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**Gastroprotective Activity of N-hexane and Chloroform Fractions of *Sesamum radiatum* Leaf Extract on Aspirin Induced Ulceration in Rat**

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**ABSTRACT**

Gastric ulcer is one of the most common gastrointestinal disorders, which causes a high rate of morbidity all over the world. Available drugs for ulcer treatment are associated with several side effects, thus the need to search for alternative treatment using plants and vegetable which are readily available and safer. The effect of n-hexane and chloroform fractions of *Sesamum radiatum* leaf extract on aspirin inducedulceration in albino rats were investigated. Ulcerative index, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA) and the extent of inhibition of lipid peroxidation were determined using standard methods. Acute toxicity study revealed that the fractions were nontoxic up to a dose of 5000mg/kg bodyweight. Animals administered with 100 and 500mg/kg bodyweight of n-hexane fraction exhibit inhibition of ulceration of 33.33% and 50.00% while those of chloroform fractions showed 50.00% and 66.0% inhibition respectively compared to the 0% of the untreated group. AST, ALT, ALP and MDA Level of the ulcer induced rats treated with both fractions were significantly (p<0.05) reduced in a dose dependent manner. The SOD and CAT activities of *Sesamum radiatum* fractions treated groups showed significant increase as compared with untreated and toxic group. It can therefore be suggested that then-hexane and chloroform fractions of *S. radiatum* may possess considerable therapeutic potential in the management of gastric ulcer.

**Keywords:** *Sesasum radiatum*, aspirin-induced ulcer, n-hexane, chloroform

**INTRODUCTION**

Ulcer is an open sore or lesion that occurs on the Ulceration of the gastric mucosa due to contact skin or membranous areas of the body. It could with gastric juice is called gastric ulcer. Where it be external or internal (Mcquaid, 2007). occurs in the lining of the stomach or duodenum,

Hamzah R. U., Busari M. B., Agboola A. R., Mohammed H. A., Sayyadi A. Momoh O. L. & Ejiro O. (2021). Gastroprotective Activity of N-hexane and Chloroform Fractions of *Sesamum radiatum* Leaf Extract on Aspirin Induced Ulceration in Rat. 2nd Faculty of

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it is referred to as peptic ulcer. Peptic ulcer is a heterogeneous group of disorder involving the upper gastrointestinal tract that shows incidences of relapse, drug interactions and side effects such as arrhythmias, impotence, gynaecomastia, haematopoietic changes, etc. (Mcquaid, 2007).

Peptic ulcer is a gastro intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus and innate resistance of the mucosal cell factors (Ramakrishnan, 2007). Normally peptic ulcer develops when aggressive factors overcome the defensive factors (Mcquaid, 2007). The major factors that disrupt the equilibrium between aggressive factors and defensive factors are Helicobacter pylori, acid–pepsin hyper secretion, non-steroidal anti-inflammatory drugs, sometimes idiopathic due to usage of tobacco, psychological stress and rapid gastric emptying (Atawodi, 2003).

Herbal medicines are considered as better alternatives for the treatment of peptic ulcer (Chukwurah, 2000). For instance, proton pump inhibitors (Omeprazole, lansoprazole) may cause nausea, abdominal pain, constipation, diarrhoea and H2 receptor antagonists (cimetidine) may cause gynaecomastia, loss of libido. Due to the occurrence of these side effects from the use of synthesized drugs for many diseases, Herbal medicines are considered safe for the treatment of ulcers with lesser adverse effects, economical, effectiveness and less toxic (Oyetayo, 2007).

*Sesamum radiatum* belongs to the family of Pedaliaceae. It is a leafy vegetable locally called *ekukugogoro* oe *Ewe atura* in Yoruba language, *beni* or gingelly in English, *ridi* in Hausa, and in Igala, *Oro dudu* (Gills, 1992). It is an indigenous vegetable that grows in small quantity in the rural areas. The plant occurs throughout the tropical Africa mainly as weed, where it is gathered in the wild and used as a potherb (Auwalu *et al*., 2007). It is one of the many neglected leafy vegetables of the tropics due to lack of knowledge of its medicinal importance.

