

ANTI-ULCER AND BLOOD-BOOSTING EFFECT OF DIET SUPPLEMENTED WITH DAEDALEA QUERCINA FROM OGBOMOSO, OYO STATE, SOUTH WEST OF NIGERIA ON INDOMETHACIN INDUCED GASTRIC ULCER IN RATS.

ABSTRACT.

Gastric Ulcer is a common ailment in Nigeria, and with synthetic drug treatment becoming less effective, herbal remedies are being sought. *Daedalea quercina* (Dq) has been shown to have significant therapeutic potential, but little is known about its anti-ulcer and blood-enhancing qualities, which is why this study was conducted.

Fifty male Wistar rats (100-110g; n=5) were divided into two sets of five groups respectively for days 7 and 14 treatments. Blood samples were collected on days 7 and 14 for full blood count. Gastric ulceration was induced in the rats using indomethacin (40 mg/kg p.o) after 24 hours of fast on days 8 and 15. Animals were euthanized 4 hours after ulceration, while stomachs were excised and analyzed for malondialdehyde, sulfhydryl, nitrite, mucin, and H^+K^+ -ATPase activity, using standard procedures while tissues from the stomach were harvested and processed for routine histology. Data were expressed as Mean \pm SEM, analyzed using analysis of variance (ANOVA), and $p \leq 0.05$ was significant.

Hematological indicators were not significantly affected by the treatment. Significant differences were observed with nitric oxide, mucin, sulfhydryl, hydrogen-peroxide, and H^+/K^+ -ATPase.

Daedalea quercina treatment groups demonstrated anti-ulcer and blood-boosting activities through the synergistic activities of increased nitrite and antioxidant pathways.

Keywords: *Daedalea quercina*, Gastric Ulcer, Blood-boosting, Antioxidant properties, Herbal remedies, and Anti-ulcer.

1. INTRODUCTION

Mushrooms are still the most primitive species of fungi known to mankind due to their widespread existence (Comandini & Rinaldi, 2020). The traditional climatic conditions of Nigeria make it a perfect location for growing a wide range of mushrooms. Factors such as microbial resistance to orthodox drugs, the high cost and inaccessibility of orthodox drugs, and consumers' concerns about chemical excesses in a foodstuff have all contributed to an increased reliance on fungi as a replacement in the management of various diseases affecting humans and animals in Nigeria (Adeniji *et al.*, 2021). Herbal medicine is the oldest type of medical treatment known to humanity, and it has been used in all cultures throughout history (Petri Jr *et al.*, 2015). Furthermore, in recent years, the use of herbal remedies for disease prevention and treatment has risen considerably in Western countries. Herbal remedies are now available not only in drug stores but also in grocery stores and supermarkets, as public acceptance and interest in natural therapies develop in both developing and industrialized countries (Ekor, 2014).

Peptic ulcer, which includes both stomach and duodenal ulcers and affects 10% of the world's population, is one of the most common gastrointestinal illnesses (Kuna *et al.* 2019). A peptic ulcer is a non-malignant mucosal lesion in the gastrointestinal or duodenal tract. It is caused by an imbalance in the protective and destructive factors in the mucosal barrier (Hussain, *et al.*, 2015). Although the exact cause of peptic ulcer is unknown, it is widely agreed that peptic ulcer occurs when aggressive factors

(such as pepsin, gastric acid secretion, and *Helicobacter. pylori*) overpower mucosal defense mechanisms such as gastrointestinal blood flow and human epidermal development (Panda and Suresh, 2015). Intestinal injury is a common side effect of nonsteroidal anti-inflammatory drugs (NSAIDs), with symptoms ranging from nonspecific stomach pain to ulcers, hemorrhage, and death (Beck *et al.*, 2000). Indomethacin causes gastric ulcers by inhibiting prostaglandin development without causing gastric damage, as well as by generating free radicals and reducing Nitric oxide levels (Haniadka *et al.*, 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) are used to relieve pain in addition to their anti-inflammatory and antipyretic effects. However, their use is often linked to the development of peptic ulcers (Lee *et al.*, 2016). Pain relief, ulcer healing, and recurrence prevention are the goals of treating peptic ulcer disease (Lanas & Chan, 2017). As a result, medicinal herbs are often good sources of pleiotropic therapeutic agents (Sharma *et al.*, 2020).

Hematological parameters have long been related to well-being indices and are important prognostic indicators in routine health status clinical evaluations (Hoeney, 1985). Assessing hematological parameters may be a marker of the harmful effects of foreign compounds on an animal's blood constituents hence blood parameter analysis is important to the assessment of hematological system alterations in humans (Arika *et al.*, 2016). Complete blood count represents the reaction of cellular immunity (Rana *et al.*, 2015), and red blood cells, hemoglobin, and Packed cell volume are indicators of anemia, estimating the increased risk of heart disease (Arika *et al.*, 2016).

