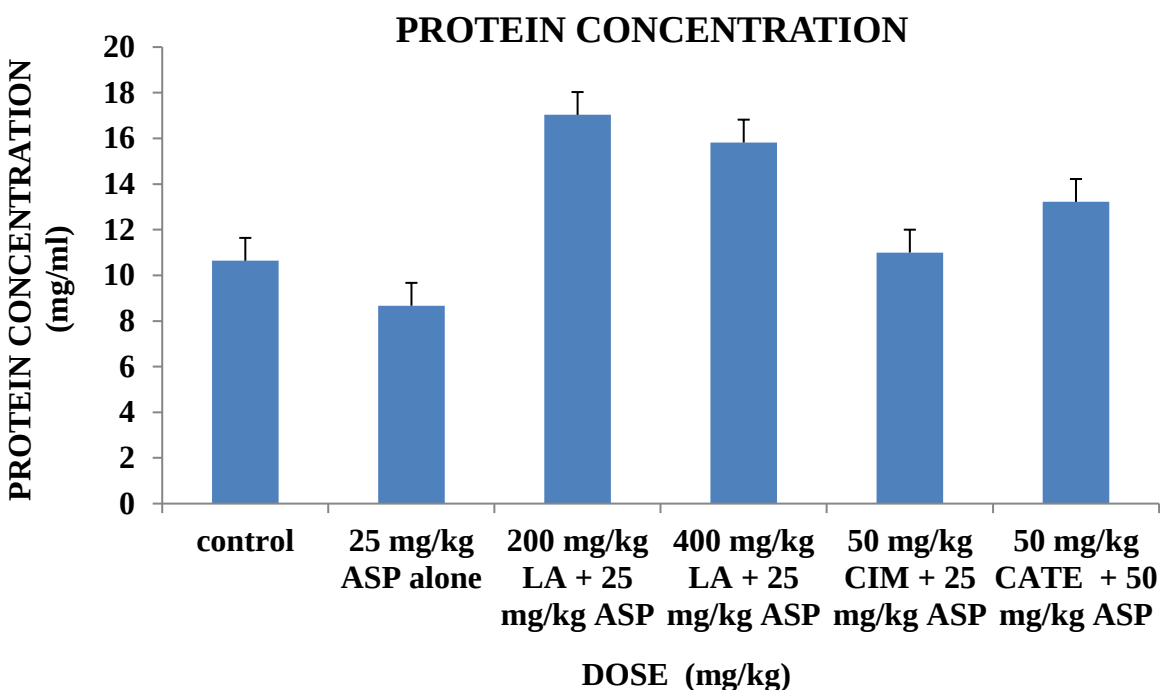


Figure 2. Shows the effect of different treatment on protein concentration in aspirin-induced gastric ulcer in male Wistar rats. Each bar represents Mean $\pm$ SD of 4 rats.

When also compared to the ulcerated, untreated group, the rats pre-treated with cimetidine and catechin showed decreased gastric volume. Pre-treatment with cimetidine was statistically significant when compared to the ulcerated, untreated group.

From the Figure 2 shown above, the group treated with 25 mg/kg ASP alone had decreased protein concentration compared to the normal control group which had increased protein concentration. Groups pre-treated with different doses of the treatment significantly increased the protein concentration compared to that of the ulcerated, untreated group with increased protein concentration while the group pre-treated with cimetidine and the group pre-treated with catechin also increased protein concentration when compared to the ulcerated, untreated group.

In Figure 3, the group treated with 25 mg/kg ASP alone showed decreased GSH activity and was statistically significant when compared to the normal control group. Groups pre-treated with different doses of the treatment, cimetidine and catechin all showed increased GSH and were statistically significant when compared to the ulcerated, untreated group.