In South - Western Nigeria the leaves are used to bring relaxation and health to the body, possibly because they relieve constipation and cure other ailments on ingestion (Odugbemi, 2008). The leaves, seeds and oil serve as food especially in farming communities in Nigeria (Akpan-Iwo *et al*., 2006). The leaves are also used for treating various stomach ailments. While the use of nonsteroidal anti-inflammatory drugs (NSAID) may be effective, they are sometimes associated with relapse and adverse effects (Dharmani & Palit, 2006). This has led to renewed interest in the search for new anti-ulcer drugs from natural sources. This study was therefore carried out to determine the effects of the chloroform and nhexane fractions of *Sesamum radiatum* on aspirin-induced ulceration in rats.

**MATERIALS AND METHODS**

**Sample Collection, Chemicals and Control drug**

*Sesamum radiatum* leaves were obtained from a private garden in Fiidi, Makurdi local Government of Benue in January of 2014.The plant was identified at the Department of Biological Science, University of Agriculture, Makurdi, Benue State.

Chemicals of analytic grade such as: methanol, chloroform, linoleic acid and Tween 20 produced by Amazon Chemical Limited, n-hexane, hydrogen peroxide, hydrochloric acid (Sigma Chemical Co. USA), ascorbic acid (Sigma Aldrich, USA) and others were used.

Aspirin (Greenfield Pharmaceutical Limited (JIANG S.U,China) and Omeprazole (Ranivan 20mg) produced by CIPLA Limited, India ) were used.

**Extraction and Fractionation of Plant Sample**

The leaves were thoroughly washed under running tap water and were air dried at room temperature for seven (7) days, grounded into powder using mortal and pestle. Fifty grams of the dried powdered leaves was weighed into a distillation flask and 400 ml of methanol solution was added to the flask. The mixture was refluxed for two hours, filtered hot using muslin cloth and subsequently evaporated using a rotary evaporator. The fractionation of the crude extract of the sample was carried out using chloroform and n-hexane to obtain the fractions. The fractions were subsequently concentrated using a rotary evaporator (Bibby Sterling Ltd, Stone Staffordshire ST 15OSA, UK) and water bath (450C). The semi-dry extracts were weighed placed in a sterile sample bottles and stored in a refrigerator until require for further analysis (Ogbadoyi *et al.,* 2007).

**Experimental Animals**

A total of forty (40) Wister albino Rats (weighing about 120 - 200 g) of both sexes were used in this experimental study. Animals were procured from the animal house of Department of Pharmacology and Clinical Pharmacy ABU, Zaria. They were transported in well ventilated plastic cages to Department of Biochemistry, Federal University of Technology Minna, Niger State. The Animals were allowed to acclimatize for a period of 3 weeks and fed with grower feed throughout this period having free access to tap water. Animal handling and experimentations complied with the principles governing the use of laboratory animals as laid out by the international standard set by the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities’ council directive of 24 November 1986 (86/609/EEC), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

**Determination of Acute Toxicity LD50**

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. Animals were observed for 14days to check for behavioral changes in animals and mortality rate (Wannang & Bichi,2004).

**Induction of Ulcer and Experimental Design**

Aspirin (25mg/kg body weight) was administered orally to all animals in the toxic and treatment groups. The animals were made to fast for 24 hours and administered with aspirin of 200 mg/ kg bw. Orally (Das & Banerjee, 1993). Animal were randomly grouped into seven groups of four animals each as follow; **Group 1:** Normal control group; **Group 2**: Positive Control (Aspirin + Omeprazole 20 mg/kg bw.); **Group 3**: Negative control (Induced with Aspirin and not treated); **Group 4:** Induced with Aspirin + 100 mg/kg bw. of chloroform fraction (CF); **Group 5:** Induced with Aspirin + 500 mg/kg bw. of chloroform fraction. (CF), Group 6: Induced + treated with 100mg/kg bw. of n-hexane fraction (NHF); Group 7: Induced + treated with 100mg/kg bw. of nhexane fraction (NHF). The experiment lasted for 14 days.

**Collection of blood and Determination of Biochemical parameters**

Collection of blood samples was done according to the method described previously by Yakubu *et al.* (2006). Rats were euthanized and plasma were collected for biochemical assays.

**Assessment of Gastric Mucosal Lesion**

The stomachs of the rats were removed and open along a greater curvature and were washed with ice-cold saline and examined for microscopic mucosal lesions. The gastric mucosal lesions were expressed in terms of ulcer index (UI) according to method described by Peskar *et al.,* (2002).

