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EFFECT OF CAPSICUM ANNUUM ON THE LIVER FUNCTION OF ASPIRIN-INDUCED WISTAR ALBINO RATS

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Abstract

Peptic ulcer disease remains one of the most prevalent gastrointestinal diseases and has been linked to *H. pylori* infection and the use of non-steroidal anti-inflammatory drugs. *Capsicum annum* is known for its anti-inflammatory, antioxidant, antibacterial, nutritional, and medicinal properties. This study aimed to determine the effects of aspirin on liver function parameters (alanine aminotransferase, alkaline phosphate, serum aspartate aminotransferase, and gamma-glutamyl transferase) in male Wistar albino rats. Twenty-five (25) male wistar albino rats were procured and acclimatized for 2 weeks under normal laboratory conditions and were divided into five groups. Group A (blank control) was fed only food and water and was neither induced with aspirin nor treated. Group B (negative control) was induced with 50 mg/kg of aspirin and was not treated. Group C (positive control) was induced with 50 mg/kg of aspirin and was treated with omeprazole. Group D (low-dose *Capsicum anuum*) was induced with aspirin and treated with 100 mg/kg of C. annum extract, and group E (high-dose control) was induced with aspirin and treated with 400 mg/kg of *Capsicum annum* ethanoic extract. Ulceration and treatment were administered orally via intubation. After 4 weeks of treatment, ulceration significantly increased the alanine aminotransferase level ($p > 0.05$) in the negative control group (28.06 ± 2.10) and decreased when treated with both low-dose and high-dose *Capsicum annum* extract (25.20 ± 2.40, 21.70 ± 1.80). Changes in other parameters were significant in the low- and high-dose treatments, indicating that the changes in these parameters are dose-dependent.

Introduction

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Peptic ulcer disease (PUD) is an illness that develops because of a mucosal break of the upper gastrointestinal tract due to the digestion of peptic acid, which results in the formation of ulceration, and this extends beyond the muscularis mucosae into the submucosa. It usually occurs in the stomach and first part of the duodenum, but can also occur in the distal esophagus, distal duodenum, and jejunum, and the Meckel's diverticulum with heterotrophic gastric mucosa (Lanas and Chan, 2017). The ulcer size varies from 5 mm to several centimeters.

According to statistics, PUD affects approximately 4.5 million persons per year in the United States (US) and causes huge healthcare costs of approximately $3.3 billion/year (Sandler *et al*., 2002). The prevalence of PUD varies with the prevalence of H. pylori infection. *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs) account for the majority of PUD cases. More than 90% of duodenal ulcers and >70% of gastric ulcers are positive for *Helicobacter pylori* (O'Connor, 1994). All NSAIDs can cause gastrointestinal injuries, including inflammation, erosions, ulcerations, and bleeding.

Peptic ulcer disease is characterized by the following signs and symptoms: loss of appetite, heartburn, bloating, and abdominal pain (Suer and Michetti, 2002). If the patient does not start treatment early, it will lead to complications. These complications are usually more serious and include gastric outlet obstruction, perforation, gastrointestinal bleeding, stomach ulcer, and intestinal wall perforation (Satoh, 2005). Peptic ulcer disease is clinically diagnosed with the following characteristic symptoms: loss of appetite, heartburn, bloating, and abdominal pain. Beyond the symptoms, it is diagnosed using esophagogastroduodenoscopy, serologic test, and breath test (Rockey and Koch, 2010). Treatment of peptic ulcers depends on the cause. Treatment usually involves using standard ulcer drugs when it is caused by *H. pylori* bacteria, which stops or reduces the use of nonsteroidal anti-inflammatory drugs and helps the ulcer heal with medications. The successful eradication of *H. pylori* is paramount for treating associated peptic ulcers and preventing recurrence; however, increasing antibiotic resistance has made this issue a global concern.