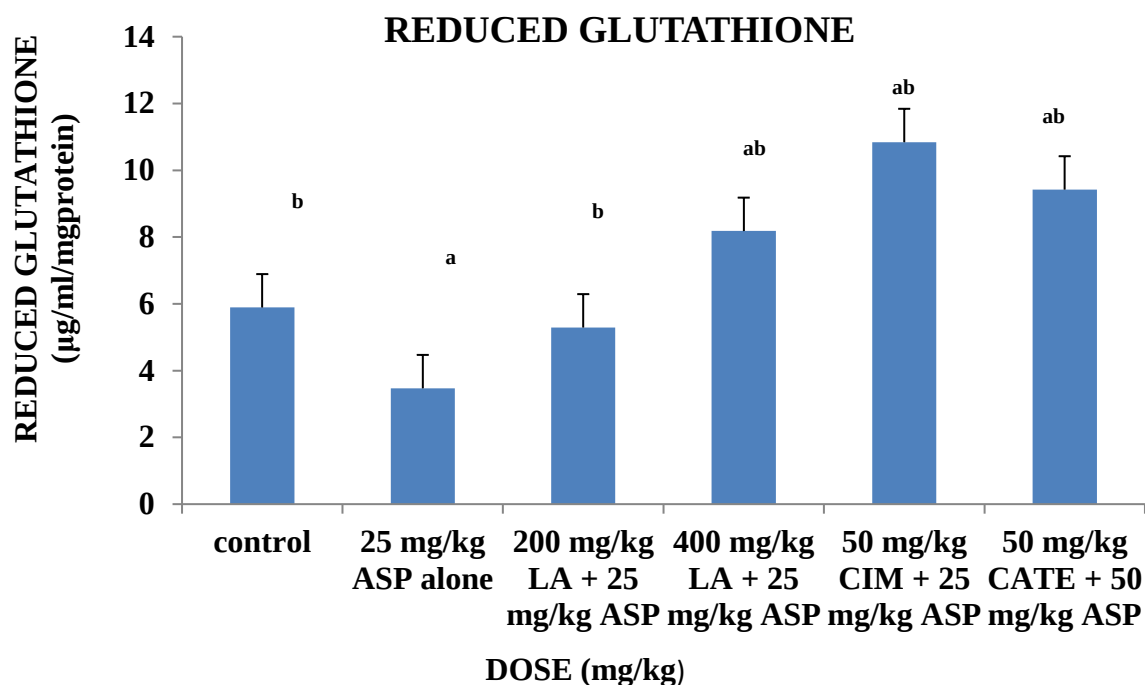


Figure 3. Shows the effect of the effect of different treatments on reduced glutathione levels (GSH) in aspirin-induced gastric ulcer in male Wistar rats. Each bar represents Mean $\pm$ SD of 4 rats. ^avalues differ from normal control group, ^b values differ from ulcerated untreated group at ($p < 0.05$).

In Figure 4, oral administration of 25 mg/kg aspirin alone showed a decrease in catechin activity which was statistically significant when compared to the normal control group. Pre-treatment with different doses of the treatment showed increased catalase activity with the group pre-treated with 200 mg/kg being statistically significant to the ulcerated, untreated group. The group pre-treated with cimetidine showed increased catalase activity and was

statistically significant from the ulcerated, untreated group. Also, the group pre-treated with catechin also showed an increase in catalase activity and was statistically significant from the ulcerated, untreated group.