Daedalea quercina is a member of Fomitopsidaceae family of the *Daedalea* genus and polypore fungi order. They are commonly known as maze-gill fungi. It is not edible due to its cork-like texture and is mostly found in Europe, Asia, Northern Africa, and Australia. Fruiting bodies of *Daedalea quercina* have been used for brushing down horses with tender skin.), some used for anesthetizing bees (Gilbertson 1980), and some for application in bioremediation (Asgher *et al.*, 2008) while some have anti-inflammatory activity (Gebhardt *et al.*, 2007). Medicinal plants and herbs are high in phytochemicals, which they release for defense, and these components are what give them their medicinal efficacy (Alamgir, 2017). Phytochemicals found in medicinal herbs and plants include saponin, which can be used to lower cholesterol in the blood; nitrogen-rich alkaloids, which can be used as stimulants; tannins, which can be used as natural antibiotics; anthraquinones, which are used as laxatives and dyes; cardiac glycosides for cardiac drugs and flavonoids; and antioxidant phenols (Oyugi, 2016). *Daedalea quercina* is recognized to be useful in the treatment of ulcers in China due to its anti-inflammatory activity, but information on antiulcer and blood-enhancing actions in Oyo State, Southwest Nigeria, has not been well explored. This study aimed to investigate the antiulcer and blood-boosting activities of *Daedalea quercina* from Ogbomoso, Southwestern, Nigeria.

2. MATERIALS AND METHODS

During the rainy season (August-September, 2017), fresh fruiting sections of the wild Higher fungi were obtained from Ogbomosho (8.1227° N, 4.2436° E) in Oyo State, South-West Nigeria. They were taken in a clean bag to the University of Ibadan's Botany Department Laboratory (7° 26' 6.453100 N, 3° 54' 51.525400 E), where they were validated. They were recognized using Alexopoulos *et al.* (1998) and Webster and Weber, (2007) descriptions of basidiocarp/sporocarp, color, spore type, and other characteristics. The polymerase chain reaction (PCR) amplification methods were used to characterize the Higher fungus at the molecular level. The hexadecyl trimethyl ammonium bromide (cTAB) technique was used to extract fungus DNA (Möller *et al.*, 1992). The internal transcribed spacer (ITS) region was amplified using the primer pair pITS4-F (5'-TCCGTAGGTGAACCTGCCG- 3') and pITS1-R (5'-TCCTCCGCTTATTGATATGC-3'). The PCR products were checked on 1% agarose gel electrophoresis and sequenced at Genewiz Inc., Suzhou, China. Generated sequences were analyzed by basic alignment search tools (BLAST) (GenBank; <http://www.ncbi.nlm.nih.gov/BLAST/index.html>) of the National Center for Biotechnology Information (NCBI) to determine the closest sequence matches, allowing taxonomic identification. The DNA sequences were deposited in GenBank under the accession number MN596945, while the basidiocarps were deposited in Jonathan Gbolagade's (personal macro-fungi collection center at the Mycology Laboratories of the Botany Department, University of Ibadan). The fungal samples were air-dried, powdered, and kept refrigerated at 4°C in an air-tight amber bottle for future use.

2.1 Feed formulations

Table 1. Composition of Basal feeds for Rodent in 10 Kg.

Table 2. Composition of 20% Higher Fungi supplemented diet for Rodent in 10 Kg.

Table 3. Composition of 40 % Higher Fungi supplemented diet for Rodent in 10 Kg.

2.2 Design of the experiment

Thirty-five male Wistar rats (100-110g; n=7) were divided into five groups: groups; 1- (non-ulcerated normal feed (CN)) 2- (ulcerated untreated feed (CU)), 3- (20 mg/kg cimetidine (Cm)), 4- (20% *Daedalea quercina* (Dq)) and 5- (40% *Daedalea quercina*) respectively for days 7 and 14 treatments. They were acclimatized for two weeks and housed in solid polypropylene bottom cages, with free access to their basal diet feeds, under normal ambient room temperature conditions (23°C-25°C) before pre-treating with Dq and cimetidine for 7 and 14 days respectively with water ad libitum. At the end of each experimental day, a complete hematological examination was performed on the Wistar rats and was then fasted for 24 hours before indomethacin administration and sacrificed after 4 hours, excised, weighed, and graded for ulceration of the stomachs. The National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Animal Care and Use Guide, 2011) were followed.

2.3 Experimental Induction of Gastric Ulceration

The indomethacin-induced gastric ulceration model used was the same as that described by [Oluwole et al. \(2007\)](#). After 7 and 14 days of pretreatment with basal and formulated feed at 20% w/w and 40%, w/w of *Daedalea quercina* (Dq) indomethacin was given orally at a dose of 40 mg/kg body weight to each animal. The animals were sacrificed after four hours of indomethacin induction via cervical dislocation. The stomachs were cut anteriorly from the esophageal end, with an incision made along the larger curvature, and then rinsed in cold phosphate buffer saline and weighed.

2.4 Ulcer rating by macroscopic measurement

A microscopic examination and a macroscopic inspection with a hand lens were used to determine the extent of the ulceration. Scoring of the ulcerated gastric region was done by opening the abdomen along the greater curvature. After rinsing with normal saline, the stomach was pinned to a corkboard. Elegbe (1976) scoring technique was used to determine the degree of gastric ulceration

Scoring method Parameters

- 0.....Normal stomach
- 0.5.....Punctuated hemorrhage/ Pin-point ulcer
- 1.0.....Two or more small hemorrhagic ulcers
- 2.0.....Ulcer greater than 3mm in diameter

The percentage of ulcer protection was obtained using the formula

$$\text{Percentage (\%) Inhibition} = \frac{UC - UX}{UC} \times 100$$

U is the percentage of animals in the group with ulcer

UC is the Control mean ulcer index

UX is the Test mean ulcer index

2.5 Histopathological studies.

Animal stomach tissue was cut into sample bottles containing a 10% ethanol-formalin solution before being stained with hematoxylin and eosin (H&E) (Avwioro, 2010).

