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EFFECT OF ALLIUM SATIVUM AND ALOE BARBADENSIS ON INDOMETHACIN INDUCED STOMACH ULCER IN MALE WISTAR RATS

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ABSTRACT

Natural compounds derived from plants materials have been used in the treatment of gastric ulcers for centuries because of their low side effects as well as significant medicinal effects. In this study, the effect of *Allium sativum* and *Aloe barbadensis* rind extracts on indomethacin induced stomach ulcer in male albino Wistar rats was evaluated. A total of 35 rats divided into seven (7) groups of five (5) animals each were used in this study. Group A received only water and feed, while the other groups received: Group B 30mg/kg of indomethacin only, Group C 200mg/kg of *Aloe barbadensis* rind extract, Group D 200mg/kg of *Allium sativum*, Group E 30mg/kg of indomethacin + 200mg/kg of *Allium sativum*, Group F 30mg/kg of indomethacin + 200mg/kg of *Aloe barbadensis rind extract*, Group G 30mg/kg of indomethacin + 200mg/kg *Allium sativum* + 200mg/kg *Aloe barbadensis rind* extract. Administration lasted for seven days only after which they were sacrificed and the stomach harvested for histopathological studies. Data were subjected to ANOVA followed by post hoc LSD and significant at $p \leq 0.05$. Result showed a significant increase in the stomach ulcer index in group B compared to group A ($p<0.05$). Groups E, F, and G had a significant decrease in the ulcer index compared to group B ($p<0.05$) and this therapeutic effect can be attributed to the strong antioxidants contained in the plant materials. In conclusion, *Allium sativum* and *Aloe barbadensis* are effective in the treatment of stomach ulcers and therefore recommended.

KEYWORDS: *Allium sativum*, *Aloe barbadensis*, indomethacin, stomach ulcer.

INTRODUCTION

Indometacin, also known as indomethacin, is a nonsteroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling from inflammation.[1] Indomethacin is frequently used as a reference drug for the formation of ulcer model in rats due to its high gastric toxicity.[2] The ulcer-forming mechanisms of indomethacin include increased oxidant parameters, decreased antioxidant parameters, and secretion of prostaglandin E2 (PGE2).[3]

NSAIDs have shortcomings because of side effects that majorly affect the gastrointestinal (GI) tract. The increased risk of developing stroke, haemorrhage, and gastrointestinal bleeding, and the formation of gastric lesions are noted, and therefore, gastric ulcer formation is related to the response to stress; furthermore, treatment with NSAIDs decreases gastric ulcer healing.[4,5]

Stomach ulcers are caused by an infection with the bacterium *Helicobacter pylori* (H. pylori) and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs). Rarely, a condition known as Zollinger-Ellison syndrome can cause stomach and intestinal ulcers by increasing the body's production of acid. Generally, it has symptoms such as dull pain in the stomach, weight loss, nausea or vomiting, loss of appetite because of pain, bloating, feeling easily full, acid reflux, heartburn and anaemia.[6]

Natural compounds derived from plants have been used in the treatment of gastric ulcers for centuries because of their low side effects as well as significant medicinal effects.[7] For instance, EGCG, an antioxidant in green tea and also quercetin known to have antioxidant properties have demonstrated beneficial effects against gastric ulcers.[8,9,10]

Dietary garlic (*Allium sativum* L.) is widely consumed in different dishes and has been an important part of folk medicine practices since ancient times.[11] Traditionally, it has been used to treat varieties of disorders such as arthritis, cardiovascular disease, toothaches and asthma; recently, garlic supplements are available as over-the-counter products.[11,12,13]

Pharmacologically reported activities of garlic are, antioxidant, antihypertensive, antidiabetic, anti-atherosclerotic, neuroprotective, nephroprotective and cancer

chemopreventive.[14,15,16,17,18,19,20] Potentially active phytochemical constituents in this plant are alliin, allicin, allinase, ajoene, arginine, selenium, germanium and tellurium.[21]