**Preparation of Stomach Tissue Homogenate**

The stomach of animals was washed in ice cold normal saline (0.9% NaCl) solution, blotted and weighed. The grounded tissues (each 0.5g) were then treated with 4.5mL of appropriate buffers, at different pH for each biochemical assay. The resulting mixture was then homogenized and centrifuged at 1000 rpm for 15 minutes then it was removed from the centrifuge and the supernatant was decanted and stored at 100C for further analysis.

**Determination of liver Enzymes and Antioxidant Activities**

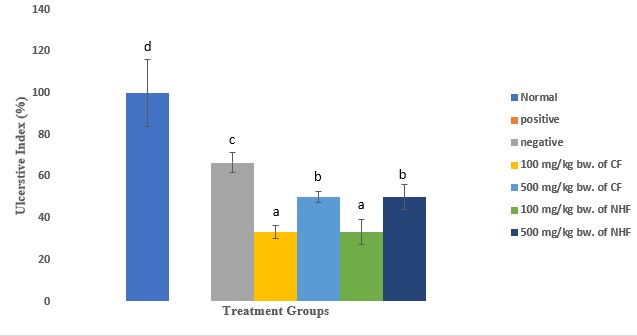
Liver enzyme activities of the rat’s plasma were determined using the methods as specified by the Randox Kit used. Superoxide dismutase and catalase activities of tissue homogenate of experimental animals were determined following the method described by (Mishra and Fridovich, 1972) and (Sinha *et al*., 1972)while lipid peroxidation was determined by measuring Malondialdehyde using the method of (Armstrong & Browne, 1994).

**Statistical Analysis**

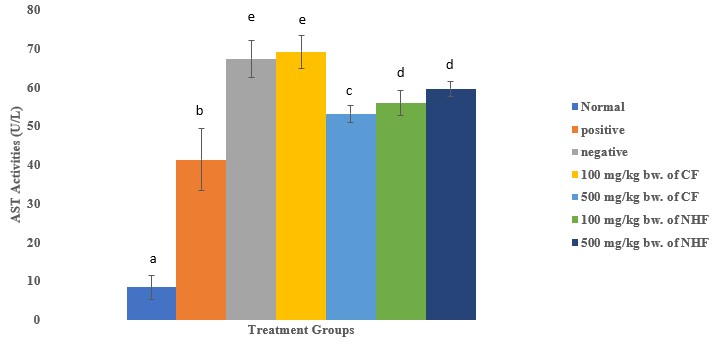
The results are given as mean ± Standard error using the SPSS 16.0 software. Group comparisons were statistically analyzed using one- way analysis of variance (ANOVA) with multiple comparisons versus control group. Values of P<0.05 were taken as significant.

**RESULTS**

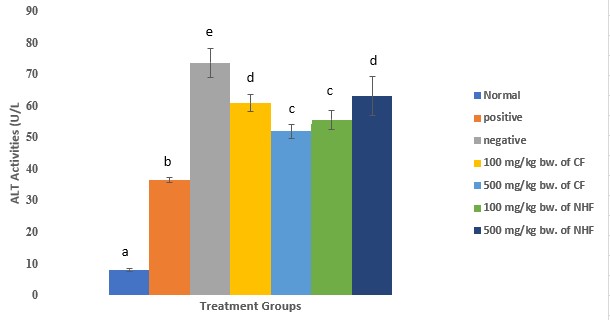
The ulcerative index, liver enzyme assay (AST, ALT, and ALP) and antioxidant assay results of the chloroform fraction and n-hexane fraction of *Sesamum radiatum* leaf extract are presented in figure 1-7.



