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ANTIMICROBIAL SCREENING AND ANTI-ULCEROGENIC EFFECTS OF *LEPIDIUM SATIVUM* EXTRACT ON *H. PYLORI* INDUCED GASTRIC ULCER IN A RAT MODEL

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ABSTRACT: Purpose: *Helicobacter pylori (H-pylori)* infection has been commonly associated with gastric ulcers. *Lepidium sativum* (*L. Sativum*) is an herbaceous edible plant, botanically related to mustard and watercress with remarkable pharmacological features. In this study, we investigate the anti-microbial and anti-oxidant effect of *L. sativum* seed methanolic extract *in-vitro* and correlate this with the gastroprotective potential of this extract against *H-pylori*–induced gastric ulcer in an *in-vivo* model. **Methods:** *L. sativum* extract was quantified for phenolic compounds using high-performance liquid chromatography, and the anti-microbial and anti-oxidant activities of the extract were evaluated *in-vitro*. Furthermore, an *in-vivo* rat model was assessed in order to explore the effect of *L. sativum extract* against *H. pylori*-induced gastric ulcer *in-vitro*. **Results:** The *L. sativum* extract exhibited significant antioxidant and anti-microbial activities against different pathogenic organisms. *In vivo*, *L. sativum* extract reduced the ulcer index, increased the percentage of ulcer inhibition, halted lipid peroxide and triggered anti-oxidant markers. Moreover, it suppressed inflammatory and apoptotic markers. The histopathological results for *L. sativum* treated rats showed the amelioration of epithelial erosion, restoration of enterocytes and an intact mucosal layer. **Conclusion:** The *L. sativum* extract exhibited antimicrobial, antioxidant, and antiulcerogenic effects and exerted significant cytoprotective activity against *H. pylori*-induced gastric ulcers.

INTRODUCTION: Peptic ulcer disease is one of the most widespread diseases affecting the gastrointestinal tract.

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This disease induces inflammatory damage to either the gastric or the duodenal mucosa and extends from the submucosa to the muscularis mucosa.

The etiology of this disease is multifactorial and is related to excessive acid secretion. The most prevalent gastroduodenal pathogen is known as *Helicobacter pylori* [1]. *H. pylori* is known to cause severe gastritis, chronic ulcers, mucosal lymphomas and gastric cancer [2].

The cytotoxic and inflammatory effects of *H. pylori* are mediated through vacuolating toxin (VacAH). Infection with *H. pylori* lipopolysaccharide (HP-LPS) induces a provoked increase in pro-inflammatory interleukin (IL) expression [3], epithelial cell apoptosis and prostaglandin and nitric oxide release, as well as marked activation in the nuclear factor-κB (NF-κB) pathway, which in turn stimulates IL-8 [4]. HP-LPS contributes to the destruction of mucosal integrity via triggering histamine and pepsinogen secretion, restriction of sulphated mucin synthesis and stimulation of destructive antibodies [5]. *H-pylori*-induced gastritis elevates oxidative and nitrosative stress production, thus depleting endogenous anti-oxidant protection [6]. *H. pylori* induces gastric ulcers, which may lead to gastric cancer. This is considered a serious health problem that requires radical treatment.

Successful treatment, therefore, should abolish the pathogen, cure the induced gastritis and prevent the recurrence of ulcers [7]. Many pharmacological approaches (*e.g.,* antibiotics) have been proven to have an effect in treating gastric ulcers; however, the induced antibiotic resistance makes it difficult to control diseases. Moreover, these pharmacological interventions confer adverse side effects. Therefore, searching for safer, cheaper, natural herbal medicines seems to be the best choice [8].

Lepidium sativum (*L. sativum*) (family Cruciferae) is an annual, herbaceous edible plant that is associated botanically with watercress and mustard. *L. sativum* is native to Egypt and Southwest Asia and is considered as a culinary vegetable all over Asia, locally known as 'Elrashad' [9]. Seeds contain 35%-54% carbohydrates, 14%-26% lipids, 8% crude fiber and 27% protein [10].

