

Histological Study of Therapeutic Effect of *P.ostreatus* on Gastric Ulcer in Male Rats

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ABSTRACT

Mushrooms extract have wide range of medical activity. Present study goaled to investigate healing role of *P.ostreatus* on aspirin induced gastric ulcer. This study carried out on rats males of *Rattus norvegicus*, rats divided into negative control group(C), positive control group (T1), standard drug treated control group (T2), group treated with alcoholic extract of *P.ostreatus* (T3), group treated with polysaccharide of *P.ostreatus* (T4) and group treated with chitin and chitosan of *p. ostreatus* (T5). Histopathological examination of stomach tissue by hematoxylin and eosin, Periodic acid Schiff and Masson's trichrome of *P.ostreatus* extract treated group (T3) at the end of the 10 day of treatment showed epithelial cell reconstitution and gastric cell repair in gastric mucosa accompanied with marked infiltration of leucocytes and congestion in the walls of the blood vessels of the submucosal layer.

At the end of 20 days, it was observed the gastric mucosa was closely no major differences compared with normal group and had better healing effect than that of omeprazole group. Histological observation of the animals treated with Polysaccharide, Chitin and Chitosan of *P.ostreatus* (T5 and T4) showed moderate disruption of the surface epithelium with leucocyte infiltration and thickening of muscularis mucosae at the first period. At second period there is still mild disruption and the repair is not complete. Present study detected a significant role of *P.ostreatus* extract in gastric ulcer healing.

Key words: *P.ostreatus*, Aspirin, Gastric Ulcer, Omeprazole

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INTRODUCTION

Peptic ulcer is a gastrointestinal tract (GIT) region damaged by gastrointestinal acid or pepsin. Gastric ulcer, duodenal ulcer, or esophageal ulcer may be referred to as a peptic ulcer. Peptic ulcer symptoms include multiple symptoms such as nausea, vomiting, appetite change, bloating, burning or gnawing pain [1]. Ulcer develops when a balance between some offensive and defensive factors is lost. Offensive factors are either endogenous or exogenous in origin. Endogenous damaging factors are HCl, pepsin, refluxed bile, leucotrienes and ROS such as OH, H₂O₂ and (O₂) [2]. Exogenous harmful factors include NSAIDs, *Helicobacter pylori* bacterial infection, intake of alcohol, uneven diet, emotional stress and smoking. Defensive variables like mucus bicarbonate barrier, prostaglandin E2 (PGE2), nitric oxide, growth factors, blood flow in mucosa, cell migration and regeneration, antioxidants enzymatic and non-enzymatic [3]. NSAIDs postpone the healing process of gastric injury arising from the suppression of angiogenesis by down-regulation of pro-angiogenic agents besides anti-angiogenic protein up-regulation.

Omeprazole is a selective and irreversible pump proton inhibitor that suppresses the secretion of stomach acid [4]. Despite the accessibility of counter-drugs for oral administration or parenteral therapy for gastric ulcers, hypersecretory illnesses and gastroesophageal reflux disease, It has a huge disadvantage because most of the drugs on the market presently have restricted efficacy against gastric illnesses and are often linked with serious side effects [5]. The use of herbal medicines as a mushroom extract for the prevention and therapy of various pathologies is ongoing global expansion [6, 7].

Medicinal mushrooms as *P.ostreatus* are believed to have many medicinal properties, including antitumor, antioxidant, immunomodulating, epidermal growth factor activation, anti-allergic, anti-inflammatory activity, collagen activity stimulation, anti-hyper cholesterolemic, antiviral,

antibacterial, anti-parasitic, antifungal, detoxification, hepatoprotective, antidiabetic and acne treatment [8,9]. *P.ostreatus* have shown therapeutic action primarily because they contain a number of biologically active compounds [10]. These include elevated molecular compounds, including lipids, polysaccharides, proteins and various low-molecular mass metabolites such as lectins, terpenoids, lactones, alkaloid phenol and sterols. [10, 11]. Present study goaled to investigate gastro-protective activity of *P.ostreatus* extract on aspirin induced gastric ulcer.

MATERIALS AND METHODS

Preparation of fungal extracts

The fresh fruiting bodies of *Pleurotus ostreatus* were supplied from the Ministry of Science and Technology-Directorate of Agricultural Research-Baghdad. The first step in preparation of mushroom extracts is cutting fresh fruiting bodies of mushroom (*P. ostreatus*) with sharp knife into small parts. The parts were well air-dried for one week and kept in a night oven at a temperature of 40 °C to prevent rot. The air-dried mushroom sample was blended into powder using a blender. The crushed biomass (100 g) was placed in 400 ml of absolute methanol and incubated for 48 hours at 200 rpm. And the temperature is 37 °C. To remove the biomass, the suspension was filtered by Whatman filter paper No.2. The supernatant was concentrated under lower pressure in a rotary evaporator at 50 °C. The results were kept at 4 °C in the dried biomass [12]. Extraction of polysaccharide was prepared according to method of Gaur *et al.*[13], extraction of chitin & chitosan according to method of Erdogan *et al.*, [14]

Aspirin preparation

Aspirin was used for the development of gastrointestinal ulcers in all experimental animals except the negative control group. In order to obtain the required dose (100 mg / kg b.w), a solution was prepared by dissolving 1 g of aspirin powder

in 100 ml of 5% Carboxy methyl cellulose to be aspirin concentration 10 mg/ml and given 1ml/100 g b.wt [15].