Preparation of tissue for biochemical assay

2.6 Determination of mucosal total protein.

The protein concentration of stomach tissue was determined using the Biuret method (Gornal *et al.*, 1949), with a modest modification in that potassium was added to the reagent to prevent Cu^{2+} ions from precipitating as cuprous oxide.

2.7 Evaluation of lipid Peroxidation (MDA level)

Lipid peroxidation (LPO) evaluation is another name for this type of antioxidant investigation. The amount of thiobarbituric acid reactive compounds (TBAR) formed during lipid peroxidation was measured. This was done using the Varsheny and Kale (1949) approach.

2.8 Estimation of mucin activities

The Winzler, (1958) test was used to assess the content of Mucin. The theory is concerned with calculating the hexose portion of mucin. The condensed carbohydrate orcinol (5-methyl resorcinol) sulfuric acid reaction is responsible for the coloration.

Procedure; 1.6 percent orcinol and 2 ml of 60 percent sulphuric acid were applied to the 1:20 diluted 0.25 ml sample, respectively. The mixture was then immersed in boiling water for 10 minutes before being cooled to ice. The optical density was estimated at 425 nm.

2.9 Determination of mucosal nitric oxide (NO)

The amount of NO in the tissues was measured indirectly as total nitrite (NO_2) using the Griess reagent, which depends on diazotization with sulfanilic acid and N-1-naphthyl-ethylene diamine to produce a colored result (Ignarro *et al.*, 1987).

2.10 Determination of Sulfhydryl level

Using the Ellman, (1959) technique, sulfhydryl levels in the tissue were determined. The values are expressed in the form of the Molar (M). One ml of the protein solution and 2 ml of the phosphate buffer is mixed (approximately 0.2 percent protein concentration). To this mixture, 0.05 ml of DTNB solution (36.9 mg of DTNB dissolved in 10 ml of 0.1, pH 7.0 phosphate buffer ionic strength) was applied. When assessing the sulfhydryl content in the presence of denaturants, 1 ml of a protein solution was combined with 2 ml of 6 M guanidine hydrochloride

Phosphate buffer. The absorbance was measured 10 min later at 412 nm because the color was formed within 5min.

2.11 Measurement of Mucosal-hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) tissue levels were analyzed using the Wolf *et al.* (1959) method. The values were represented as U / mg protein. In small test tubes, ranging from 10 to 100 μmoles , different quantities of H_2O_2 were taken and 2ml of dichromate / acetic reagent was applied to each. The reagent's application immediately produced an unstable blue precipitate per chromic acid. Subsequent heating in a boiling bath of water for 10 minutes changed the color of the solution to permanent green due to the apparent formation of chromic acetate. The volume of the reaction mixture was transformed to 3 ml after cooling at room temperature and the optical density was measured using a 570 nm spectrophotometer.

2.12 Estimation of H^+/K^+ ATPase activities

Using Bewaji *et al.* (1985), this study was carried out. A reaction mixture containing 200mM NaCl/ 40mM KCl/ 60mM tris buffer (pH.7.4), 80mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 20mM EGTA and an enzyme source was incubated at 37°C. 8 mM of ATP was then applied, then incubated for 30 minutes at the same temperature. Then 5 percent SDS was applied at 3000rpm at 40°C for 5min to avoid the reaction and centrifuge. The reagent mixture (H_2SO_4 -Ammonium molybdate-Ascorbate) was added and permitted

color formation to stand at room temperature for 20 minutes, after which absorption was read at 725 nm.)

2.13 Full Blood Cell Count Determination.

Full blood count analysis was carried out on all the test groups by the method of Dacie and Lewis (1994). All measurements were done in triplicate using the mean values for statistical analysis.

2.14 Statistical analysis

Results were presented as Mean \pm SEM, calculated with Graph Pad Prism 7.0 using Analysis of Variance (ANOVA) and significant at $p = 0.05$.

3. RESULTS AND DISCUSSION

The macro-fungi used in this study (Figure 1) was identified as *Daedelia quercina* and was documented on the NCBI with accession number MN596945.

Figure 1.

Table 4 shows the effect of *Daedelia quercina* (Dq) on gastric ulcer score and gastric ulcer percentage inhibition of indomethacin-induced Gastric ulcerated rat. It was observed that both treatments of 20%w/w *Daedelia quercina* and 40%w/w *Daedelia quercina* decreased in mean gastric ulcer score, and increased percentage inhibition by

Day 7 and 14 when compared with CU. However, the highest inhibition of gastric ulcers was observed on day 14 for both 20Dq and 40Dq treatments. The pre-treated groups (Cm, 20Dq, and 40Dq) increased the percentage of ulcer inhibition of the indomethacin gastric ulcer-induced rats when compared with CU on both days of the treatments. This increase must have been a result of the presence of some phytochemicals that possess antiulcer properties. This is in support of the findings of Buswel *et al.* (2004) who reported that phytochemicals present in mushrooms are their source of antiulcer properties.

Table 4

As observed in figure 2, no significant difference was observed in their total protein value across all treatment groups on day 7 treatment whereas, on day 14, a significant increase ($p < 0.05$) was observed with 20Dq and 40Dq when compared with the corresponding control ulcer untreated (CU). Comparing treatment days 7 and 14 treatments, a significant increase ($p < 0.05$) was observed with CN, and 20Dq. The existence of high protein content in mushrooms, which is primarily essential for cell regeneration and inflammatory repair, could explain the observed increase in total protein levels observed on day 14 of the treatment. This is in agreement with the results of Jonathan *et al.* (2006), who reported that mushrooms are high in protein content.

