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Cytoprotective And Anti Ulcer Activities of The Ethanolic Leaf Extract of *Irvingia Gabonensis* on Aspirin-Induced Ulcer

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Abstract: *This study evaluated the gastroprotective and hepatoprotective effects of ethanolic leaf extract of Irvingia gabonensis on aspirin-induced ulcer in albino rats. The animals were grouped into 5 groups of 5 rats each with Group 1 (Control), Gp 2 (Aspirin alone ASA 400mg/kg), Gp 3 (Omeprazole 20mg/kg + ASA 400mg/kg), Gp 4 (200mg/kg Extract + ASA 400mg/kg(and Gp 5 (400mg/kg Extract + ASA 400mg/kg). The result of the study showed a significant decrease ($p \leq 0.05$) in ulcer score, ulcer index, volume of gastric content, total acidity and pepsin activity. There was an increase observed in ulcer percentage inhibition in wistar rats pretreated with 20 mg/kg omeprazole, 200 mg/kg and 400 mg/kg of ethanolic leaf extract of Irvingia gabonensis when compared with the untreated group. The liver enzyme levels were also estimated using standard analytical method. There was a significant decrease on serum alanine transaminase (ALT) and serum aspartate transaminase (AST) but an increase in alkaline phosphatase (ALP) of the group pretreated with ethanolic leaf extract of Irvingia gabonensis when compared to omeprazole and untreated group. The findings of the present study showed that ethanolic leaf extract of Irvingia gabonensis may possesses bioactive ingredients that elicited the hepatoprotective effect and antiulcer activity against the ulceration caused by aspirin.*

Keywords: *Aspirin, Gastroprotective, Hepatoptotective, Irvingia gabonensis, Ulcer*

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I. Introduction

Plants have long played tremendous role in growth and maintenance of life. Prior to the introduction of chemical medicines, man depends on the healing properties of medicinal plants. They have remained a vital source of medicines for a large proportion of the world's population, particularly in the developing countries[1] . Ethnomedicinal treatments utilize the bark, kernels, leaves, or roots for a variety of ailments[2] . A good number of plant materials have been used in traditional medicine to treat so many disorders including gastro intestinal disorders. Mucosal surface epithelium is prone to attack by physical, chemical or microbiological agents acting from the gastric lumen leading to a lot of pathologies such as gastritis, peptic ulcer or even gastric cancer. The gastric mucosal layers play an important role in creating a barrier that limits an exposure of the gastric mucosal cells to all these exogenous and endogenous agents. Pretreatment with different substances could effectively prevent gastric mucosa from the development of erosions and ulcerations. Recent studies found that different substances from plant sources, not only afford gastroprotection but also accelerate ulcer healing. They may also possess anti-inflammatory action by suppressing the neutrophil/cytokine cascade in gastrointestinal tract (GIT)[3] promoting tissue repair through expression of various growth factors[4] exhibiting antioxidant activity on reactive oxygen species (ROS)[5,6] , showing anti-nucleolytic, cytochome P450 2F1 inhibitory acitivity, anti-necrotic and anti-carcinogenic activities[7,8] .

Irvingia gabonensis (O'Rorke) baill Var. Excelsa[9] is one of such often spoken medicinal plants used in Asia and West Africa including Nigeria. The bark of this plant is mixed with palm oil for treating diarrhea and for reducing the breast-feeding period. The shavings of the stem bark are consumed by mouth to treat hernias, yellow fever, and dysentery, and to reduce the effects of poison in French Equatorial Africa[10] . The antibiotic properties of the bark help heal scabby skin, and the boiled bark relieves tooth pain[2] . The Mende tribe in Sierra Leone grinds the bark into a paste mixed with water and applies the product to the skin for pain relief [10]. In certain parts of Africa, the bark extract is ingested to produce an analgesic effect [11]. The stems of the tree have been used as chewing sticks to help clean the teeth[2]. The ethanolic leaf extract has been reported pharmacologically to possess diuretic properties, antihypertensive effect and contraction of uterine smooth muscle [12,13,14] . The powdered kernels act as an astringent and are also applied to burns[10] . The phytochemical screening revealed the presence of flavonoids, tannin, alkaloids, carbohydrate, volatile oils, terpenoids, saponins and cardiac glycosides[16] . Plant-originated flavonoid substances have been shown to be highly

gastroprotective[14] . Based on these claims and the revealed flavonoid content, this present study therefore investigated the gastroprotective and hepatoprotective effect of ethanolic leaf extract of *Irvingia gabonensis* in wistar rats.