Omeprazole preparation

Omeprazole was used as a standard drug in the treatment of ulcer will developed in animals. In order to obtain the required dose (20 mg / kg bw), a Stock solution was prepared by dissolving (0.2 g) of Omeprazole powder in 100 ml of distilled water to make its concentration (2 mg / mL) and given 1 ml /100 g body weight [16].

Animals

In this study, 60 males rats of *Rattus norvegicus* were divided into six groups each group with 10 animals, these groups included: negative control group (C) which given the standard diet and distilled water only along study period and remaining rats were treated by aspirin (100 mg/kg body weight) for induction of ulcer by oral dosage for one week then these animals were divided into: T1 (positive control group or aspirin ulcerated group which developed peptic ulcer through oral dosage for one week and remain without treatment for 10 and 20 day), T2 (ulcerated males rats were treated with 20 mg/kg of omeprazole), T3 (ulcerated males rats were treated with alcoholic extract of *P. ostreatus* (200mg/kg bw) for 10 and 20 days), T4 (ulcerated males rats were treated with polysaccharide of *P.ostreatus* (200mg/kg bw) for 10 and 20 days) and T5 (ulcerated males rats were treated with chitin and chitosan of *P.ostreatus* (200mg/kg bw) for 10 and 20 days). Each group was anesthetized after the end of 10 days and 20 days.

Histological study

Ten samples of stomach from each group were fixed in 10% formalin and the solution was 1:10 of the size of specimen and left for 24 hours and processed in routine methods according to Suvarna *et al.*, (2018) [17]. After fixation, the tissue specimens were washed by tap water for 3-4 hours to remove the formalin solution and transferred to dehydration, clearing, infiltration and embedding then sectioning stained by hematoxylin & eosin (H&E) stain to demonstration the general component of the tissue and the staining steps preformed according to Dey, *et al.* (2018) [18]. Periodic acid Schiff (PAS) also used to demonstrate mucopolysaccharide. In additional, Masson trichrome (many colors dye) were used to differentiate the collagen fibers.

RESULTS

In Figure (1), histological sections of stomach of male rats which stained by hematoxylin and eosin in negative control group (C) showed normal histological structure and revealed that the stomach consisted of four main layers, respectively from the inside to outside: the mucosa layer in which the lining epithelium modified in the form of a gastric pits and gastric glands situated at the base of these pits. At the bottom of these glands was the presence of Muscularis mucosae, which was based on a loose connective tissue representing the submucosa layer, which is bounded externally by muscularis externa. The outer wall of the stomach is covered with the Serosa layer. On the other hand, histological examination using special stain (Periodic Acid-Schiff) for mucosal glycoprotein analysis showed normal magenta color of PAS staining in the apical epithelial cells of gastric mucosa. The second special stain (Masson's Trichrome) for collagen

fibers showed that the control group contained normal amount and distribution of collagen and fibers (fig.1).

Histological examination of hematoxylin and eosin stained sections after the first period (10 days) of the first treatment group with aspirin only treated group (T1) showed clear ulcers characterized by severe lesion and extensive damage to the gastric mucosa (Fig.2). At the second period (20 days) the results showed the occurrence of histological changes similar to changes in the first period, which included the presence of erosion in some parts of the entire mucosa and infiltration of inflammatory cells well as the presence of disintegration in the muscularis mucosae and (Fig.3). It has to be mentioned that there is no any sign indicating a marked regeneration and healing in ulcerated parts and characterized by the destruction of the mucosa surface were obvious. Stomach tissue sections stained with PAS staining showed decreased in magenta color in first period (10 days) compare with control group (Fig.2) but it is slightly increased in second period (20 days) (Fig.3). Masson's Trichrome in (T1) group showed less amount of collagen fibers in submucosa and indicated fragmented and disorganized collagen fibers in the ulcer bed. The collagen fibers were more obvious in second period (Figs.2 &3).