Figure 2.

Figure 3 shows the effects of 20Dq and 40Dq on Malondialdehyde levels in rats for 7 and 14-day exposure periods. A significant difference ($p < 0.05$) was observed only with 40Dq when compared with CU on day 7 whereas no significant difference ($p < 0.05$) was observed with both 20Dq and 40Dq as compared with CU treatment on day 14. Significant differences ($p < 0.05$) were observed with both 20Dq, and 40Dq when comparing both days of treatments. After day 14, the treatments with 20Dq and 40Dq showed a decrease in gastric MDA. This oxidative activity test, which used the malondialdehyde assay as a marker, revealed that the treatment had no effect on lipid stores on the cell membrane. It also demonstrated the ability of the feed treatment to prevent an increase in free radical production, which could have exacerbated the gastric ulcer.

Figure 3.

As shown in figure 4, 20Dq and 40Dq significantly increased ($p < 0.05$) the mucin content in indomethacin-induced ulcerated rats on days 7 and 14 when compared with the corresponding CU. Meanwhile, only the 40Dq treatment group significantly increased ($p < 0.05$) when days 7 and 14

were compared. The increased mucin content in the *Daedalea quercina* supplemented diets conferred antiulcer properties by assisting in the maintenance of homeostasis through the mucosal defense mechanism. This finding supports Sabiu *et al.* (2015)'s argument, that drugs that increase intracellular mucin secretion speed up ulcer healing.

Figure 4.

Figure 5 shows the effect of 20Dq and 40Dq supplemented diets on nitric oxide content of indomethacin-induced Gastric ulcerated rats for 7 and 14-day exposure periods. A significant increase ($^j p < 0.05$) was observed with 40Dq and Cm, when compared with CU on day 7 while on day 14, 20Dq, and 40Dq, significantly increased ($^k p < 0.05$) relative to CU. However, a significant increase ($^z p < 0.05$) was observed with, 20Dq, and CN treatment when days 7 and 14 were compared. On both treatment days, both treatment groups supplemented with *Daedalea quercina* showed an increase in gastric nitric oxide (NO). The increase in NO conferred anti-ulcer properties to the feeds, which is consistent with the findings of Moura *et al.* (2010), who reported that Nitric oxide (NO) helps to preserve the integrity of the mucus membrane and stomach epithelium, which helps mediate gastric blood flow as a vasodilator and inhibits acid secretion while promoting the production of mucus and bicarbonate, thus protecting the gastrointestinal tract.

Figure 5.

As observed in figure 6, 20Dq and 40Dq did not have any significant difference in sulfhydryl content in the indomethacin-induced rat when compared with control ulcer untreated (CU) after day 7 whereas a significant increase ($^k p < 0.05$) was observed with only 20Dq after day 14 treatment. Comparing treatment day 7 and day 14, both 20Dq, CN, and 40Dq were significantly different ($^z p < 0.05$). The increase in sulfhydryl level observed with treatment 20Dq, which is useful for the formation and maintenance of gastric mucus through the growth of disulfide bridges by restricting the growth of reactive oxygen species associated with tissue injury while maintaining gastric integrity, suggested that the supplemented diets with *Daedalea quercina* had gastro-protective effects.

Figure 6.

As observed in figure 7, a significant increase ($^j p < 0.05$) in their mucosa H_2O_2 content was seen with 20Dq treatment on day 7 while on day 14, a significant increase ($^k p < 0.05$) was observed with the treatment 40Dq when compared with the corresponding CU. The antioxidant properties of the supplemented feeds were also tested using the hydrogen peroxide assay, the increase was seen here may be due to the presence of bioactive agents like flavonoids

that allowed the mushroom to confer antiulcer properties. This is in support of Oyedemi *et al.* (2010) findings, that artificial and biological antioxidants are needed to prevent unpaired radicals from causing damage.

Figure 7.

As shown in figure 8, a significant decrease ($^j p < 0.05$) in the Hydrogen/Potassium anti-pump activities with Cm, CN, and 40Dq was observed on day 7 treatment while on day 14 treatment, a significant decrease ($^k p < 0.05$) was also observed with Cm, CN and 40Dq when compared with their corresponding CU. Meanwhile, a significant difference ($^z p < 0.05$) was observed with CN and 40Dq when comparing both days of treatment. A significant decrease in gastric H^+/K^+ ATPase was observed in most treatment groups as compared to CU. The large reduction in H^+/P^+ ATPase observed in the treatments acted as a proton pump inhibitor, giving the rat a gastro-protective effect. This is consistent with the findings of Strand *et al.* (2017), who stated that physicians' primary goal in the treatment of peptic ulcers is to treat drugs that help suppress gastric acid secretion via proton pump inhibitors

Figure 8.

As shown in Table 5, on both days of the treatments with 20Dq and 40Dq, no significant differences were observed in the above hematological variables when compared with CN. After 14 days of treatment, 20Dq and 40Dq increased red blood cell content, packed cell volume, platelets,

reticulocyte, hemoglobin, and erythrocyte sedimentary rate. This indicates that erythropoiesis, blood clotting, and oxygen transport are all improved. This is in line with Omeonu *et al.* (2020) findings on the blood-boosting effects of a mushroom-supplemented diet in Wistar rats.