II. Materials And Methods

Collection and Identification of Sample: The leaves of Irvingia gabonensis were collected from the Herbarium Keeper of the Forestry Research Institute, Ibadan, Oyo State, Nigeria. A voucher specimen of the plant has been deposited with the Institute's Herbarium under voucher number FHI 103947 for future reference.

Experimental Animals: Twenty five (25) healthy male wistar rats of weighing between 120-180 g were purchased from the animal farm of University of Nigeria Nsukka and kept in metallic cages in the animal house of the Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu. The animals were allowed to acclimatize for 14 days under standard laboratory conditions with free access to commercial grower's mash and water *ad libitum* and 12 h/ 12 h light/darkness cycle prior to the inception of this study.

Preparation of Plant Extract: The plant leaves were obtained in large quantities and left to dry at room temperature for two days after which they were dried in an oven at $35 - 40^0$ C for 36 hrs. After that some of the leaves were ground into a coarse powder. The powdered leaves were kept in an air-tight glass container and stored in a dry place. 200g of the powder was subjected to Soxhlet extraction for 20 hrs at $50 - 55^0$ C. The extract was concentrated and dried under vacuum.

Experimental Design: The animals were randomly grouped into five (5) groups of five (5) rats each

Group I: Normal control rats that received 0.2 ml of distilled water

Group II: Negative control (Aspirin alone (400mg/kg))

Group III: Reference drug (omeprazole 20mg/kg + Aspirin 400mg/kg)

Group IV: Treatment group (200 mg/kg Extract + Aspirin 400mg/kg)

Group V: Treatment group (400 mg/kg of Extract + Aspirin 400mg/kg)

Induction of Ulcer with Aspirin (ASA)[17]

Procedure:

The rats were starved for 24 h prior to the commencement of this study, but had access to clean drinking water. Groups I and II rats were orally pretreated with 0.2 ml distilled water using a gavage tube. Group III animals were pretreated with omeprazole (a reference ulcer drug) at 20 mg/kg body weight of rat. Groups IV and V received orally, ethanolic leaf extract of *Irvingia gabonensis* at 200 mg/kg and 400 mg/kg respectively using gavage tube. Thirty minutes after these pretreatment, all animals in groups II to V were administered 400mg/kg *p.o* of aspirin for ulcer induction, but none was given to the group I rats (Normal control). One hour after this induction; all the animals were anaesthetized with chloroform and sacrificed. The stomach of each of the animals was carefully isolated and incised along the greater curvature. Their stomachs were spread and pinned flat on plywood using thumb tacks. With the aid of a magnifying glass, their stomachs were observed using Main and Whittle[18] method as described below:

Normal stomach	= 0
Red coloration	= 0.5
Spot ulcer	= 1
Hemorrhagic streaks	= 1.5
Ulcer >3mm< 5mm	= 2
Ulcer >5mm	= 3

The total score divided by a factor of 10 was designed as ulcer index for their stomach which is

$$\text{Ulcer index} = \frac{UA + US + UP}{10}$$

Where:

UA= Average number of ulcers per animal

US= Ulcer severity score

UP=% of animals with ulcers

The percentage ulcer inhibition was calculated using the formula[19] as follows:

$$\% \text{ ulcer inhibition} = 1 - \frac{\text{ulcer index for the test agent}}{\text{Ulcer index for the control}} \times 100$$

Collection of Gastric Juice: Gastric juice was collected from pylorus ligated rats. The gastric juice collected was centrifuged at 60 rpm for 10 min. and the volume of gastric juice was measured. The gastric juice was used for biochemical estimation.