Aloe vera is a plant species of the genus Aloe. It is the oldest and the most applied medicinal plant worldwide. The Aloe vera plant is a member of lily plant known as *Aloe Barbadenesis* which is full of juice and closes similar to a cactus.[22] So far, 75 known compounds have been identified in Aloe vera, including 20 amino acids, 20 minerals, vitamins, and water.[23,24] It is widely used for its antibacterial, anti-viral, anti-inflammatory effects and has been considered in medical sciences.[24,25,26]

The aloe vera rind (AVR) is a discarded solid biomass after extraction of gel and finds no commercial importance but could serve as a potent lignocellulosic feedstock for bioethanol production that requires an efficient pretreatment to remove the lignin (16.4%, w/w).[27]

However, the effect of aloe vera rind extract on gastric ulcer has not yet been shown. Therefore, this research work is designed to elucidate the effects of aloe vera rind, garlic and their co-administration on indomethacin induced gastric ulcer in male wistar rats.

MATERIALS AND METHODS

Collection and Preparation of plant materials

Aloe vera was obtained from Okofia in Nnewi, Anambra state. Garlic was obtained from Nkwo market, Nnewi. Both plants were authenticated by a botanist and deposited at the herbarium, Botany Department, Nnamdi Azikiwe University Awka.

Aloe vera rind and garlic were air dried under ambient temperature and crushed using laboratory blender. Two hundred and fifty (250) gram of the dried *Aloe barbadenesis* and *Allium sativum* were macerated in 1500mls of lukewarm water for 24-hours. It was then filtered using a clean porcelain cloth and further filtrated using filter paper (Whatman Qualitative Filter paper, No. 1, Sigma Aldrich; WHA1001042, USA). The filtrate obtained was concentrated using a rotatory evaporator (Digital TT-52; Techmel & Techmel, USA), further dried using a laboratory oven (DGH-9023A, PEC MEDICAL, USA) at 45ºC into a gel-like form. The extract was preserved in a refrigerator (Nexus) for further usage. The extract procedure was done according to the method described by Attar and Abu-Zeid (2013) with modifications.[28]

Experimental animals

Thirty five (35) male wistar rats weighing 100-160g were purchased from the animal House, Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (Grower feed) and water *ad libitium*. The animals were acclimatized for two weeks and kept on 12hours light and dark cycles.

Experimental protocol

A total of 35 rats were used in this research and were divided into seven (7) groups of five (5) animals each.

Group A received only water and feed

Group B received 30mg/kg of indomethacin only

Group C received 200mg/kg of *Aloe barbadensis* rind extract

Group D received 200mg/kg of *Allium sativum*

Group E received 30mg/kg of indomethacin + 200mg/kg of *Allium sativum*

Group F received 30mg/kg of indomethacin + 200mg/kg of *Aloe barbadensis rind extract*

Group G received 30mg/kg of indomethacin + 200mg/kg *Allium sativum* + 200mg/kg *Aloe barbadensis rind* extract

The extracts were administered once daily between the hours of 6am and 8am for 7 days, through oral gavage.

Induction of ulcer

Gastric ulceration was induced in the animals according to the method described by Sabiu *et al*., (2015) and Brito *et al.*, (2018).[29,30] Rats were administered a single oral dose of indomethacin of 30 mg/kg body weight. They were deprived of food but had free access to water 24 hours prior to ulcer induction. The animals were anaesthetized with diethyl-ether and stomach was incised along the greater curvature and ulceration were scored.

Measurement of ulcer index

Ulcers in the acute models were scored with the help of magnascope under 5X magnification using the ulcer scoring criteria.[29,30]

Normal stomach= 0

Red coloration= 0.5

Spot ulcer= 1

Haemorrhagic streak= 1.5

Ulcers= 2

Perforation= 3

Mean ulcer score for each group will be expressed as Ulcer index.

Ulcer index was calculated from scorings described as follows: UI = (UN + US + UP) × 10–1.