Traditional medicine and herbal remedies have always been an integral part of Nigerian culture and have been used to treat several ailments (Rainsford, 2007). *Capsicum annuum* is a plant genus native to southern North America, the Caribbean, and northern South America. It is an herb or small shrub that grows to a height of 0.3–1.2 m (1–4 feet) and a width of 15–30 cm (6–12 inches). It is commonly known as bell pepper or chili pepper (Perry *et al.,* 2007). *Capsicum annuum* is known for its high nutritional value. They are rich in vitamins A and C, dietary fiber, and antioxidants. These nutrients contribute to the health-promoting properties of bell peppers (Anaya *et a*l., 2021). *Capsicum annuum* contains capsaicin, a bioactive compound responsible for its spicy taste. Capsaicin has been studied for its potential health benefits, including pain relief, weight management, and anti-inflammatory properties (Chaiyasit *et al.,* 2009). Capsaicin inhibits acid secretion and stimulates alkali and mucus secretions, particularly gastric mucosal blood flow, which helps in the prevention and healing of ulcers (Kim and Hong, 2014). Therefore, this research aimed to use the medicinal plant *Capsicum annum* for the cure of peptic ulcer disease because the tannins present in *capsicum* act as an astringent and benefit inflammation and other problems of the gastrointestinal tract, such as dysentery, diarrhea, and other microbial disorders. It also acts as an agent of gastric mucus formation, ensuring that peptic ulcers do not develop.

Materials and Methods

Animal model and experimental design

A total of 25 mature male Wistar albino rats were used in this research. These rats were grouped into five groups, i.e., from Groups A to E.

Group A (Blank Control): This group contained five male Wistar albino rats that were not induced or treated.

Group B (Negative control): This group comprised five male Wistar albino rats that were induced with 50 mg/kg of aspirin but left untreated.
Group C (Positive Control): This group contained five male Wistar albino rats that were induced with 50 mg/kg of aspirin and then treated with omeprazole (20 mg/kg of body weight).
GROUP D (Low dose of extract): This group had five male Wistar albino rats that were induced with a high dose (50 mg/kg) of aspirin and treated with a low dose (100 mg/kg) of *Capsicum annuum* extract.
GROUP E (High dose of extract): This group contained five male Wistar albino rats that were induced with a high dose (50 mg/kg) of aspirin and then treated with a high dose (500 mg/kg) of *Capsicum annuum* extract.

Animal procurement selection and grouping

Twenty-five (25) mature male Wistar albino rats were purchased from the University of Nigeria, Nsukka (UNN). The rats were transported to the Power-Tech Analytical and Scientific Research Laboratory in the town of Enugu. The rats were allowed to acclimatize for 14 days under normal water, food, and temperature conditions. After the acclimatization period, the rats were weighed and grouped into five (5) according to their relative body weight in grams, with a maximum difference of five (5) grams.

Induction of peptic ulcer

Male Wistar albino rats were induced with 50 mg/kg aspirin through oral intubation for 3 days.

Collection of plant extracts

C. annuum was plugged from a garden in Nkanu West LGA in Enugu state. The plant was identified and authenticated by Prof. C. S. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria

Preparation of *C. annuum*

The fruit *of Capsicum annuum* was rinsed in clean water, after which it was pounded in a mortar with a pestle with a small amount of water added. The bell pepper was then sieved to separate and obtain the part that was most needed for this experiment.

The *Capsicum annuum* pepper was carefully selected and washed to ensure hygiene, after which it was pounded in a mortar with a pistil with the addition of a small amount of water. The mixture was then sieved with a filter to extract the nutritional component of *C. annuum*.

Method of *Capsicum annuum* Administration

The administration of *Capsicum annuum* as treatment to Groups D and E was done orally through intubation. Group D was administered a low dose (100 mg/kg) of *Capsicum annuum* extract once daily, whereas Group E was treated with a high dose (500 mg/kg) of *Capsicum annuum* extract once daily.

Evaluation of the parameters

Evaluation of ALT

One hundred micro liter (100µl) or 0.1 ml of the individual serum sample was pipetted into a clean test tube properly labeled and (100µl) or 0.1 ml of distilled water to another test tube labeled blank.500µl or 0.5 ml of ALT reagent 1 (R1) was added, and the mixture was incubated in an electro-thermal oven at 37ºC for 30 min. Thereafter 500µl or 0.5 mL of ALT reagent 2 (R2) was added, and the mixture was incubated for another 20 minutes at 25 ºC. After that, 5.0 mL of 0.4 mol/L sodium hydroxide was added. The absorbance of pyruvate hydrazone, which is equivalent to the level of alanine amino transferase reaction, was measured at 546 nm against the reagent blank. The ALT level estimation was by tracing the absorbance of the sample to the enzyme activity U/L from the standard ALT graph.