**Figure 1: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on Ulcerative Index in Aspirin-Induced Ulcered Rats.**



**Figure 2: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on AST Activity in Aspirin-Induced Ulcered Rats.**

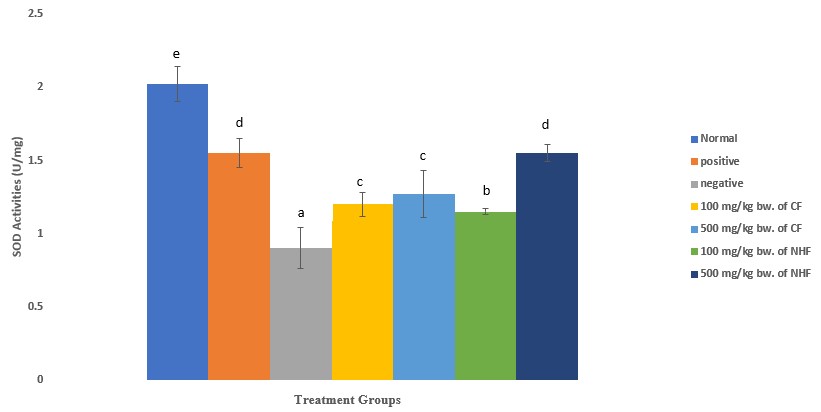


**Figure 3: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on ALT Activity in Aspirin-Induced Ulcered Rats**

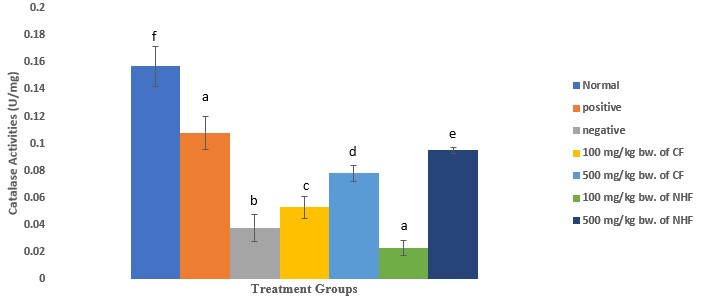


**Figure 4: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on ALP**

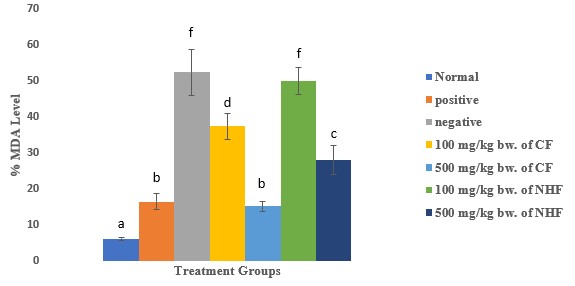
**Activity in Aspirin-Induced Ulcered Rats**



**Figure 5: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on Superoxide Dismutase (SOD)Activity in Aspirin-Induced Ulcered Rats**



**Figure 6: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on Catalase (CAT)Activity in Aspirin-Induced Ulcered Rats**



**Figure 7: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions ofLeaf Extract on**

**Malondialdehyde (MDA) Level in Aspirin-Induced Ulcered Rats**

**DISCUSSIONS**

The result of this study revealed that the chloroform and n-hexane fractions at 100 and 500mg/kg body weight led to the rapid healing in aspirin-induced gastric ulcer therefore significantly reducing the ulcerative index when compared to the untreated group. This result is compatible with a previous research result in which *Bauhinia racemosa*, *Moringa pterygosperma* and *Trianthema pentandra* were reported to cause a significant reduction in the ulcerative index of experimental rats (Akhtar & Ahmad, 1995), but is not in agreement with the report of Akhtar and Ahmad 1995, that showed that *Cordia latifolia* did not cause a reduction in ulcerative index. (Mozafar & Hossein, 2006) also reported a similar result in their experiment where they concluded that extract of *Falcaria vulgaris* reduced the ulcerative index in ethanol induced gastric ulcer in rats. Other works that agree with this result include that of Omojola *et al.* (2019), which reported that coconut milk reduced the ulcerative index in ethanol induced rats, Manowar *et al.* (2015) also showed the reduction of ulcerative index by *Sesamum indicum* in rats induced with ulcer

An increase in the liver enzymes were observed in the negative group and this indicate hepatic injury occurred due to aspirin-induction causing leakage of the enzymes into the blood (Myagmar *et al.,* 2004). *S. radiatum* administration significantly decreased the levels of AST, ALT and ALP that shows its tissue damage preventing action and this may be attributed to the presence of phytochemicals capable of healing hepatic injury.

Catalase activity (CAT) and Superoxide dismutase activity (SOD) level were significantly decreased whereas there was significant increase in Malondialdehyde (MDA) activity in the in the negative group when compared with treated and control group. This decrease could be due to generation of free radical leading to lipid peroxidation. Administration of chloroform and extracts of *S. radiatum* at 100 and 500mg/kg bw were able to improve the antioxidant parameters. This result is in agreement with previously published work by Vinodhini *et al*., (2007) which show bale leaf extracts showed positive effect on the status of antioxidant parameters.

**CONCLUSION**

In conclusion, the results shows that chloroform and n-hexane fractions of *Sesamum radiatum* leaf extract possess gastro protective activity .and provides support for the traditional use of this plant in the treatment of gastric ulcer. Further studies are ongoing to isolate the active components and to elucidate their mechanism of action.

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