Pepsin activity: This was determined by the method described by Lowry *et al.,*[20] and Debnath *et al.,*[21] .

Free acidity and total acidity: This was determined by the method described by Kulkarni [22]. The acidity was calculated using the formular:

Acidity MEq/L= vol. of NaOH x Normality x l00/0.1

Liver Function Estimation

Determination of serum alkaline phosphatase: The serum activity of alkaline phosphatase (ALP) was determined using Randox test kits. (Randox laboratories, UK)

Calculation

The ALP activity of the serum (U/L) =2760x ΔA405nm/min

Determination of serum aspartate transaminase activity: The serum activity of aspartate transaminase (AST) or glutamate oxaloacetate transaminase (GOT) was determined using Randox test kits (Randox laboratory, UK).

Calculation

The AST activity was obtained according to manufacturer's instructions by comparison to the table provided in the kits leaflet.

Determination of serum alanine transaminase activity : The serum activity of alanine transaminase (ALT) or glutamate pyruvate transaminase (GPT) was determined using Randox test kits (Randox Laboratories, Crumlin, England).

Calculation

The ALT activity was obtained by comparison to the table provided in the kit's leaflet

III. Results

Table no1: Hepa-enzymatic effect of ethanolic leaf extract of *Irvingia gabonensis* on aspirin-induced ulcer in Wistar rats

Parameter	Group I	Group II	Group III	Group IV	Group V
ALT (U/L)	35.33±0.58^{a}	58.33±3.79^{b}	41.67±1.53^{b}	43.67±2.08^{ab}	35.00±1.00^{ab}
AST (U/L)	33.00±1.00^{a}	66.00±3.61^{b}	58.67±1.53^{bc}	66.67±5.13b	57.33±3.79^{bc}
ALP (U/L)	80.00±1.00^{a}	103.33±3.21^{b}	101.33±8.39^{ab}	109.33±6.51^{b}	123.67±11.02^{b}

Values represent the mean ± s.e.m for n = 5. Values in the same row bearing the same alphabets are not significantly different from each other. Alanine Transaminase (ALT); Aspartate Aminotransferase (AST);AlkalinePhosphatase(ALP)

Table no2: Gastroprotective effect of ethanolic leaf extract of *Irvingia gabonensis* on aspirin-induced ulcer

Parameters	Group I	GroupII(Aspirin (ASA) only)	GroupIII (ASA+Omp)	GroupIV 200mg/kg Ext	GroupV 400mg/kg Ext
Ulcer score	-	12.00±0.50^{b}	5.00±0.60^{a}	8.00±0.50^{b}	3.00±0.50^{c}
Ulcer index	-	11.80±0.10^{b}	11.00±0.20^{a}	11.40±0.20^{b}	10.70±0.10^{ab}
% inhibition	-	-	6.78	3.39	9.32
pH	4.86±0.24^{b}	1.98±0.32^{ac}	3.28±0.45^{bc}	2.33±0.48^{ac}	2.88±0.21^{ac}
Gastric volume	0.63±0.06^{b}	1.36±0.06^{a}	0.61±0.01^{b}	1.05±0.13^{a}	0.78±0.02^{b}
Total acidity	3.37±0.03^{a}	5.04±0.10^{b}	2.87±0.16^{a}	4.69±0.62^{a}	3.37±0.03^{a}
Pepsin activity	286.33±0.58^{a}	314.37±14.04^{a}	243.77±31.59^{a}	281.55±23.06^{a}	208.70±0.53^{b}

Keys: Values are mean± s.e.m for n = 5. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P \geq 0.05$). Gastroprotective effect of ethanolic leaf extract of *Irvingia gabonensis* on aspirin- induced ulcer was shown in **Table no2**. From the result, group IV and V pretreated with 200 mg/kg and 400 mg/kg of the extracts had a low mean ulcer score and index, but groups III and V showed a higher percentage protection when compared with the negative control. Also, there was a significant decrease ($P \leq 0.05$) in the volume of gastric content, total acidity, pepsin activity and increase in pH in Wistar rats pretreated with 20 mg/kg omeprazole, 200 mg/kg and 400 mg/kg ethanolic leaf extract of *Irvingia gabonensi* when compared with the untreated group (negative control).