Where US = mean severity of ulcer score; UP = percentage of animals with ulcer incidence and UN = Average number of ulcers per animal.

Percentage protection index is calculated as follows: Percentage protection index = (Uc - Ut) C–1 × 100.

Where Uc = ulcer index in control group; Ut = ulcer index in treated group.

Sample collection

The animals were anaesthetized with chloroform in an enclosed container 24 hours after the last administration. The stomach was harvested through abdominal incision, and stored in 10% formal saline prior to histopathological studies.

Data analysis

Data were analysed using SPSS version 25 (IBM, USA, 2018). Data obtained for relative organ weight and Ulcer index were subjected to ANOVA, followed by post hoc LSD. Body weight were analysed using dependent t-test and values were considered significant at $p<0.05$.

RESULT

Physical observations

There was loss of weight during the administration of indomethacin and its treatment with *Aloe barbadensis* (aloe vera) (rind) and *Allium sativum* (garlic). Frequent and watery stool was also observed after indomethacin administration. The animals also showed signs of weakness and loss of appetite.

During administration of garlic and aloe vera rind, the animals in group C - G increased in weight. Their feeding habit improved and their faeces were no longer watery. Not all the animals used for the experiment survived till the end. The animals in group A (control) were healthy throughout the experiment.

Table 1: **Effect of Garlic and Aloe vera on bodyweight following indomethacin-induction.**

		Mean	±SEM	P-value	T-value
Group A	Initial body weight (g)	117.50	±8.53	0.02*	-4.36
	Final body weight (g)	146.50	±5.37		
Group B	Initial body weight (g)	140.00	±11.54	0.71^{NS}	0.44
	Final body weight (g)	134.66	±22.58		
Group C	Initial body weight (g)	125.00	±10.40	0.05*	-4.28
	Final body weight (g)	165.66	±5.78		
Group D	Initial body weight (g)	116.66	±±8.81	0.16^{NS}	-2.16
	Final body weight (g)	133.33	±2.40		
Group E	Initial body weight (g)	145.00	±12.58	0.73^{NS}	0.40
	Final body weight (g)	144.33	±11.66		
Group F	Initial body weight (g)	131.66	±6.01	0.33^{NS}	-1.28
	Final body weight (g)	139.66	±0.33		
Group G	Initial body weight (g)	126.66	±3.33	0.65^{NS}	-0.53
	Final body weight (g)	136.33	±17.26		

Data was analyzed using t-test and values were considered significant at $p<0.05$

*: significant; NS: not significant; SEM: standard error of mean

Table 1 result revealed a significant ($p<0.05$) increase in bodyweight in-group A and C; group D, F, and G had a non-significant ($p>0.05$) increase, and group E had a non-significant decrease ($p>0.05$) when initial weight was compared to final weight.

Table 2: Effect of Garlic and Aloe vera on stomach ulcer index following indomethacin induction.

		Mean	±SEM	P-value	F-value
Ulcer index stomach	Group A	0 0 0			
	Group B	1.59	±0.35		5.84
	Group C	0 0 0	0 0 0		
	Group D	0 0 0	0 0 0		
	Group E	0.63	±0.03	0.002*	
	Group F	0.35	±0.02	0.000*	
	GroupG	0.15	±0.03	0.000*	

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison, and values were considered significant at $p<0.05$.

Table 2 result showed a significant increase in the stomach ulcer index in-group B compared to group A. Group E, F, and G had a significant decrease ($p<0.05$) in the ulcer index compared to group B.