L. sativum seeds also contain 20%-25% oil, with linolenic acid being the main fatty acid (32%-35%). Tocopherols and carotenoids are natural anti-oxidants that are found in the seeds of *L. sativum*, which prevent the rancidity of oil. Sinapic acid, sinapin imidazole alkaloids, monomeric alkaloids, and lepidine were also reported to be present in the seeds of *L. sativum* [11]. *L. sativum* also contains an unsaponifiable matter, which is composed of β-sitosterol and α-tocopherol [12]. Seeds also possess antioxidant, antitumor and anti-inflammatory properties and are helpful as poultices for sprains and in ophthalmopathy, leprosy, scurvy, leucorrhoea, bronchial asthma, seminal weakness, hemorrhoids and cough [13].

The aim of this study was to investigate the feasibility of using the crude extract of *L. sativum* in eradicating *H. pylori* infections and curing gastric ulcer complications. To achieve that, the extracted *L. sativum* seeds were subjected to the following:

a. An *in-vitro* investigation for examining the anti-bacterial, anti-fungal, and antioxidant potential of the extract.

b. An *in-vivo* biochemical investigation for assessing the anti-inflammatory, anti-oxidant, anti-apoptotic and anti-ulcerogenic effects of the extract on *H. pylori*-induced gastric ulcer in an experimental rat model.

MATERIALS AND METHODS:

In-vitro Study:

Plant Materials: *L. sativum* seeds were obtained from a local market in Egypt. The plant seeds were washed, dried, and crushed.

Microorganisms: The microorganisms used are as follows: *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus,* and *Penicillium* sp.

Culture Media: Nutrient broth, potato dextrose, and nutrient agar were used in the preparation of enriched culture media.

Preparation of Plants Extracts: *L. sativum* seeds were dried, powdered and soaked in methanol for three days. The filtrates (crude extracts) obtained were then concentrated in a rotary evaporator while keeping the water bath at 55 °C – 60 °C. The isolated extracts were then preserved in separate containers at 5 °C for further experiments.

High-Performance Liquid Chromatography (HPLC) Separation and Quantification of Phenolic Compounds in the *L. sativum* Extract: The content of phenolic compounds in the extract was analyzed using an HPLC (Agilent 1260 series) according to Kingsbury and Wagner [14].

Antibacterial Activity Assay of the *L. sativum* Extract: Extracts at concentrations of 10, 20 and 30% were prepared in methanol. The anti-bacterial activity was estimated following [15]. A 100 µL aliquot from different concentrations of the extract was placed in a well using an automatic pipette, and then the plate was left for 1 h at room temperature to allow the distribution of the extract into the agar. Then, the plate was incubated for 18 h at 37 °C.

The diameters of the inhibition zones were measured to the nearest millimeter. Every test was performed twice and the average was calculated. The negative control used was the extraction solvent [16].

Antifungal Activity Assay of the *L. sativum* Extract: Wells with a 6 mm diameter were made in the agar using a sterile cork borer, and then 10%, 20%, and 30% extracts were inserted into the wells. The control solvent used was methanol. After 48 h of incubation, the anti-fungal activity was determined by measuring the diameter of the inhibition zone against the test microorganisms [17].

Determination of the Antioxidant Activity of the *L. sativum* Extract: A 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical trapping assay was conducted to evaluate the antioxidant activity of the extract. This test was performed according to Marque-Williams *et al.* [25] The percentage inhibition of DPPH was calculated by the formula proposed by Sharififar *et al.* [18]

***In-vivo* Study:** 40 healthy male Wistar albino rats, weighing between 160 and 200 g, were recruited for the study. They were housed under controlled. temperature (23 ± 2°C) and humidity (55 ± 5%), with 12 h light/dark cycles. The animals were fed a standard pellet diet and water *ad libitum*. All animal procedures were in accordance with the guidelines of the guide for the care and use of laboratory animals (publication no. 85-23, revised 1996).

Preparation of HP-LPS: *H. pylori* strain 26695 was prepared by the conventional method. The bacteria were first pelleted by centrifugation, washed with 0.9% NaCl, heat-inactivated and then suspended in 1 mL of 0.9% NaCl and lyophilized. They were then stored at 4 °C until further use.

Gastric Ulcer Induction: Gastric ulcers were induced in the animals *via* an orogastric administration (P.O.) of HP-LPS (50 µg/kg/day) for four consecutive days [19].