Histological observation of hematoxylin and eosin stain sections from rats treated with standard drug (Omeprazole) (T2) at a concentration of 20 mg /kg body weight at the end of the first period (10 days) showed a less superficial erosion of the gastric epithelial lining in comparison with positive group. The inflammatory response (leucocytes infiltration) was observed but it was slight accompanied by congestion and dilation of blood vessels (Fig.4). In the second period (Fig.5) gastric mucosa was relatively intact, mucosal columnar epithelial cell arranged neatly and the inflammatory response was observed obviously in comparison with the first period. There is also minor congestion layer was observed in the blood vessels of submucosa. PAS of the mucosal glycoproteins revealed a noticeable increase in the level of PAS staining of the gastric mucosa in the omeprazole treated group (T2) in comparison with negative and positive groups (T1 and T2). There is an obvious increase in the interaction of the stain with the apical mucous cells in the second period (Figs.4,5). Collagen can be stained blue through Masson staining, by which we can roughly evaluate the healing effect at the macro level. Histological examination of stomach sections of the T2 group showed the synthesis of new collagen at the first period (Figs.4). On 20 day, obviously contained less collagen than 10 day (Fig.5).

The results of extract of *P.ostreatus* group (T3) at the end of the 10 day of treatment showed epithelial cell reconstitution and gastric cell repair in gastric mucosa accompanied with marked infiltration of leucocytes and congestion in the walls of the blood vessels of the submucosal layer (Fig.6). At the end of 20 days, it was observed the gastric mucosa was nearly no major differences compared with normal group and had better healing effect than that of omeprazole group (Fig.7). It must be mentioned that the differences is existed. The *P.ostreatus* extract had a certain inflammatory response (infiltration) in early time (10 days) which nearly disappeared from 20 day period. On the other hand, the detection of mucous secretion using (PAS) stain indicated the presence of interaction at the end of the first period (Fig.6). The intense magenta color in the apical epithelial cells increased at the second period which

indicated the increase in glycoprotein accumulation in the gastric mucosa (Fig.7). The content of collagen deposition evaluate by using Masson's Trichrome stain. The results showed more dense deposition of collagen fibers in first period than the second period (Figs.6 & 7).

Histological observation of the animals treated with Polysaccharide of *P.ostreatus* (T4) stained with hematoxylin and eosin showed moderate disruption of the surface epithelium with leucocyte infiltration and thickening of muscularis mucosae at the first period (Fig.8). At second period there is still mild disruption and the repair is not complete (Fig.9). The special stains of both PAS and Masson's Trichrome showed progressive results in accumulation of both mucus secretion (Figs.8&9) and

collagen fibers content. The sixth group of rats treated with Chitin and Chitosan of *P.ostreatus* (T5) revealed similar results to the Polysaccharide group (T4) in hematoxylin and eosin stain and also there is moderate and mild disruption in the superficial mucosal surface in the two period respectively. There were similar observations of inflammatory response and polymorphonuclear leucocytes infiltration (Figs.10 & 11). Also the histological examination using special stain (Periodic Acid – Schiff) for mucosal glycoprotein detection showed similar intensity with Polysaccharide group (T4) group of magenta color of PAS staining in the apical epithelial cells of gastric mucosa in the two periods. The Masson's Trichrome stain gave the same reaction for collagen fibers in Polysaccharide group (T4) (Figs.10 & 11).

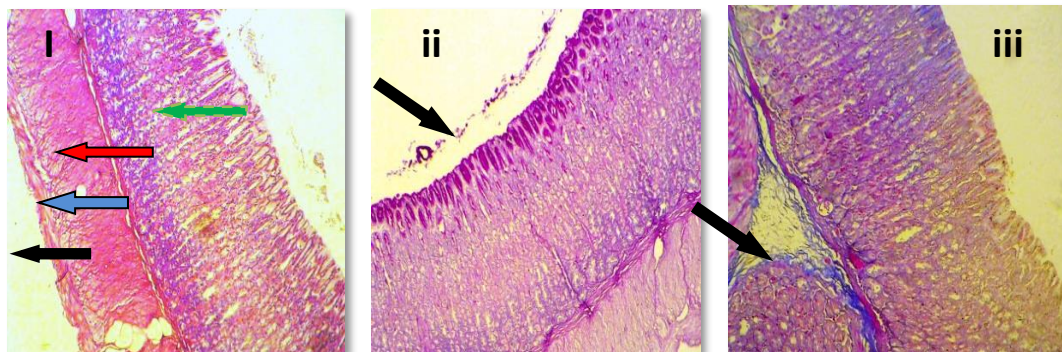


Fig (1): Section in rat stomach in negative control group (C). i: shows normal structure of the four main layers: mucosa layer (green arrow), Submucosa layer (red arrow), Muscularis externa layer (blue arrow), serosa layer (black arrow) (H&E X100). ii: shows normal intense magenta color in the apical epithelial cells (black arrow) (PAS X100). iii: shows normal amount and distribution of collagen fibers (black arrow) (Masson's trichrome X100)