Histopathological findings

ME

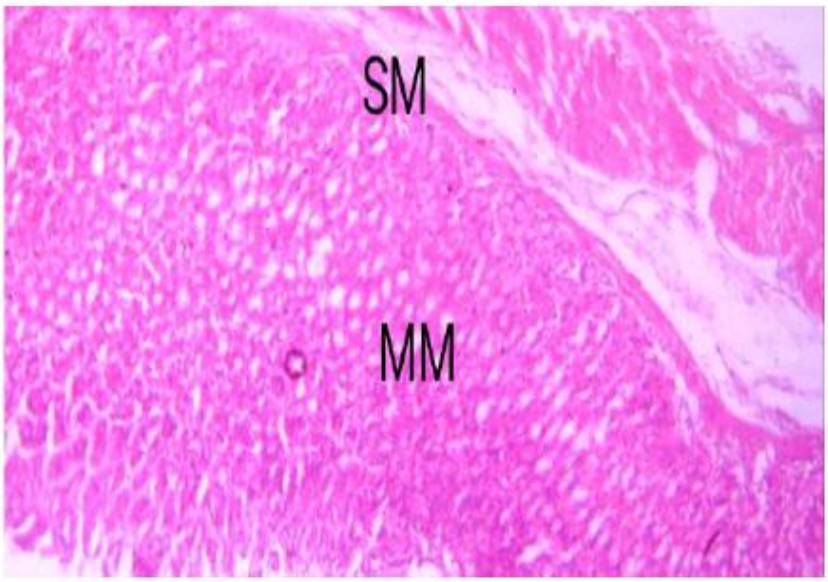

Plate A: **Photomicrograph of control section of stomach ((x150) (H/E) shows normal stomach with muscularis externa (ME), muscularis mucosa (MM) and submucosa (SM).**

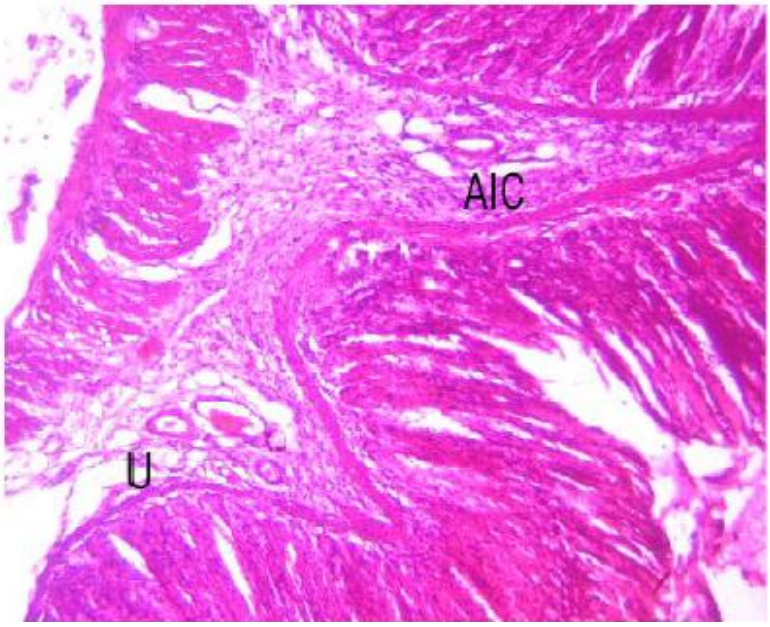

Plate B: Photomicrograph of group B section of stomach administered indomethacin only (x150)(H/E) shows severe degeneration of the lining of the stomach with severe ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the ulceration (U).