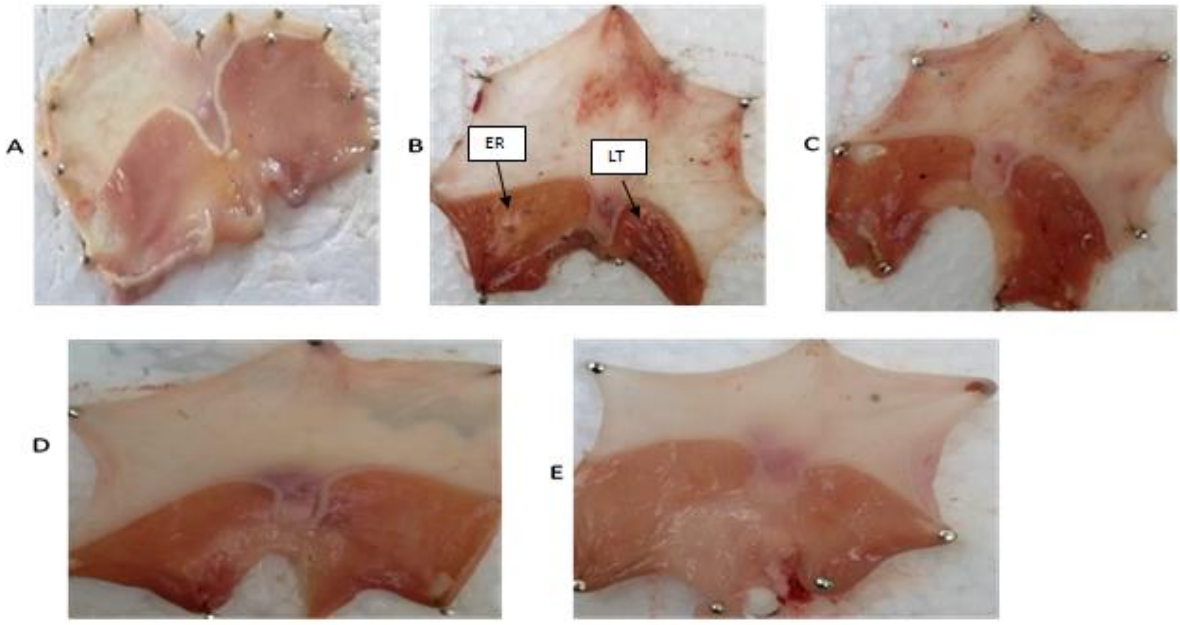

Fig no1: Pictorial section of incised aspirin ulcerated stomach obtained from untreated (control), induced, not treated (negative control) showing Laceration (LT) and Erosion (ER) and pretreated wistar rats with omeprazole and different concentration of ethanolic leaf extract of *Irvingia gabonensis*. (A) Normal control, (B) negative control, (C) wistar rats pretreated with omeprazole 20 mg/kg, (D) wistar rats pretreated with 200 mg/kg of ethanolic leaf extract of *Irvingia gabonensi*, (E) wistar rats pretreated with 400 mg/kg of ethanolic leaf extract of *Irvingia gabonensis*

IV. Discussion

Several reports have shown that large doses of Aspirin have hepatotoxic effects which lead to increase in the liver biomarkers (ALT, AST and ALP). There was a significant increase ($p \leq 0.05$) observed in the ALT and AST in Gp 2 when compared with the other groups though there was a significant decrease in groups pretreated with the ethanolic leaf extract of *Irvingia gabonensis* and the Omeprazole when compared with the negative control. This is an indication that the ethanolic leaf extract of *Irvingia gabonensis* may possess bioactive compounds that eliited the hepatoprotective effect without causing harm to the liver.