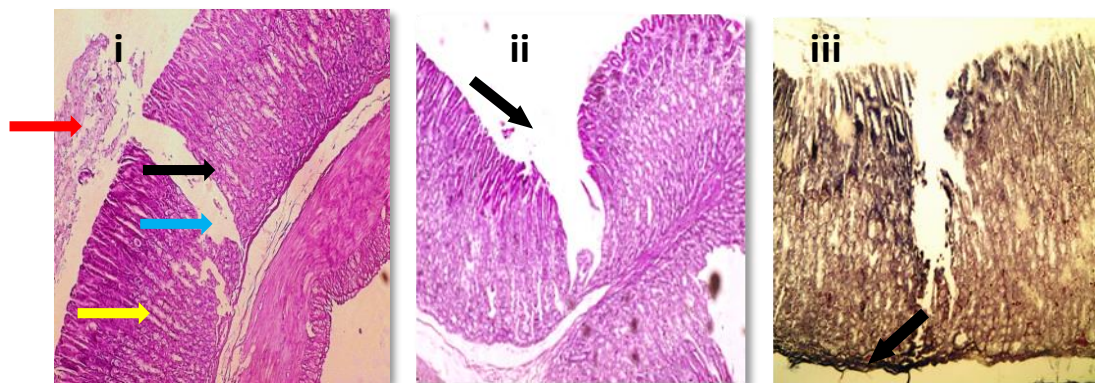


Fig (2): Section in rat stomach in positive control group (T1) at 10 days. i: shows severe lesion in surface epithelium (red arrow), leukocytes infiltration (black arrow), congestion of blood vessel (yellow arrow) and edema in submucosa (blue arrow) (H&E X100). ii: shows weak intense reaction in apical epithelial cells (black arrow) (PAS X100). iii: shows less amount of disorganized collagen fibers (black arrow) (Masson's trichrome X100)

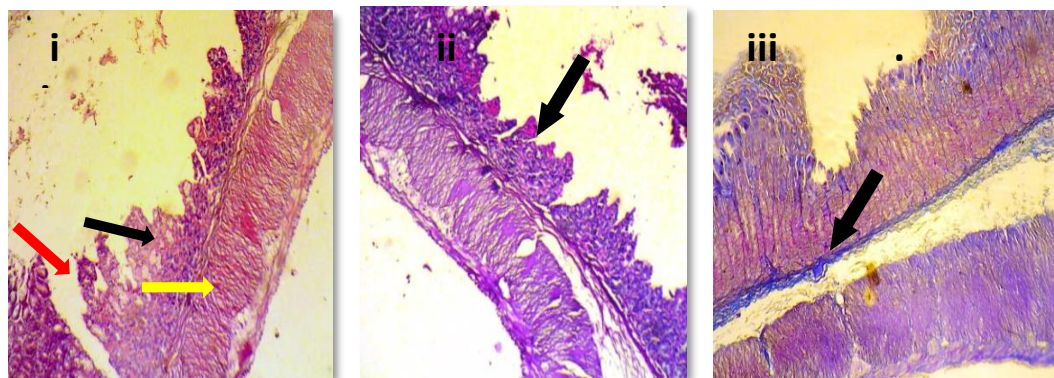


Fig.(3): Section in rat stomach in positive control group (T1) at 20 days. i: shows erosion of the surface epithelium (red arrow), leukocytes infiltration (black arrow) and disintegration of muscularis mucosa (yellow arrow) (H&E X100) ii: shows slightly intense reaction in apical epithelial cells (black arrow) (PAS X100), iii: shows slight increase amount of collagen fibers (black arrow) (Masson's trichrome X100)



Fig.(4): Section in rat stomach in Omeprazole(20mg/kg) treated group (T2) at 10 days. i: shows less erosion in surface epithelium (red arrow), slight leukocyte infiltration in mucosa & submucosa (black arrow), blood vessels dilation (yellow arrow) and muscular thickening (green arrow) (H&E X100), ii: shows moderate reaction of apical epithelial cells with stain (black arrow) (PAS X100), iii: shows more amount of collagen fibers (black arrow) (Masson's trichrome X100)

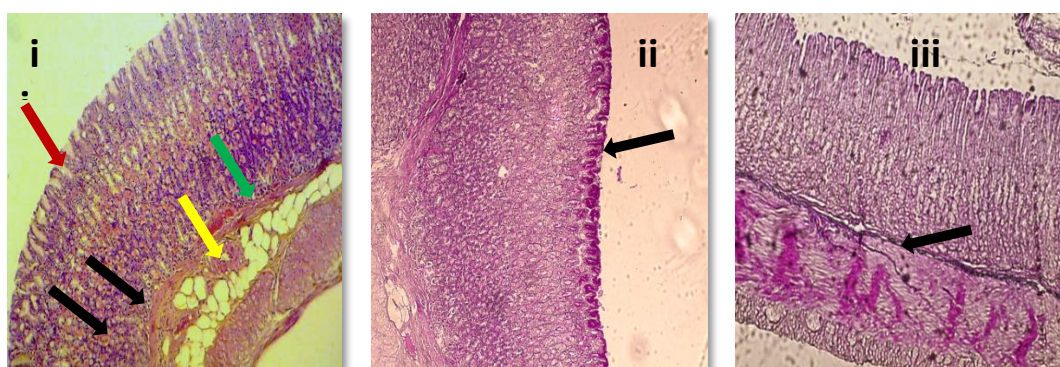


Fig.(5): Section in rat stomach in Omeprazole(20mg/kg) treated group (T2) at 20 days. i: shows intact of surface epithelium (red arrow), more leukocyte infiltration (black arrow) and blood vessels dilation in submucosa (yellow arrow) and muscular mucosa thickening (green arrow) (H&E X100), ii: shows increase intense reaction in apical epithelial cells (black arrow) (PAS X100), iii: shows less amount of collagen fibers (black arrow) (Masson's trichrome X100)