Experimental Design: The animals were randomly assigned into four groups, 10 animals each, and received the following treatments for 30 days: Group 1 (control group): The rats received saline P.O. for 30 days. Group 2 (drug control group):

The rats received the *L. sativum* extract (400 mg/kg, P.O.) for 30 days (28). Group 3 (induced group): The rats received HP-LPS for four days and then saline for 30 days. Group 4 (reference group):

The rats received HP-LPS for four days and then ranitidine (20 mg/kg, P.O.) for 30 days. Group 5 (treated group): The rats received HP-LPS for four days and then the *L. sativum* extract (400 mg/kg, P.O.) for 30 days [20]. At the end of the experiment, all rats were sacrificed, and their stomachs were dissected.

Macroscopic Evaluation and Ulcer Scoring: The obtained stomachs were opened along the greater curvature and rinsed with saline. The ulceration degree in all groups was determined as per the method of Szabo and Hollander [21].

Gastric Tissue Analysis: The gastric mucosa of the dissected stomachs was rapidly scraped using two glass slides from the gastric wall of each rat, weighed, homogenized with 0.1 M phosphate-buffered saline at pH 7.4, to give a final concentration of 10% w/v and centrifuged at 3,000 × *g* for 15 min at −4 °C. The obtained supernatant was used for subsequent biochemical investigations.

Determination of Oxidants and Antioxidant Markers: The harvested gastric mucosa was weighed and homogenized for subsequent analysis of mucosal malondialdehyde (MDA) according to Ohkawa *et al.* [22]

The superoxide dismutase (SOD) activity was determined as described by Marklund [23]. The catalase (CAT) activity was estimated according to Clairborne [24]. The reduced glutathione (GSH) concentration was assayed according to Ellman [25].

Determination of Inflammatory and Apoptotic Markers: Mucosal tumor necrosis factor-alpha (TNF-α), IL-1β, monocyte chemoattractant protein-1 (MCP-1) and caspase- were estimated using enzyme immunolinked assay kits obtained from Glory Science Co., Ltd. (Hangzhou, China).

Histopathological Investigation: Stomach specimens were fixed in 10% formol-saline, embedded in paraffin, sectioned (5 µm thick) by a microtome (Leica, Berlin, Germany), mounted on glass slides, stained using hematoxylin and eosin stain and examined under a light microscope.

Statistical Analysis: Results were expressed as the mean ± standard error of the mean. Data were analyzed using a one-way analysis of variance using the Statistical Package for the Social Sciences version 11, followed by Duncan's post hoc test to compare the significance between groups. Differences were considered significant when $p>0.05$.

RESULTS AND DISCUSSION:

HPLC Separation and Quantification of Phenolic Compounds in the *L. sativum* Extract: HPLC, coupled with a multi-wavelength detector, was used to identify and quantify the distinctive phenolics of the examined plant by performing a comparison between the retention times of the detected peaks and the peaks of the standard compounds. Six phenolics were isolated and identified: catechin, syringic acid, vanillin, naringenin, cinnamic acid, and propyl gallate. Their concentrations were 19,701.90, 303.06, 40.03, 179.79 and 24.65 µg/g, respectively, and the results are shown in **Table 1**.

The peaks of the isolated compounds were recorded at 280 nm and were identified by performing a comparison with standards **Fig. 1A** and **1B**. Catechin was found in the highest concentration in *L. sativum* (339.9 mg/100 g), whereas syringic acid was found at a concentration of 2.3 mg/100 g in *L. sativum* [26].