Gastritis has been a widely known gastrointestinal disorder, characterized by disruption of the mucosal integrity caused by various aggressive factors (acid, pepsin, stress, *Helicobacter pylori* and NSAIDs) and protective factors (mucus, bicarbonate, blood flow and prostaglandin) [23]. Non-steroidal anti-inflammatory drugs (NSAIDs) are pharmacological agents that induce gastric lesions by reducing the level of endogenous prostaglandin biosynthesis which is known to be cytoprotective in the gastric mucosa[24] . Acetyl salicylic acid (Aspirin) which is a well known NSAID produced ulcer lesions in the grandular parts of the rats' stomach. The result of the ulcer scores, ulcer index and percentage inhibition as observed in this study showed that the extract at doses of 200 mg/kg and 400 mg/kg showed a significant decrease in values in the ulcer severity score and ulcer index, but showed a marked increase in the percentage inhibition when compared to the un-pretreated group (negative control). Additionally, standard reference drugs (20 mg/kg omeprazole) group III also decreased the levels when compared to the un-pretreated group (negative control), but the extract at dose 400 mg/kg decreased more than that of standard reference drug (20 mg/kg omeprazole) group III. There was a decrease in the volume of gastric content, total acidity, pepsin activity and increase in pH in aspirin - induced in dose dependent manner as compared with ulcerated control rats. These decreases are statistically different ($P \leq 0.05$). A lot of non-steroidal anti-inflammatory drugs like aspirin are known to induce gastric damage by suppression of prostaglandins [23] . In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Considering the results obtained from the pretreatment of wistar rats with ethanolic leaf extract of *Irvingia gabonensi* on the aspirin ulcer model, which showed a decreased response of the extracts with increased dose is in consonant with findings of studies on some other plants as reported by other authors[24] . This observation showed the potency of ethanolic leaf extract of *Irvingia gabonensis* in protecting the gastric mucosa on aspirin-induced ulcer model. The extract may have enhanced the prostaglandin synthesis which offered it this

cytoprotective property and also the effect of its flavonoid content[15] which has been shown to exhibit gastroprotective properties [16].

V. Conclusion

The ethanolic leaf extract of Irvingia gabonensis may possess some bioactive ingredients that elicited the hepatoprotective effect and antiulcer activity against the ulceration caused by aspirin.

References

[1]. Enemchukwu BN, Onyedinma EI, Ubaoji KI, and Ngele KK. (2014). Anti-ulcer effects of aqueous extract of unripe plantain peels on male wistar (albino) rats. *Indian journal of Biotechnology* 2014;9(12):511 - 515

[2]. Ainge L. and Brown N. (2001). *Irvingia gabonensis* and *Irvingia wombolu*. A State of Knowledge Report undertaken for The Central African Regional Program for the Environment (CARPE).

[3]. Alarcon de la Lastra AC, Martin MJ, Motilva V, et al. Gastroprotection induced by silymarin, the hepatoprotective principle of Silybum marianum in ischemia-reperfusion mucosal injury: role of neutrophils. *Planta Med* 1995; 61 (20): 116-119.

[4]. Kim SC, Byun SH, Yang CH, et al. Cytoprotective effects of Glycyrrhizae radix extract and its active component liquiritigenin against cadmium-induced toxicity (effects on bad translocation and cytochrome c-mediated PARP cleavage). *Toxicology* 2004; 197(3):p. 239- 251.

[5]. Pastrada-Bonilla E, Akoh CC, Sellappan S, Krewer G. Phenolic content and antioxidant capacity of muscadine grapes. *J Agric Food Chem* 2003; 51(18): 5497-5503.

[6]. Liu CF, Lin CC, Lin MH, Lin YS, Lin SC. Cytoprotection by propolis ethanol extract of acute absolute ethanol-induced gastric mucosal lesions. *Am J Chin Med* 2002; 30(2-3): 245-254.