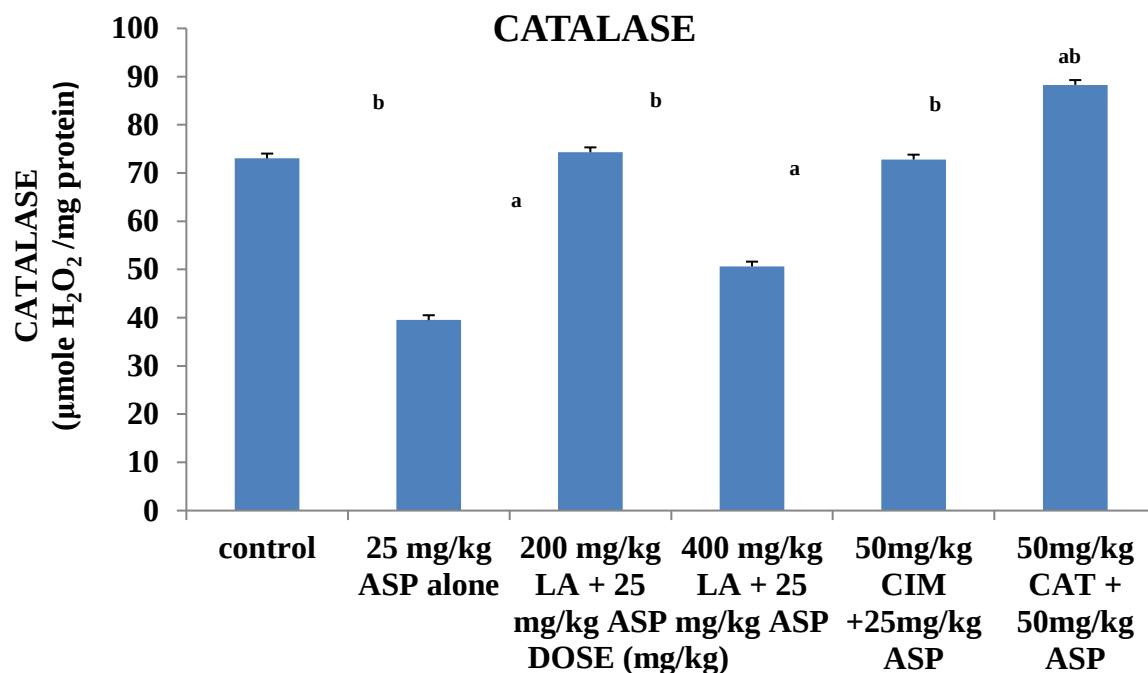


Figure 4. Shows the effect of different treatment on catalase activity in aspirin-induced gastric ulcer in male Wistar rats. Each bar represents Mean $\pm$ SD of 4 rats. ^a value differ from normal control group. ^b values differ from ulcerated untreated group at ($p < 0.05$).

This study was designed to explore and evaluate the anti-oxidant and anti-ulcerogenic properties of the ethanol extract of *L. aestuans* against aspirin-induced gastrointestinal ulcers. *L. aestuans* is a medicinal plant with several phytochemicals possessing anti-oxidant properties which can aid in the scavenging of free radicals which cause oxidative damage. Different doses of the extract was administered to the animals and tested against aspirin-induced gastric ulcer. The extract was found to be very effective in inhibiting gastric ulceration. This is evident from the significant reduction in ulcerogenic parameters assayed for such as gastric volume and protein concentration. The anti-ulcer activity of the extract can be attributed to the inhibition of ulcerogenic parameters and strengthening of the gastric mucosal barrier. Oral pre-treatment of cimetidine, a reference drug and catechin was also administered to the animals to test against aspirin-induced gastric ulcer.

They were found to be effective as they significantly reduced ulcerogenic parameters and increased antioxidative activities. The group treated with aspirin alone had increased gastric juice volume when compared to the normal control group. The gastric mucosa secretes large quantity of mucus which acts as a mechanical barrier resulting in the protection and strengthening of the gastric mucosa⁸. Treatment with aspirin alone reduced catalase activity when compared to the normal control group, while pre-treatment with different doses of the extract of *L. aestuans*, cimetidine and catechin all increased catalase activity when compared

to the ulcerated, untreated group. Reduced Glutathione (GSH) is a biological antioxidant that defends the stomach lining from generation of reactive oxygen species such as free radicals and peroxides. Treatment with aspirin alone showed depletion of reduced glutathione levels, this result is in accordance with the findings as a result of altered glutathione metabolism in aspirin-induced oxidative stress and mitochondrial dysfunction in HepG2 cells¹⁷, this is because the glutathione is used up to defend the stomach lining against free radicals which can cause oxidative and cellular damage. Pre-treatment with different doses of *L. aestuans*, cimetidine and catechin showed higher reduced glutathione level which is due to their contributing antioxidant properties. The most common causes of ulceration are stress, Non-steroidal anti-inflammatory drugs and *Helicobacter pylori*²². A decrease in the mucosal blood flow and regeneration are important in the pathogenesis of ulcer²³⁻²⁴ with the extract of *L. aestuans* containing potent antioxidants with powerful antiulcer activities.

4. CONCLUSIONS

In conclusion, the ethanolic extract of *L. aestuans* can be said to be used as an anti-ulcerogenic agent against aspirin-induced gastric ulcer which is due to the presence of phytochemicals in the plant.

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