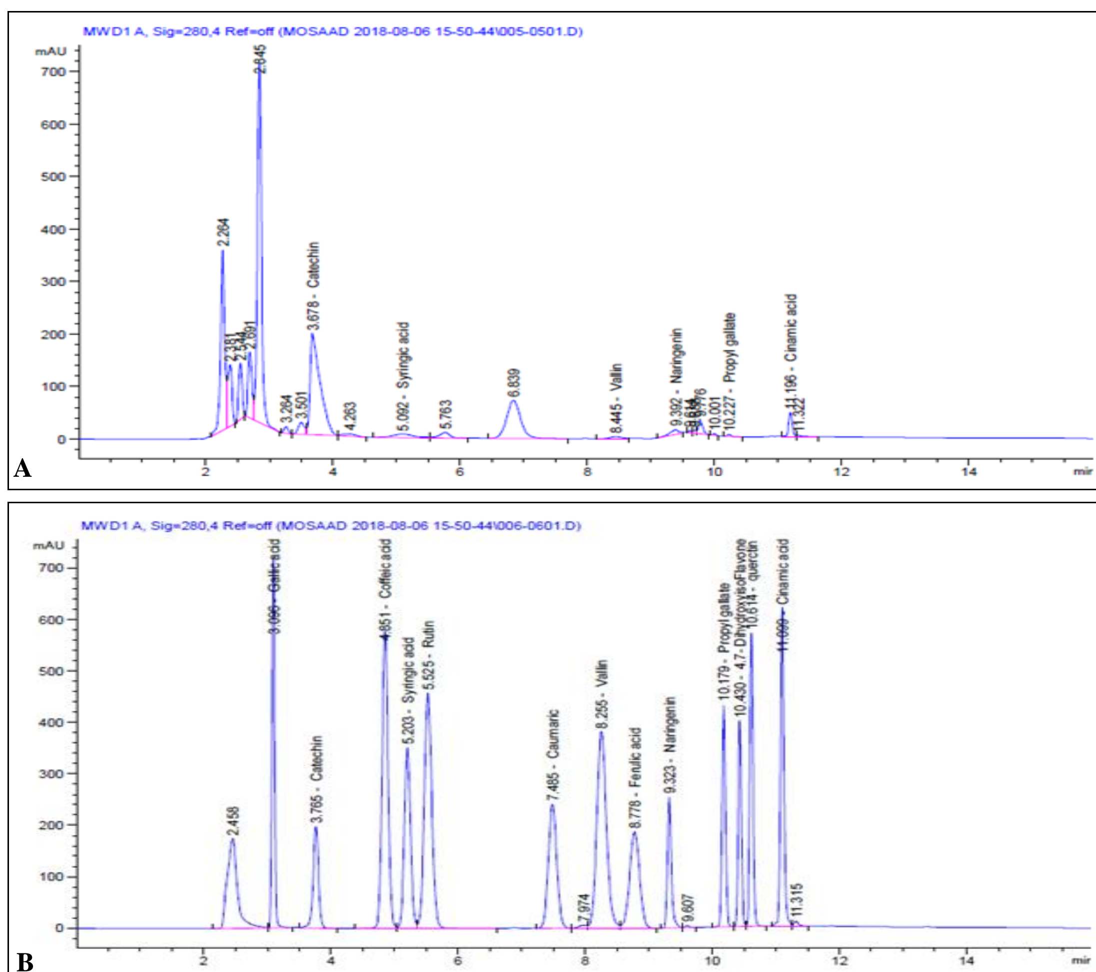

FIG. 1: HPLC CHART OF MeOH EXTRACT OF *L. SATIVUM* SEEDS (A) AN HPLC CHART OF STANDARDS OF PHENOLIC COMPOUNDS (B)

TABLE 1: PHENOLIC CONTENTS IN *L. SATIVUM* SEEDS EXTRACTED SOLUTION

Phenolic compounds	Conc. (µg/ml)	Conc. (µg/g)
Gallic Acid	0.00	0.00
Catechin	224.60	19701.90
Coffeic Acid	0.00	0.00
Syringic Acid	3.45	303.06
Rutin	0.00	0.00
Coumaric Acid	0.00	0.00
Vanillin	0.46	40.03
Ferulic Acid	0.00	0.00
Naringenin	2.05	179.79
Querectin	0.00	0.00
Cinnamic Acid	0.98	85.78
Propyl Gallate	0.28	24.65
4.7-DihydroxyisoFlavone	0.00	0.00

Antibacterial Activity of *L. sativum* Extract against Tested Microorganisms: The results in **Fig. 2** indicate that the concentrations 10, 20 and 30% of *L. sativum* extracts were active against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. The methanolic extract of *L. sativum* exhibited the highest zone of inhibition against *E. coli* (19.5 mm) at a concentration of 10%, followed by *P. aeruginosa* (10.0 mm) at a concentration of 30%; *S. aureus* (8.0 mm) at concentrations of 10, 20 and 30%; and *B. subtilis* (8.0 mm) at a concentration of 30%.