Table 5.

All values were not significantly different from control normal (CN) at $p < 0.05$. The rise in white blood cells observed on day 7 may be attributed to the rats' switch to the formulated feed, which is foreign to the body, where the bioactive mushroom compounds functioned as biological response modifiers by stimulating the body against the foreign body and disease, as seen in the results (Table 6). This backs up the findings of Kim *et al.* (2006), who found that many extracted polysaccharides from mushrooms strengthened immune responses and can thus be used as biological response modifiers. The immunomodulatory properties of the supplemented feeds were demonstrated by the decrease observed on day 14. This is consistent with the findings of Al-Obaidi (2016), who concentrated on the immunoregulatory properties of higher fungi in therapeutic applications.

Table 6.

From the results of serum biochemical studies, as shown in table 7, it was observed that on both days 7 and 14 of treatments, there was no significant difference in albumin, globulin, blood urea nitrogen, and creatinine counts with Dq supplemented diets when compared with CN. The results of the creatinine and blood urea nitrogen tests, which were not significantly different from the effects of *Daedalea quercina* supplemented diets as compared to normal feed (CN), suggested that the feeds were safe to consume. This is in line with the findings of Olorunisola *et al.* (2012), who reported that renal and hepatic function analyses are very useful in screening for the toxicity of medicinal and herbal extracts, as both are essential for an organism's survival. The biochemical parameters of the serum showed no signs of toxicity in any of the diets supplemented with *Daedalea quercina*, implying that sub-chronic feed intake does not affect the function of hepatocytes in rats.

Table 7.

Plate 1: Photomicrograph of stomach section (Day 7 stained by Haematoxylin and Eosin stain MAG. X 100) showing 7CN: There is no observable lesion in the gastric mucosa, 7CU: There is marked ulceration, necrosis of chief cells and inflammation in the gastric mucosa (U), 7Cm: There is the erosion of the gastric mucosa (E), 7Dq20: There is erosion and necrosis of the gastric mucosa (E), 7Dq 40: There are coagulation necrosis mucous and gastric epithelial cells.

Plate 2: Photomicrograph of stomach section (Day 14 stained by Haematoxylin and Eosin stain MAG.X100) showing; 14CU: There is ulceration of the mucosa with hemorrhagic exudate (U), 14CN: There is no observable lesion, 14Cm: There is loss of surface mucous cells (SMc), 14Dq20: There is necrosis of parietal cells, 14Dq40: There is no observable lesion.

4. CONCLUSION.

Gastric ulcer is a common disease of the gastrointestinal tract with anemia as a complication. Prevention using conventional anti-ulcer treatments showed incidences of drug ineffectiveness and adverse effects hence recent management is geared towards the use of supplemented diets with medicinal herbs. *Daedalea quercina* (Dq) has been suggested to exert gastro-protective activities in herbal medicine. In this work, the anti-ulcer and blood-boosting activities of *Daedelia quercina* (Dq), Higher fungi from the Basidiomycota division were studied.

Daedalea quercina treatment groups revealed anti-ulcer effectiveness through the synergistic actions of mucin, nitrite, H^+/K^+ ATPase activity, and the antioxidant pathways, whereas nitrite, sulfhydryl, and hydrogen-peroxide activities are responsible for the blood-boosting seen. This particular Higher fungus can be put into feeds for human consumption in both concentrations studied for therapeutic purposes without adverse effects as seen in the hematological studies.

Ethical Approval

The Animal Care and Use Research Ethical Committee (ACUREC), University of Ibadan, granted approval (Ref. No: UI- ACUREC/19/0039).

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CONFLICT OF INTEREST

There are no competing interests among the writers of this work.

AUTHORS' CONTRIBUTIONS

Francis Chukwumma Omeonu designed the study, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Segun Gbolagade Jonathan and Adeola Temitope Salami supervised and managed the analyses of the study. Michael Dare Asemoloye managed the literature searches. All authors read and approved the final manuscript.

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NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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LIST OF TABLES

Table 1: Composition of Basal feeds for Rodent in 10 Kg.

SN	CLASS OF FOOD	WEIGHT(Kg)	% COMPOSITION
1	Maize	5.4	54
2	GNC	2.4	24
3	Soya beans	0.8	8
4	Wheat	1.0	10
5	Fish	0.4	4
6	Vitamins; A. Premix = 0.0002kg B. Lysin = 0.00014 kg C. Metione=0.00014 kg D. Salt = 0.0002 kg		

Table 2: Composition of 20% *Daedalea quercina* supplemented diet for Rodent in 10 Kg

SN	CLASS OF FOOD	WEIGHT(Kg)	% COMPOSITION
1	Maize	4.32	43.2
2	GNC	1.92	19.2

3	<i>Daedalea quercina</i>	2.00	20.0
4	Wheat	0.80	8.0
5	Soya beans	0.64	6.4
6	Fish	0.32	3.2
7	Vitamins;		
	A. Premix = 0.0002kg,		
	B. Lysin = 0.00014 kg		
	C. Metione=0.00014 kg,		
	D. Salt = 0.0002 kg		

Table 3: Composition of 40 % *Daedalea quercina* supplemented diet for Rodent in 10 Kg

SN	CLASS OF FOOD	WEIGHT(Kg)	% COMPOSITION
1	Maize	3.24	32.4
2	GNC	1.44	14.4
3	<i>Daedalea quercina</i>	4.0	40.0
4	Wheat	0.6	6.0
5	Soya beans	0.48	4.8
6	Fish	0.24	2.4
7	Vitamins:		
	A. Premix = 0.0002kg,		
	B. Lysin = 0.00014 kg		
	C. Metione=0.00014 kg		
	D Salt = 0.0002 kg		

Table 4: Effects of *Daedalea quercina* supplemented diets on ulcer score, and ulcer percentage inhibition of Indomethacin- Induced Gastric Ulcerated rats.