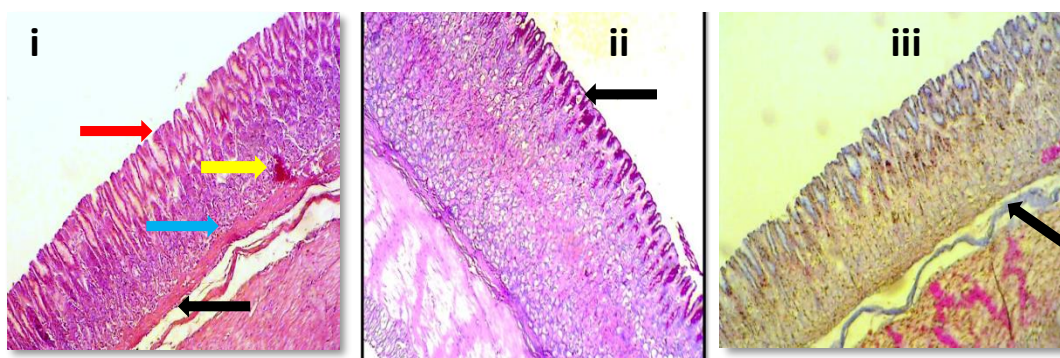
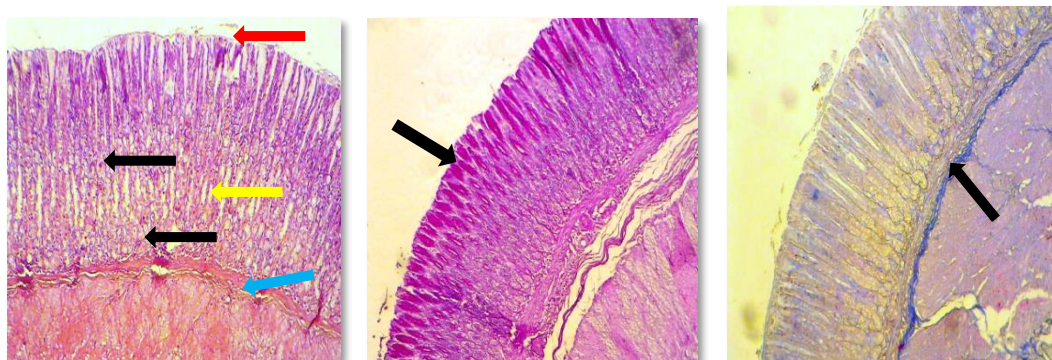


Fig.(6): Section in rat stomach in *P. ostrateus* (200 mg/kg) treated group (T3) at 10 days. i: shows roughly intact epithelium (red arrow), leukocyte infiltration (black arrow), congestion of blood vessels (yellow arrow) and thickening (blue arrow) (H&E X100), ii: shows increase intense reaction in apical epithelial cells (black arrow) (PAS X100), iii: shows less amount of collagen fibers (black arrow) (Masson's trichrome X100)



Fig(7): Section in rat stomach in *P. ostreatus* (200mg/kg) treated group (T3) at 20 days. i: shows intact epithelium (red arrow), less leukocyte infiltration (black arrow), blood vessel congestion (yellow arrow) and thickening of muscularis mucosa(blue arrow)(H&E X100), ii: shows a more reaction of apical epithelial cells (black arrow) (PAS X100), iii: shows less deposition of collagen fibers (black arrow) (Masson's trichrome X100)

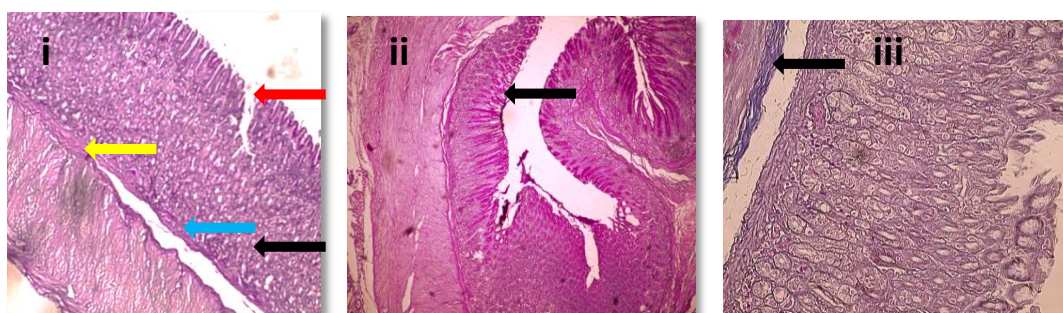


Fig (8): Section in rat stomach in *P. ostreatus* (polysaccharide 200mg/kg) treated group (T4) at 10 days. i: shows mild lesion in surface epithelium (red arrow), leukocyte infiltration (black arrow), congestion of blood vessels (yellow arrow) and thickening in muscularis mucosa (blue arrow) (H&E X100), ii: shows moderate reaction of apical epithelial cells (black arrow) (PAS X100), iii: shows moderate amount of collagen fibers (black arrow) (Masson's trichrome X100)