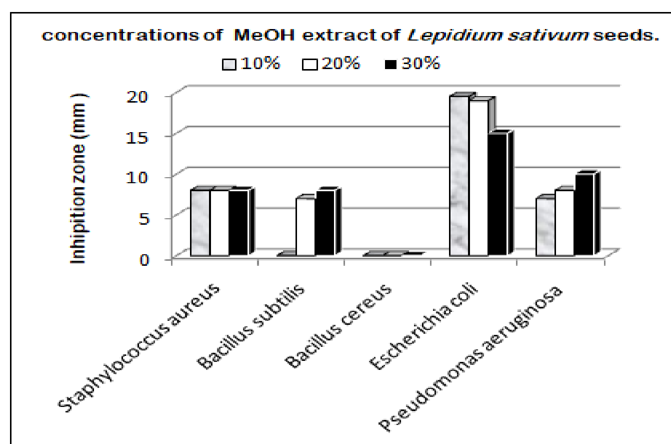

FIG. 2: ANTIBACTERIAL ACTIVITY OF (IN TERMS OF ZONE OF INHIBITION (IN MM DIAMETER) METHANOLIC EXTRACT OF *L. SATIVUM* SEEDS AT THE DIFFERENT CONCENTRATIONS OF 10, 20 AND 30% AGAINST FIVE PATHOGENIC ORGANISMS, *STAPHYLOCOCCUS AUREUS*, *BACILLUS SUBTILIS*, *BACILLUS CEREUS*, ESCHERICHIA *COLI* AND *PSEUDOMONAS AERUGINOSA*

Gupta *et al.*, [27] investigated the anti-microbial activity of *L. sativum* against food borne bacteria using methanol and performed their evaluations using the agar well diffusion method. Rahimi *et al.*, [28] carried out *in-vivo* studies on *L. sativum* for the treatment of inflammatory bowel disease (IBD). The ethanolic seed extract of this plant was found to be effective against IBD and possessed anti-inflammatory properties, whereas *in-vitro* studies on the *L. sativum* ethanolic seed extracts showed their effectiveness against Gram-positive and Gram-negative bacteria. The bioactive substances from this plant can, therefore, be employed as anti-microbial agents for the treatment of various bacterial and fungal infections, including gonorrhea, pneumonia, eye infections, and mycotic infections.

Phytochemical analysis of the seeds of *L. sativum* demonstrated the presence of alkaloids, flavonoids, tannins, sterols, triterpenes and glucosinolates [29, 30]. All of these compounds were recognised by their biological activity. Tannins bind with proteins rich in proline to form irreversible complexes [31], inhibiting protein synthesis within the cell. Medicinally, this effect is important in treating inflamed or ulcerated tissues [32]. Tannins are considered to be stable, potent antioxidants [33], used as an astringent for treating intestinal disorders, such as diarrhea and dysentery thus exhibiting an anti-microbial activity [34].

Antifungal Activity of the Methanolic Extract of *L. sativum*: Methanolic extract of *L. sativum* seeds in different concentrations (10, 20, and 30%) exhibited potent antifungal activity against four pathogenic fungi (*C. albicans*, *A. niger*, *A. flavus* and *Penicillium* sp.). The methanolic extract of *L. sativum* exhibited the largest zone of inhibition against *C. albicans*, *A. niger* and *A. flavus* (14.0 mm) at a concentration of 30% and against *Penicillium* sp. (14.0 mm) at a concentration of 20% **Fig. 3**. All concentrations of the extract inhibited fungal growth at different concentrations, with the inhibition zone diameter ranging from 10 to 14 mm across different fungal species. The results indicated that the methanolic extract of *L. sativum* seeds has strong anti-microbial activity.

Sharma *et al.*, [35] demonstrated the anti-fungal activity of the ethanolic extract of *L. sativum* seeds against *A. flavus*, *Alternaria alternata,* and *Fusarium equiseti*, using different concentrations of *L. sativum* seed extracts (2%-8%) in PDA. They concluded that fungal growth was inhibited at all

concentrations of the extract. These investigations showed that the seeds of *L. sativum* have strong antimicrobial activity [36].