Evaluation of aspartate transaminase

One hundred micro liter (100µl) or 0.1 ml of the individual serum sample was pipetted into a clean test tube properly labeled and (100µl) or 0.1 ml of distilled water to another test tube labeled blank.500µl or 0.5 ml of AST reagent 1 (R1) was added, and the mixture was incubated in an electro-thermal oven at 37ºC for 30 min. Thereafter 500µl or 0.5 ml of AST reagent 2 (R2) was added, and the mixture was incubated at 25°C for another 20 minutes at 25 ºC. After that, 5.0 mL of 0.4 mol/L sodium hydroxide was added.

The absorbance of oxaloacetate hydrazone, which is equivalent to the level of aspartate amino transferase, was measured against the blank reagent at 546 nm. The AST level estimation was by tracing the absorbance of the sample to the enzyme activity U/L from the standard AST graph.

Evaluation of ALP

The alkaline phosphate concentration was determined using the Randox kit manual method, which is based on the principle that serum alkaline phosphate quantitatively determines p-nitriphenol liberated from the catalytic activity of ALP on p-nitrophenylphosphate. The composition of R1a, buffer diethanolamine ((1 mol/L, pH 9.8), and R1b, substrate (p-nitrophenylphosphate) (10 mmol/L) was as follows: 10 ml of R1a was added to R1b to dissolve the substrate, which was used for the analysis. 0.05mls of 50µl of the blood serum was added into a clean test tube, and three (3 ml) of reagent R1b mixed were added. The contents were mixed thoroughly, and the first absorbance A1 was read as 1 after mixing. The second absorbance A2 was read exactly 2 minutes later, while the third absorbance was read 3 minutes later at a wavelength of 405 nm.

Calculation

ALP concentration U/L = 2760 × ΔA 405nm/min

Evaluation of gamma-glutamyl transferase

The substrate L-γ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine was converted by γ-GT in the ample to 5-amino-2-nitrobenzoate, which can be measured at 405 nm.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine γ-GT L-γ-glutamylglylcylglycine + 5-amino-2-nitrobenzoate.

Procedure: The GGT enzyme R1b was diluted with 3.0 ml of R1a. 0.1ml or 100µl of the serum samples were pipette into clean test tubes labeled accordingly, and 1.0 ml of the reconstituted reagent was added. The mixture was mixed properly, and the initial reading was taken. After 1-, 2-, and 3-min intervals, the absorbance reading was measured at 405 nm.

Calculation

The GGT activity was calculated using the formula: U/L = 1158× Δ absorbance.

Statistical Analysis

All information is expressed as mean ± SEM. The Statistical Program of Social Science (SPSS) for Windows version 21 was used to conduct the one-way ANOVA statistical analysis. Differences in means were deemed statistically significant ($P<0.05$)) at the 5% level.

Results

As shown in Table 1, the ameliorative effect of *Capsicum annum* on the alanine aminotransferase level of male wistar albino rats induced with aspirin was dose-dependent. Group D treated with a low-dose extract of *Capsicum annum* had a significant increase ($p > 0.05$) of (25.200 ± 2.40) compared with Group B (negative control). However, there was no significant difference in the serum alkaline phosphate level of the male wistar albino rats induced with asprin at low dose (28.30 ± 1.50) when compared with the negative control. In the serum aspartate aminotransferase level, Group C was induced and treated with a standard drug (omeprazole), and a significant reduction ($p<0.05$) (25.70 ± 1.80) was observed when compared with the negative control. In the serum gamma

glutamyl transferase level, the administration of the extract of *Capsicum annuum* at low dose (27.46 ± 1.90) had no significant difference (P<0.05) when compared with the administration of the plant extract at high dose (21.87 ± 1.80); therefore, the decrease was dose dependent.