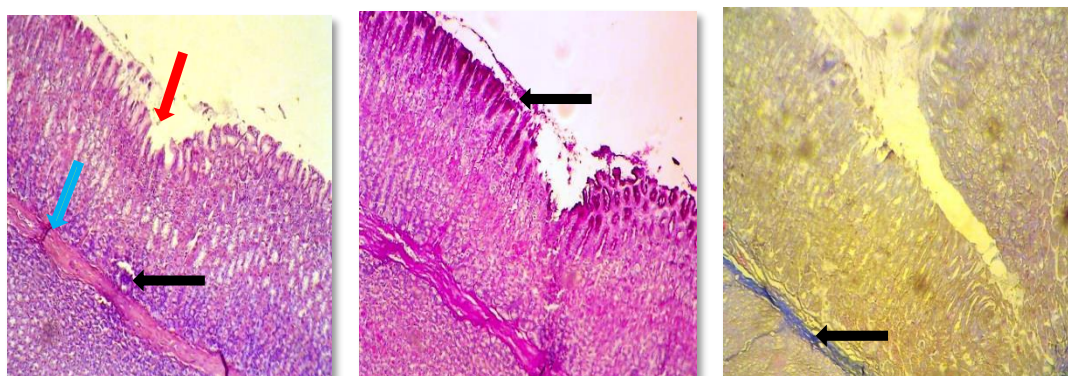


Fig (9): Section in rat stomach in *P. ostreatus* (polysaccharide) (200mg/kg) treated group (T4) at 20 days. i: shows less erosion of the surface epithelium (red arrow), leukocyte infiltration (black arrow) thickening in muscularis mucosa(blue arrow) (H&E X100), ii shows strong reaction of apical epithelial cells (black arrow) (PAS X100), iii: shows dense presence of collagen fibers (black arrow) (Masson's trichrome X100)

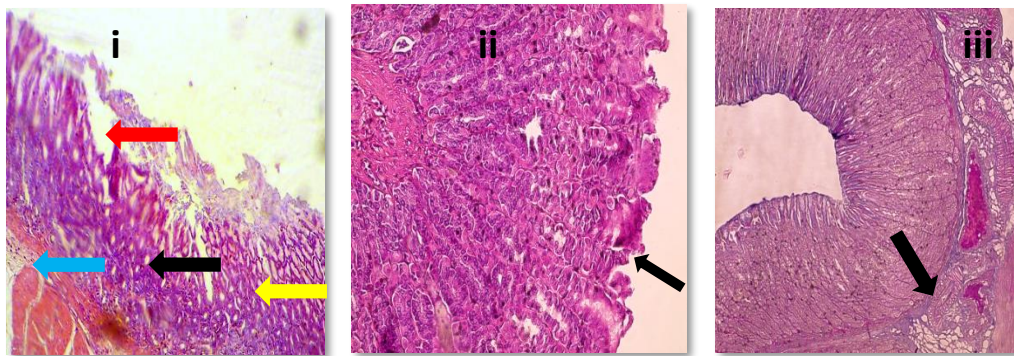


Fig (10): Section in rat stomach in *P. ostreatus* (chitin&chitosan) (200mg/kg) treated group (T5) at 10 days. i: shows moderate erosion in surface epithelium (red arrow), leukocytes infiltration (black arrow), congestion of blood vessels (yellow arrow) and edema in submucosa (blue arrow) (H&E X100), ii: shows mild reaction of apical epithelial cells (black arrow) (PAS X100), iii: shows moderate amount of collagen fibers (black arrow) (Massons trichrome X100)