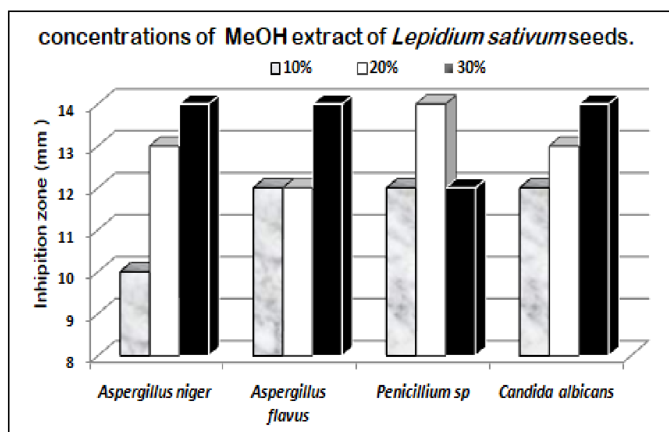

FIG. 3: ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF *L. SATIVUM* SEEDS IN DIFFERENT CONCENTRATIONS (10, 20 AND 30%) AGAINST 4 PATHOGENS FUNGUS (*CANDIDA ALBICANS, ASPERGILLUS NIGER, ASPERGILLUS FLAVUS,* AND *PENICILLIUM* SP.)

DPPH Radical Scavenging Activity of the Methanolic Extract of *L. sativum*: The data in **Fig. 4** demonstrated that the methanolic extract of *L. sativum* exhibited a maximum DPPH radical scavenging activity of 46.6, 80.4, 84.9 and 86.9%, respectively, at different concentrations of 500, 1000, 1500 and 2000 µg/mL, after 120 min in the dark, whereas the concentration of 2500 µg/mL showed the highest scavenging activity after 60 min (89.3%). These results clearly depict that the methanolic extract of *L. sativum* showed high DPPH radical scavenging activity. Owing to its hydrogen-donating capabilities, it can act as an anti-oxidant.

The scavenging activity might be due to the presence of total polyphenolic compounds, flavonoids, saponins, tannins, alkaloids, and terpenoids [37-38].

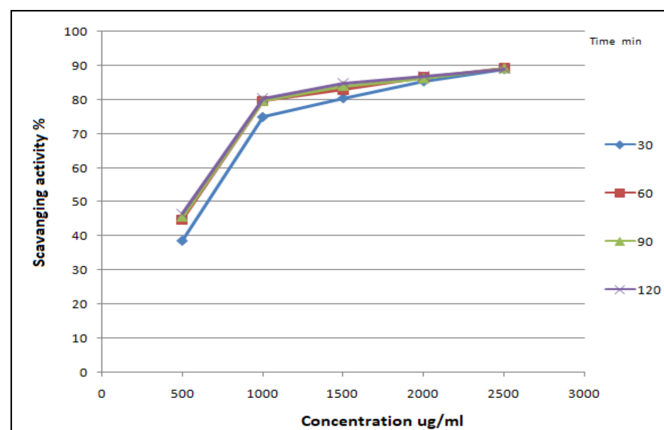

FIG. 4: THE % INHIBITION OF THE FREE RADICAL DPPH BY MeOH EXTRACT OF *L. SATIVUM* SEEDS

Antiulcerogenic Activity of the *L. sativum* Extract against HP-LPS-Induced Gastric Ulcer: Oral administration of 50 µg/kg of HP-LPS induced a significant (p<0.05) elevation in the degree of ulceration in rats. The ulcer index significantly decreased after the administration of *L. sativum* (1.8 ± 0.3) as compared to HP-LPS (6.3 ± 0.5). The percentage of ulcer inhibition in the *L. sativum* group (71%) was higher than in the rats treated with ranitidine (66%) but did not reach the level of significance **Fig. 5A** and **5B**, respectively.