Values were expressed as Mean \pm SEM. (n = 3).

20Dq= 20% w/w of *Daedalea quercina* in feed, 40Dq = 40%w/w of *Daedalea quercina* in feed, Control normal (CN),-control group not ulcer-induced. Ulcer untreated control (CU). (Cm)- Control treated with 20 mg/kg of cimetidine

Treatment	Mean ulcer score (mean \pm SEM)		Ulcer index (mm ²)		Percentage inhibition (%)	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
CN	0.0 \pm 0	0.0 \pm 0	0.0	0.0	0	0
CU	6.0 \pm 2.3	6.0 \pm 2.3	0.18	0.18	0	0
Cm	3.5 \pm 1.2	1.83 \pm 0.4	0.11	0.06	41.67	69.44
20Dq	3.67 \pm 1.1	4.67 \pm 1.35	0.11	0.14	41.67	22.17
40Dq	3.67 \pm 1.12	4.67 \pm 0.8	0.11	0.14	41.67	22.17

Table-5: Effects of *Daedalea quercina* supplemented diets on Packed Cell Volume (PCV), Erythrocyte Sedimentary Rate (ESR), Reticulocyte (RECTIC), Haemoglobin (HB), Red Blood Cell (RBC) and Platelets counts

Values are expressed as Mean \pm SEM.(n = 3). All values were not significantly different from control normal (CN) at p<0.05.

Gr	PCV (%)		ESR (mm/hr)		RECTIC (x 10 ⁹ /L)		HB (g/dl)		RBC (x10 ¹² /L)		PLATELETS (x 10 ⁹ /L)	
	Day	Day1	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	41 \pm	49.3 \pm	1.13 \pm 0.7	1.43 \pm 0.07	2.4 \pm 0.2	3.3 \pm 0.4	13.8 \pm 0.4	14.77 \pm 0.1	7.23 \pm 0.4	7.49 \pm 0.15	104333 \pm 15	94666 \pm 5
20Dq	40 \pm	50.3 \pm	0.83 \pm 0.03	1.17 \pm 0.08	2.6 \pm 0.15	2.97 \pm 0.03	13.5 \pm 0.12	16.1 \pm 0.7	6.63 \pm 0.14	8.38 \pm 0.2	102000 \pm 13	135333 \pm 6
40Dq	44 \pm	53.7 \pm	1.17 \pm 0.2	1.17 \pm 0.08	3.03 \pm 0.1	3.17 \pm 0.2	14.8 \pm 0.3	17.7 \pm 0.3	7.61 \pm 0.04	8.81 \pm 0.04	124333 \pm 12	156000 \pm 12

20Dq = 20% w/w of *Daedalea quercina* in feed, 40Dq = 40%w/w of *Daedalea quercina* in feed, Control normal (CN),-control group not ulcer-induced.

Table 6: Effect of *Daedalea quercina* supplemented diets on White Blood Cell, Lymphocyte, Neutrophils, Monocyte, and Eosinophils counts in wistar rat.

Group	WHITE BLOOD CELL(10 ⁶ / μ L)		LYMPHOCYTE S (10 ³ / μ L)		NEUTROPHILS (10 ³ / μ L)		MONOCYTE (10 ³ / μ L)		EOSINOPHILS (10 ³ / μ L)	
	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	4183 \pm 44	5733 \pm 20	71.3 \pm 1.8	77.7 \pm 0.4	26 \pm 1.5	13.7 \pm 0.3	1.3 \pm 0.33	1.7 \pm 0.67	1.33 \pm 0.88	0.33 \pm 0.33
20Dq	4250 \pm 57	3750 \pm 23	67 \pm 4.6	81 \pm 0.7	29.7 \pm 2.6	16.3 \pm 0.52	2 \pm 0.6	1.3 \pm 0.35	1.33 \pm 0.88	0.67 \pm 0.05
40Dq	3833 \pm 33	3550 \pm 25	72 \pm 3.0	81 \pm 0.6	25.3 \pm 0.88	15.7 \pm 0.5	2 \pm 0.57	1.7 \pm 0.2	0.67 \pm 0.33	1.67 \pm 0.03

Values are expressed as Mean \pm SEM.(n = 3). All values were not significantly different from control normal (CN) at p<0.05.

20Dq= 20% w/w of *Daedalea quercina* in feed, 40Dq = 40%w/w of *Daedalea quercina* in feed, Control normal (CN),-control group not ulcer-induced.

Table 7: Effects of *Daedalea quercina* supplemented diets on Albumin, Globulin, Blood Urea Nitrogen, and Creatinine on rat

Groups	ALBUMIN (g/dL)		GLOBULIN (g/dL)		BUN (g/dL)		CREATININE (g/dL)	
	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	2.87±0.3	2.93±0.18	4.5±0.2	4.17±0.3	17.27±0.35	16.67±0.18	0.77±0.03	0.83±0.09
20Dq	2.63±0.2	3.6±0.12	4.53±0.2	3.9±0.25	17.13±0.3	17.93±0.5	0.73±0.03	1.07±0.13
40Dq	3.03±0.2	3.53±0.09	4.6±0.1	4.03±0.13	17.57±0.3	17.33±0.38	0.8±0.0	1.23±0.03

Values are expressed as Mean±SEM.(n = 3). All values were not significantly different from control normal (CN) at p=0.05.