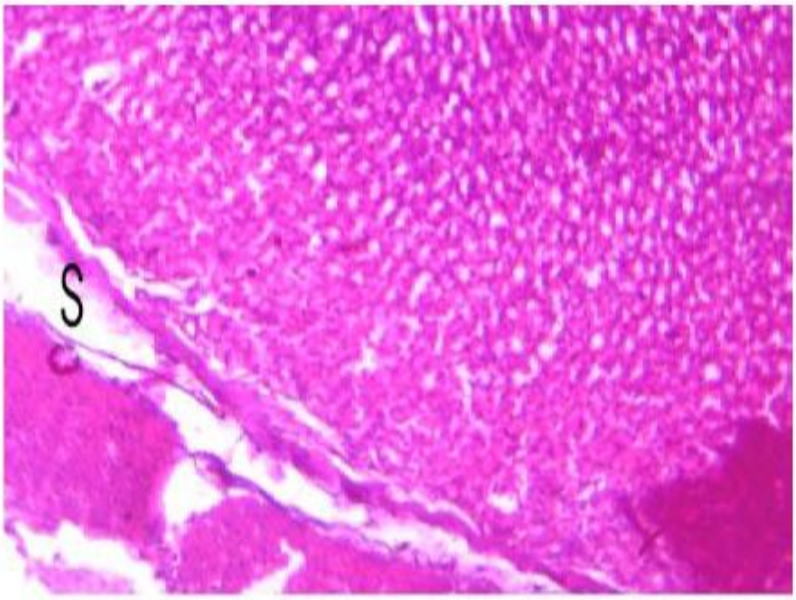

Plate C: **Photomicrograph of group c section of stomach administered aloe vera rind only (x150)(H/E) shows mild separation of the submucosa otherwise normal.**

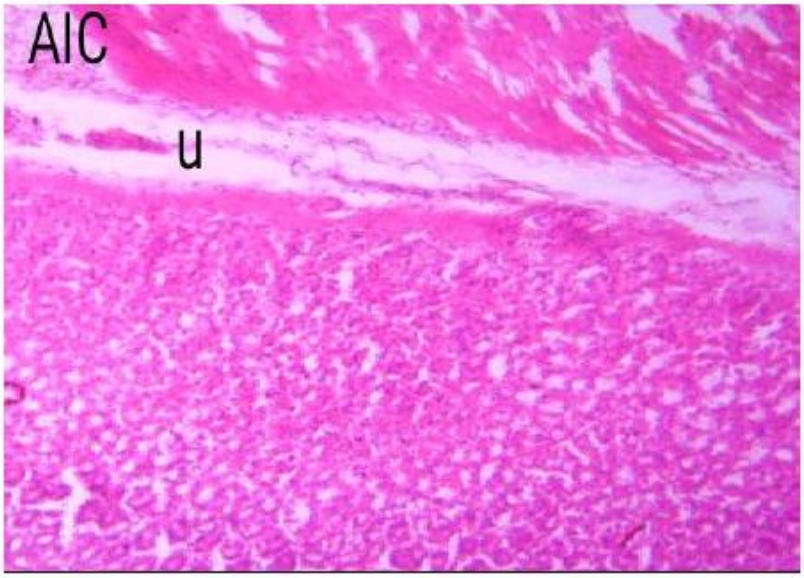

Plate D: Photomicrograph of group D section of stomach administered garlic only (x150)(H/E) shows mild ulceration of the submucosa with aggregate inflammatory cells otherwise normal.

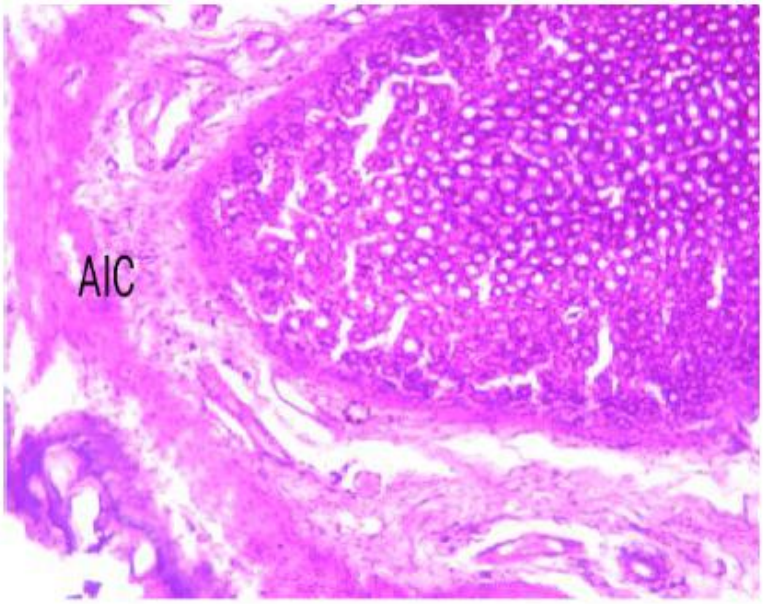

Plate E: Photomicrograph of group E section of the stomach administered indomethacin and garlic (x150)(H/E) shows mild regeneration with mild aggregate of inflammatory cells (AIC) within the submucosa layer.