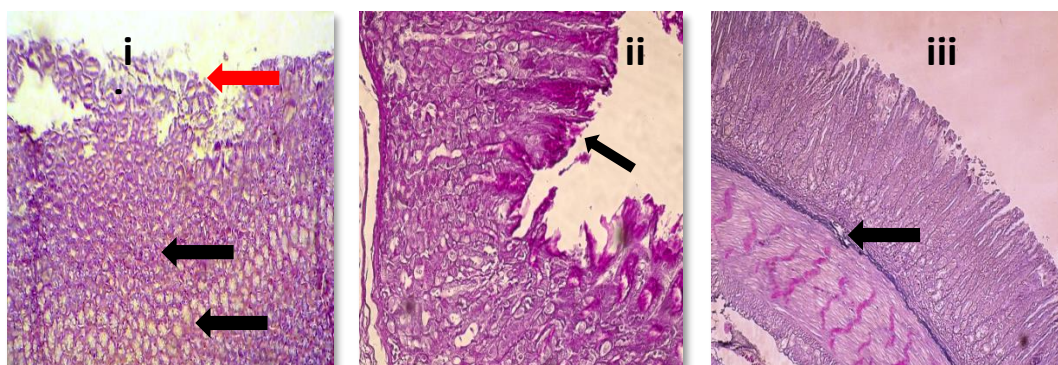


Fig (11): Section in rat stomach in *P. ostreatus* (chitin&chitosan) (200mg/kg) treated group (T5) at 20 days. i: shows less erosion of the surface epithelium (red arrow), leukocytes infiltration (black arrow) (H&E X100), ii: shows moderate reaction of apical epithelial cells (black arrow) (PAS X100), iii: shows dense deposition of collagen fibers (black arrow) (Massons trichrome X100)

DISCUSSION

The current study evaluated the therapeutic effect of *P. ostreatus* edible mushrooms and its compounds against aspirin-induced ulcer. Gastric ulcer was induced by oral administration of aspirin (100mg/kg bwt) for seven days. The results of the microscopic examination in the positive control group (T1) showed clear pathological changes caused by aspirin these changes came in agreement with previous studies, that showed typical histologic modifications of the atrophic gastric mucosa with reduction of glandular tissues, reduction of thickness of the mucosa, erosion of the surface epithelial cells, loss of the integrity of the gastric mucosa, submucosal edema, inflammatory cell infiltration and muscular layer thickening [19,20].

Aspirin is a drug that used for both therapeutics (to reduce pain, inflammation and fever) and prophylactics (to prevent thrombotic events). Serious side effects on the gastrointestinal system, particularly peptic ulcers and gastrointestinal hemorrhage are the cause of major limitations of its clinical application [21]. The pathogenesis of aspirin-induced mucosal injury involves COX-dependent mechanisms. COX-1 maintain gastric mucosal homeostasis and integrity, and its inhibition decreases the mucous layer. Inhibition of COX-2 results in reduced angiogenesis and increased leukocyte adherence, leading to microvascular occlusion, which results in weakening the protective barrier of the stomach which is

composed of mucus and bicarbonate, that lead to the generation of free radicals and increased infiltration of neutrophil white blood cells, causing tissue damage and ulcers formation [22]. The result were also similar to the findings of the Hajrezaie *et al.*, (2015) whose observed multiple ulcers with bleeding in rat stomach treated with aspirin because it inhibits the formation of prostaglandine from the mucous layer of the stomach in addition to inhibit thromboxane of platelets and thus reduce accumulation of platelets and causes bleeding (Sorensen *et al.*, 2009) [23, 24].

The PAS staining of stomach and duodenum tissues in aspirin group (T1) showed weak reaction with this stain as a result of damage in apical epithelial cells of stomach, this was evidenced by the decrease of the estimated gastric mucus content in the current experimental group. Wallace, (2008) explained these findings by the direct cytotoxic effect of aspirin while Kwiecie, *et al.* (2012) demonstrated that the absence of gastric mucus protective layer was due to the accumulation of oxygen free radicals, stated that the non-steroidal anti-inflammatory drugs (NSAIDs) cause suppression of cyclo-oxygenase enzyme and inhibition of prostaglandin synthesis which was the cause of mucous layer loss from the gastric mucosa [25,26]. Seleem, *et al.* (2010) had another explanation that aspirin which causes gastric injury might lead to decrease in the number of mucous cells [21]. Helal, *et al.* (2011) demonstrated that stomach tissues stained with PAS stain showed evidence of loss of gastric mucosa and mucosal

synthesizing cells with reduced mucin content in ulcer-induced groups [27].

Masson's Trichrome staining of aspirin group (T1) showed less amount of collagen fibers as collagen is the primary component of extra-cellular matrix, ulceration seemingly due to differences in ECM turnover, driven by a balance between deposition and collagen degradation in gastric tissue. Current results were in accordance with those of Xue *et al.* (2019) who saw reduced collagen in various models of gastric ulcer [28].