Table 1: Effect of *Capsicum annum* on liver serum enzyme activities of male wistar albino rats induced with aspirin

Groups	ALT (µ/l)	ALP (µ/l)	AST (µ/l)	GGT (µ/l)
A (Blank Control)	23.06 ± 2.10^{a}	24.26 ± 1.50^{a}	23.26 + 2.00^{a}	18.33 + 2.10^{a}
B (Negative control)	28.60 ± 3.40^{b}	33.50 ± 2.50^{b}	41.50 + 2.10^{b}	59.31 + 3.20^{b}
C (positive control)	21.45 ± 1.80^{c}	25.40 ± 1.90^{a}	25.70 + 1.80^{a}	22.70 + 1.80^{a}
D (low-dose extract)	25.20 ± 2.40^{d}	28.30 ± 1.50^{d}	28.20 + 1.70^{c}	27.46 + 1.90^{c}
E (high-dose extract)	21.70 ± 1.80^{c}	27.50 ± 1.90^{c}	27.40 + 1.80^{c}	21.87 + 1.80^{a}

Data are presented as mean ± SEM. Mean values with different superscript letters were significantly different (p<0.05).

Discussion

Capsicum annum, commonly known as chili pepper, is a renowned spice worldwide. In addition to its dietary significance, its medicinal importance has also been established in folklore. Studies have demonstrated that this spice exerts a significant impact on the renal function of male Wistar rats treated with *Capsicum annum* extract or capsaicin (the active constituent present in chili peppers) after being induced with aspirin. This improvement in liver function can be attributed to various mechanisms, such as enhanced blood flow to the kidneys and reduced inflammation in kidney tissue. Additionally, this effect appears to be more pronounced in male albino Wistar rats than in females or non-albino rats.

This improvement can be attributed to several possible mechanisms, including increased blood flow to the kidneys and reduced inflammation in kidney tissue. Additionally, this effect appears to be more pronounced in male albino Wistar rats than in female or non-albino rats.

The findings from the experiment showed that *Capsicum annum* has an ameliorative effect on the alanine aminotransferase level of male Wistar albino rats induced with aspirin as the treatment group, which was induced and treated with *Capsicum annum* showed a significant reduction in the alanine aminotransferase level when compared to the non-treated group, which is in accordance with the findings of (Bjornsson, 2016). A high dose of aspirin increased the alkaline phosphate level. The high-dose administration of *Capsicum annum* extract showed more reduction when compared with the low-dose administration; therefore, the decrease is dose dependent, as discovered by Sachs in 2008.

The results also showed no significant difference in the administration of the extract of *Capsicum annuum* at low dose compared with the administration of the plant extract at high dose.

The ulcer index decreased significantly on treatment with *Capsicum annum* compared to the non-treatment groups.

These findings propose that *C. annum* could be used as a therapeutic agent for improving liver health and function in certain populations.

Conclusion

Capsicum annum contains various phytochemicals, such as phenols, flavonoids, tannins, anthraquinone, and terpenoids. These compounds have been scientifically proven to have ameliorative effects on liver function parameters, including alanine aminotransferase (ALT), alkaline phosphate, serum aspartate aminotransferase (AST), and serum gamma-glutamyl transferase.

Recommendations

Further studies are recommended to investigate the protective effects of *Capsicum annuum* on liver function in aspirin-induced Wistar albino rats using varied doses and treatment durations. Comparative evaluation with standard hepatoprotective agents will help establish its relative efficacy. Long-term safety assessments are necessary to rule out potential hepatic or systemic toxicity. Mechanistic studies focusing on antioxidant, anti-inflammatory, and enzyme-modulating pathways are encouraged.

Declaration

We, the authors, declare that this manuscript titled " Effect of *Capsicum annuum* on the Liver Function of Aspirin-Induced Wistar Albino Rats" is original and has not been published or submitted elsewhere for publication. All data were collected and analyzed following the ethical guidelines for animal research. There are no conflicts of interest to declare, and all authors have approved the final version of the manuscript for submission.

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Conflict of Interest

The authors declare no conflict of interest regarding the manuscript titled “Effect of *Capsicum annuum* on the Liver Function of Aspirin-Induced Wistar Albino Rats”.

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