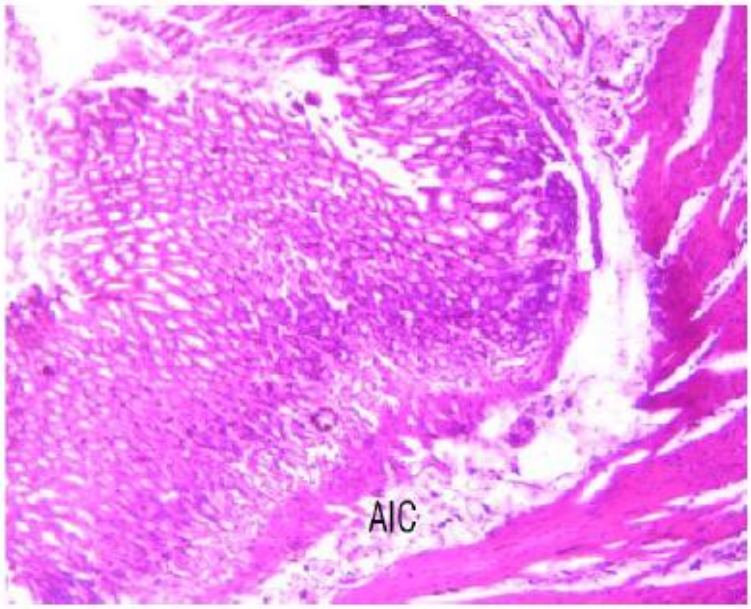

Plate F: Photomicrograph of group F section of stomach administered indomethacin and aloe vera rind (x150)(H/E) shows moderate regeneration with mild aggregate of inflammatory cells (AIC) within the submucosa layer.

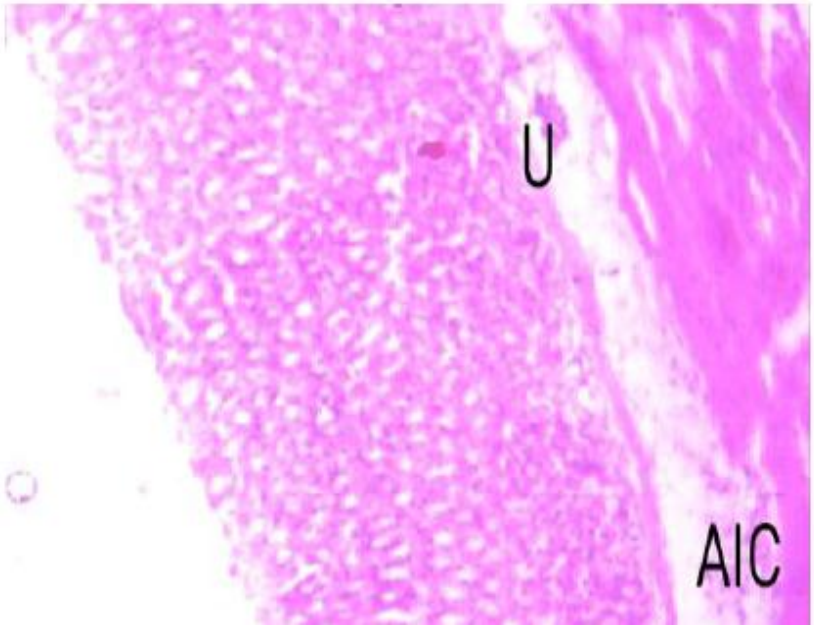

Plate G: Photomicrograph of group G section of stomach administered indomethacin, aloe vera and garlic (x150)(H/E) shows moderate regeneration with mild aggregate of inflammatory cell (AIC) within the ulcerated (U) submucosa layer.