20Dq= 20% w/w of *Daedalea quercina* in feed, 40 Dq = 40%w/w of *Daedalea quercina* in feed, Control normal (CN),-control group not ulcer-induced.

LIST OF FIGURES



Figure 1: *Daedalea quercina* with accession number MN596945 collected from Ogbomoso

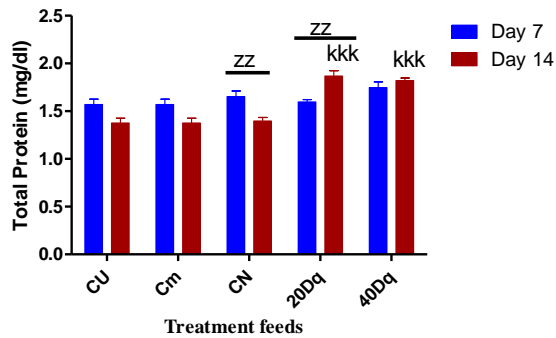


Figure 2: Effects of *Daedalea quercina* supplemented diets on total protein of Indomethacin- Induced Gastric Ulcerated rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^jp < 0.01, ^{jj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

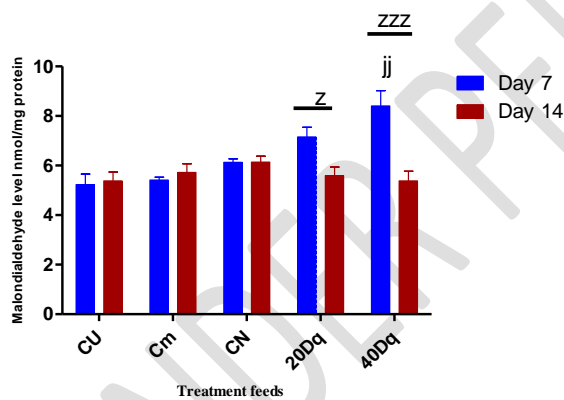


Figure 3: Effects of *Daedalea quercina* supplemented diets on Malondialdehyde (MDA) Level of Indomethacin- Induced Gastric Ulcerated rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^jp < 0.01, ^{jj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

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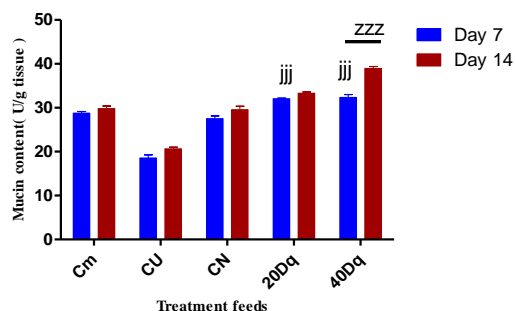


Figure 4: Effects of *Daedalea quercina* supplemented diets on Mucin content of indomethacin induced ulcer in rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^{jj}p < 0.01, ^{jjj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

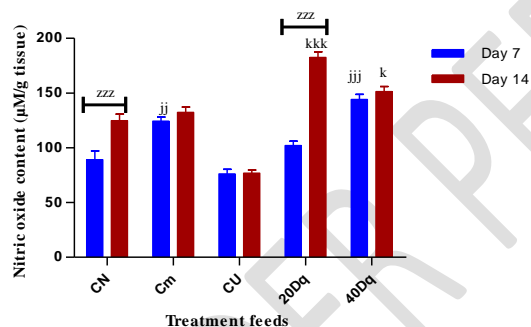


Figure 5: Effects of *Daedalea quercina* supplemented diets on Nitric Oxide (NO) Content of indomethacin induced ulcer in rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^{jj}p < 0.01, ^{jjj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

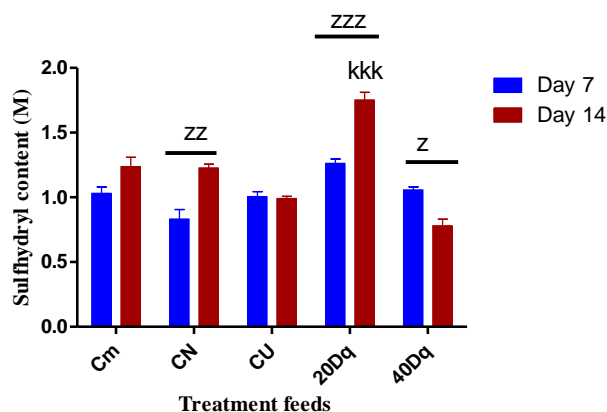


Figure 6: Effects of *Daedalea quercina* supplemented diets on sulfhydryl content of indomethacin induced ulcer in rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^{jj}p < 0.01, ^{jjj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

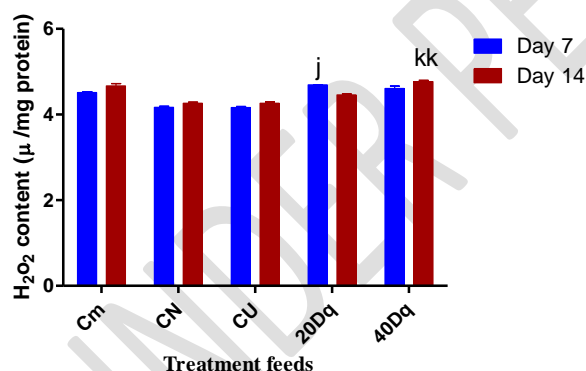


Figure 7: Effects of *Daedalea quercina* supplemented diets on hydrogen peroxide content of indomethacin induced ulcer in rats for 7 and 14 day exposure periods.