In this study, oral administration of *p.ostreatus* (200 mg/kg) after ulcer induction improved the gross morphology, histopathology of gastric ulcers better than other groups. Curring effects on mushrooms have also been shown to be accompanied by an increase in prostaglandin, the enzyme and nonenzyme antioxidant systems (GSH, SOD, and CAT), the reduction in the levels of lipid peroxidation marker (MDA) and inflammatories (TNF- α , IL1B) . In comparison to the positive control group (T1), the score of ulcer and ulcer index also decreased significantly. Previous studies reported therapeutic effects of mushrooms in gastrointestinal pathological models, Bilay *et al.*, (2011) confirmed role of mushrooms species as antiulcer treatment when study effect of mushroom species (*Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus*) have been shown that treatment with these mushrooms inhibited ulcer formation [29]. These extracts had protection effects of increased mucus production and reduced formation of free radicals in the body on the gastric mucosa. [30, 31]. The effectiveness these mushroom for ulcer healing can be due to the presence of micro- and macro nutrients and other nutraceuticals. Shaik, (2018) has shown that mushroom is a good source of vitamin A and vitamin C. To maintain healthy mucus membrane, vitamin A is required. Vitamin C has many essential functions, such as free radicals scavenger, immune booster and anti-inflammatory substances. [32].

Akila and Priya, (2012) were founded that treatment of gastric ulcer with chitin and chitosan extracted from mushroom at dose 200mg/kg have been showed significant healing of ulcer and this may be due to its neutralization effect on H⁺ ions and pepsin in the gastric juices and exert its protective effect by coating the ulcerated area. The mushroom extract gave therapeutic effect and relieved ulcer healing by enhancing antioxidant defensive lines via dietary supplementation to eliminate oxidative stress levels. [33]. A bioactive substances, such as phenols, flavoids, polysaccharides, vitamins, carotenoids, minerals lycopene, ascorbic acid, tocopherols, and ergosterols have aligned them with important antioxidant properties, which show excellent antioxidant capacity [34, 35]. Özdal *et al.* (2019) illustrated that every one of the five species of *Pleurotus* (*P. ostreatus*, *P. sajor-caju*, *P. florida*, *P. citrinopileatus*, and *P. eryngii*) have shown antioxidant activity as a result of containing phenolic and flavonoid compounds. [36].

PAS staining showed increase intense with increase duration of treatment due to improve of mucous barrier formation. Masson's trichrome showed less deposition of collagen fibers in 20 days than 10 days because these mushrooms complete healing of ulcer damage at 20 days.

There was less erosion of the mucosa and mild inflammation in the group treated with Omeprazole 20mg/kg B.Wt at 10 days and the mucosa relatively intact at 20days accompanied with increase intensity of PAS staining and increase deposition of collagen fibers when staining with Masson Trichrome. Omeprazole was documented to have anti-ulcerative activity through alpha-2 adrenergic receptor, which has a direct correlation with gastroprotective cyclooxygenase-1 (COX-1) and PGE2 [37]. In addition, omeprazole upregulates cyclooxygenase-2 (COX-2) and PGE2, which play an important role in ulcer healing through re-epithelization [38].

Omeprazole was improved ulcer healing but less than mushrooms extract (*P. ostreatus*), current results also in line with study of Ajayi *et al.*, (2016) which implied that treatment with *Talinum triangulare* at a dose of 100 mg/kg improved healing of peptic ulcers better than omeprazole [39]. These histological (H and E staining) evaluations revealed that rats treated with *Talinum triangulare* demonstrated the ability to regenerate breached or ulcerated gastric mucosa faster than other treatment groups. Ezemagu, *et al.* (2019) who found that Omeprazole could not protect the gastric mucosa from damage induced by aspirin [40].

Treatment with compounds extracted from mushrooms (polysaccharide and Chitin & chitosan) in T4 and T5 groups, also revealed curative histological effect but less than crude extract and Omeprazole treated groups. This study consistent with previous studies of Yang, *et al.* (2012) showed that acetic acid-induced gastric lesions have been significantly inhibited by oral administration of polysaccharide derived from *Pleurotus ostreatus* in rats as well as significant increase in the mucus synthesis and prostaglandins production. Furthermore, glutathione (GSH) levels and superoxide dismutase (SOD) activity could also be considerably increased [41]. Cipriani, *et al.* (2009) demonstrated that polysaccharides extracted from plants also exhibited, anti-inflammatory and antiulcer activities [42]. Helal, *et al.* (2011) showed that pretreatment of rats with polysaccharide extracted from gum arabic for 10 days before induction of stress resulted in significantly decreased gastric lesions [27]. Kim, *et al.* (2004) conducted study that showed that polysaccharides extracted from *Pleurotus* showed protective effect against auto-oxidation in a linoleic acid model system [43]. Zhang, *et al.* (2016) recorded that Chitosan can improve gastric mucosa tissue, exert significant influences on oxidative and antioxidant enzyme activities and neutrophil infiltration [44].

PAS staining and masson trichrome showed that progress deposition of mucus and collagen fibers with duration of treatment as collagen fiber is mainly secreted by fibroblasts, which is the final product for tissue repair and healing. On the other hand, previous study indicated the importance of colloidal fibers in healing from other conditions such as oral ulcers [45]

CONCLUSION

The histopathological examination with H&E, PAS, masons trichrom stains of stomach tissues of rats showed that *P.ostreatus* compounds especially extract of

P.ostreatus (T3) have curative effect on the aspirin damage tissues and preserved the normalcy histological architecture of the stomach.

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