[7]. Bagchi D, Ray SD, Bagchi M, Preuss HG, Stohs SJ. Mechanistic pathways of antioxidant cytoprotection by a novel IH636 grape seed proanthocyanidin extract. *Indian J Exp Biol* 2002; 40(6): 717-726.

[8]. Kyogoku K, Hatayama K, Yokomori S, et al. Anti-ulcer effect of isoprenyl flavonoids. II. Synthesis and anti-ulcer activity of new chalcones related to sophoradin. *Chem Pharm Bull* 1979; 27: 2943- 2952.

[9]. Keay RW Trees of Nigeria Clarendon Press Oxford London 1989, 330 - 333

[10]. George IN, and Zhao Y. Pharmacological activity of 2,3,8-tri-O-methyl ellagic acid isolated from the stem bark of *Irvingia gabonensis* . *Afr J Biotechnol*. 2007; 6(16):1910-1912.

[11]. Okolo CO, Johnson PB and Abdulrahman EM. Analgesic effect of irvingia gabonensis stem bark extract . *Journal of ethnopharmacology* 1995;45(2): 125 - 129

[12]. Nosiri CI, Abdu-Aguye I, Hussaini IM and Abdulrahman E. Leaf extracts og Irvingia gabonensis increase urine output and electrolytes in rats. *Internet journal of Alternative Medicine* 2009;(8);2

[13]. Nosiri C*, Hussaini I, Abdu-Aguye I and Abdurahaman E Pharmacological Effects of *Irvingia gabonensis* Leaf Extracts on Cat Blood Pressure . *Internet journal of Pharmacology.* (2011), Vol. 9 Number 1

[14]. Nosiri C*, Hussaini, I.M and I. Abdu-Aguye, I. Effect of *Irvingia gabonensis* leaf extracts on non pregnant rat uterus. The *Internet Journal of Alternative Medicine.* (2011) *Volume 8 Number 2.*

[15]. Obianime AW and Uche FI Effects of aqueous extracts of *Irvingia gabonensis* seeds on the hormonal parameters of male guinea pigs *Asian Pacific Journal of Tropical Medicine* (2010) 200-204

[16]. Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, and Ghegotsky MR Gastroprotective Effects Of Flavonoids In Plant Extracts. *Journal Of Physiology and Pharmacology 2005; 56, Suppl 1, 219-231*

[17]. Goel RK, Govinda Das D, Sanyal AK. (1985). Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved mucosubstances in gastric juice. *Indian Journal of Gastroenterology* 1985; 4, 249–251.

[18]. Main JH and Whittle NB. Investigation of vasodilator and antisecretory role of prostaglandin in the rat mucosa by the use of NSAIDs. *British journal of pharmacology.* 1975; 53:217 - 224

[19]. Suzuki Y, Hayachi M, Itom M and Vamagani T. Anti ulcer effects of 4'- (2-carboxyetyl) phenyl trans-4-aminomethylcyclohexanecarboxylate hydrochloride (cetraxate) on various experimental gastric ulcers in rats *j. pharmacology.* 1976;26:471 - 80

[20]. Lowry OH, Rosebrough NL, Parr AL and Randall RJ. (1951). Protein measurement with folin phenol reagent. *Journal Biological Chemistry*, 1951; 193:265-275.

[21]. Debnath PK, Gode KD, Govinda D, Sanyal AK. Effects of propranolol on gastric secretion in albino rats. *British Journal of Pharmacology* 1974; 51: 213–216.

[22]. Kulkarni, S. K. Handbook of Experimental Pharmacology, 3rd ed. New Delhi, India, Vallabh Prakashan, 1999. Pp. 148–150.

[23]. Bhatnagar M, Sisodia SS and Bhatnagar R. Antiulcer and antioxidant activity of *Asparagus racemosus* WILLD and *Withania somnifera* DUNAL in rats. *Ann. N. Y. Acad. Sci.,* 2005;1056: 261-278.

[24]. Wallace, J. L. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. *Best Practical Research and Clinical Gastroenterol* 2001;15(5):691-703

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