DISCUSSION

In this study, the antiulcer effect of garlic, aloe vera rind and their co-administration was examined in indomethacin induced gastric ulcer model.

Following indomethacin administration, there was a significant increase in ulcer index in groups B, E, F and G when compared to group A. This is in agreement to the assertions by Garcia-Rayado *et al*., and Halici *et al*., which states that chronic administration of NSAIDs (indomethacin) causes ulcer.[31,32] For the histopathological finding of Group B (indomethacin only), the present study showed severe degeneration of the stomach linings with severe ulceration which is in line with the work done by Nahla *et al*.(2016) which revealed that oral administration of indomethacin to rats caused significant ulceration in the glandular region of the rat stomach as evident by histopathological assessment.[33]

The significant increase in ulcer index in the present study may be attributed to either free radicals formation or inhibition of prostaglandin synthesis. Decreased prostaglandin level has been attributed to impair gastric ulcer healing and increased gastric acid secretion which are important events in the aetiology of mucosal ulceration. This correlates with the works done by Onabanjo *et al*, Ruiz-Hurtado *et al*, and Seo *et al*.[34,35,36]

A significant reduction in ulcer index was observed in group E (indomethacin + garlic) in comparison to group B which shows that garlic administration has a potent ulcer healing effect. This is in line with the findings of El-Ashmawy *et al*., whose findings shows that administration of aged garlic extract reduces indomethacin-induced gastric ulcers in rats by increasing prostaglandin E2 levels in the gastric tissue.[37] This is also in agreement with the work by Khosla *et al*., 2004 in which garlic oil pre-treatment was seen to decrease ulcer index, lipid peroxidation and improve antioxidant enzymes in ethanol induced gastric ulcer.[38] The histopathological finding of indomethacin + garlic treated group (Plate E) revealed moderate regeneration with mild aggregate inflammatory cells within the submucosa layer in the stomach which suggests that treatment of garlic has a beneficial effect on the treatment of ulcer which may be attributed to the presence of powerful antioxidant property that is contained in aqueous extract of garlic which is in accordance with the study done by Khosla *et al*.[38]

In group F (Indomethacin + aloe vera rind treated) the result revealed a significant decrease in ulcer index which is in agreement with the work done by Borra *et al*., and Eamlamnam *et al*.[39,40] From Histopathological finding of same group, an improvement in ulcer healing was observed which correlates with the work done by Keshavarzi *et al*., (2014) whose result shows that the aqueous extract of aloe vera leave (rind) has an healing effect in ulcer treatments.[41] It was reported that aloe vera could promote burn wound healing in rats (Duansaks *et al*., 2003), in addition Aloe vera could induce angiogenesis in vivo (Moon, 1999) which plays an important role in wound healing.[42,43] It was also reported that aloe vera rind has protective functions and synthesize carbohydrate and protein.[44] Aloe vera can result in reduced vasoconstriction and improve perfusion of gastric mucosa capillaries, thus promoting ulcer healing.[45,46]

Histopathological finding of indomethacin + aloe vera rind + garlic (Group G) showed significant improvement in the reduction of ulcers which is in contrast with the previous work done by Frederick and Eze, (2016) which observed that combination of Allium sativum, Brassica oleracea and Aloe barbadensis cleared all visible ulcer when compared with control.[47] The difference in the observation could be as a result of the third extract administered and also dose differences (300mg/kg weight of the animals in the treatment groups) whereas in this study they were given 200mg/kg body weight. The ulcer index was observed to have decreased significantly when compared to group B. It was also observed to

have decreased more than that of group E and F which is suggestive of either an increased antioxidant aggregation or different mechanism of action of both garlic and aloe vera rind extracts at play.

CONCLUSION

From these observations, it is evident that aqueous extract of aloe vera rind and garlic individually and in combination accelerates ulcer healing which is due to the presence of antioxidants which suppresses the oxidative stress caused by indomethacin.

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