Following one –way Anova,

ⁱp < 0.05, ^{jj}p < 0.01, ^{jjj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

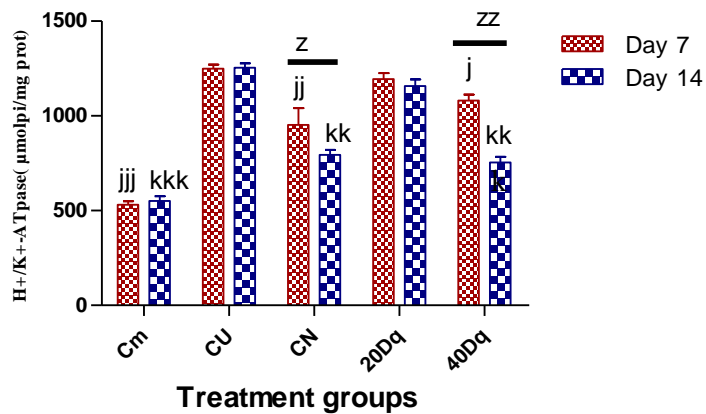


Figure 8: Effects of *Daedalea quercina* supplemented diets on Hydrogen Potassium pump activities of indomethacin induced ulcer in rats for 7 and 14 day exposure periods.

Following one –way Anova,

^jp < 0.05, ^{jj}p < 0.01, ^{jjj}p < 0.001 at 7 days and ^kp < 0.05, ^{kk}p < 0.01, ^{kkk}p < 0.001 at 14days compared with the corresponding controls (CU) .

Using two-way Anova .

^zp < 0.05, ^{zz}p < 0.01, ^{zzz}p < 0.001, between 7 and 14 days exposure periods.

20Dq = 20% of *Daedelia quercina* in feed, 40Dq = 40% of *Daedelia quercina* in feed, CN= Control with normal feed not ulcerated, Cm= control treated with 20 mg/kg of cimetidine, CU= ulcer untreated control.

LISTS OF PLATES

Effect of higher fungi supplemented diets on the Histological changes in the gastric mucosa of Indomethacin Induced Gastric Ulcerated

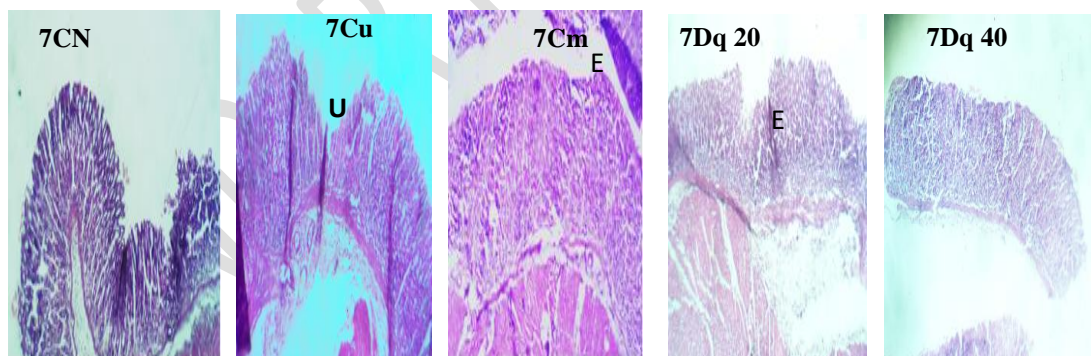


PLATE- 1

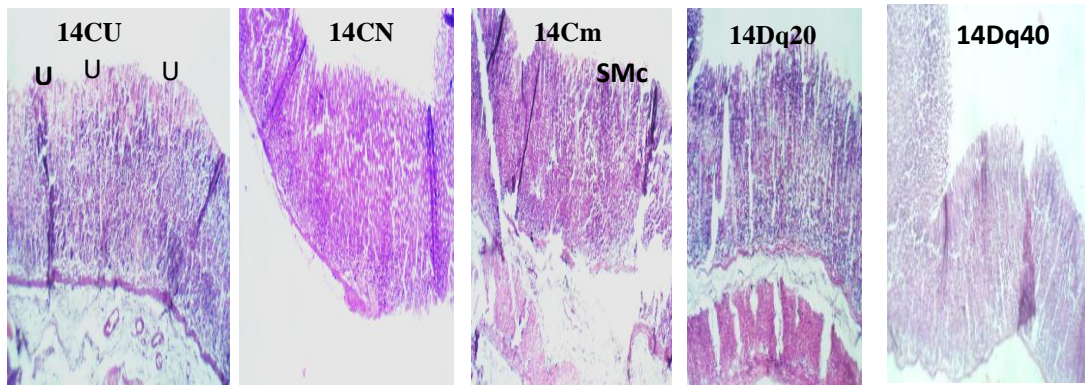


PLATE- 2

UNDER PEER REVIEW