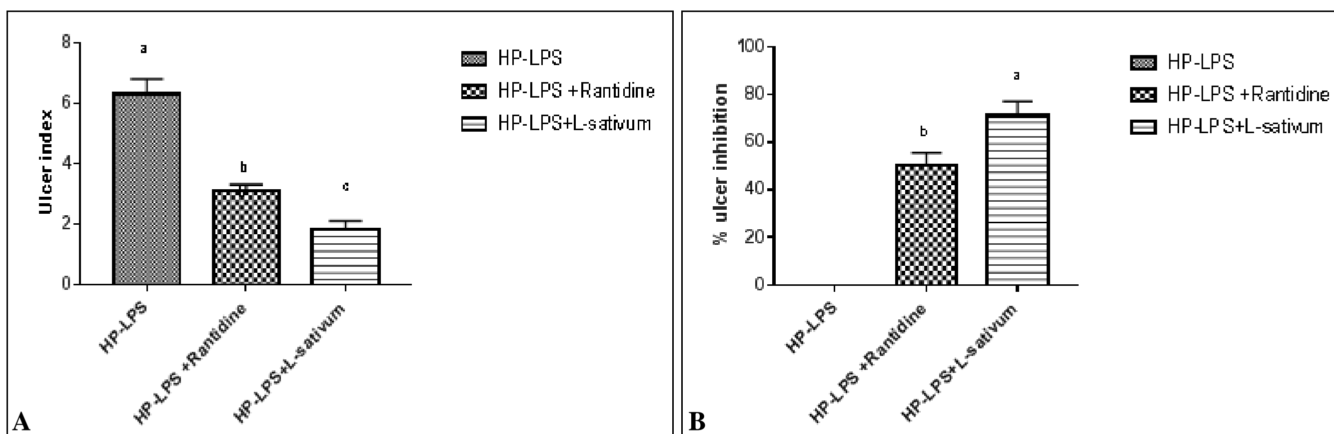

FIG. 5: EFFECT OF *L. SATIVUM* EXTRACT ON ULCER INDEX (A) AND PERCENTAGE OF INHIBITION (B) IN HP-LPS INDUCED GASTRIC INJURY IN RATS. (n = 10, X ± SEM). BARS WITH DIFFERENT SUPERSCRIPTS [a, b, c,] FOR THE PARAMETER ARE SIGNIFICANTLY DIFFERENT (p<0.05). HP-LPS: *HELICOBACTER PYLORI* LIPOPOLYSACCHARIDE: (50 µg/kg b.w.), RANITIDINE (20 mg/kg b.w.), *L. SATIVUM*: *LIPEDIUM SATIVUM* EXTRACT (400 mg/kg b.w)

Antioxidant Effect of the *L. sativum* Extract: HP-LPS treatment induced a significant elevation in oxidative stress, as evidenced by the significant elevation in the mucosal MDA level by 116% as

compared to the control group. Administering the *L. sativum* extract significantly attenuated the MDA level by 52% in comparison with HP-LPS-treated rats. Meanwhile, the mucosal anti-oxidant parameters (SOD, CAT, and GSH) were significantly reduced after HP-LPS ingestion by 64, 63 and 55%, respectively, as compared to the control rats. The administration of *L. sativum* significantly increased the mucosal level of SOD (120%), CAT (152%) and GSH (88%), as compared to HP-LPS-treated rats **Fig. 6**.

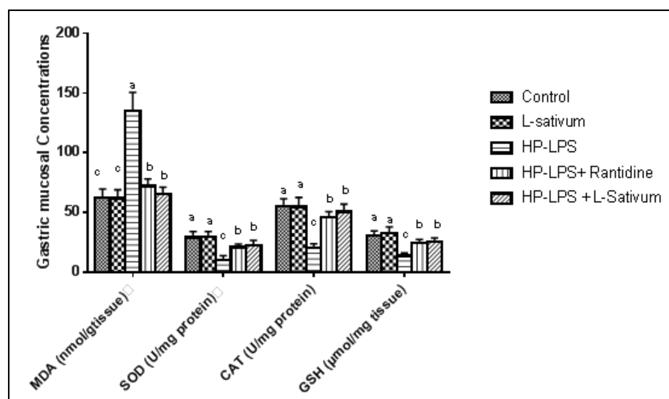

FIG. 6: EFFECT OF *L. SATIVUM* EXTRACT ON OXIDANT AND ANTIOXIDANT MARKERS IN HP-LPSs INDUCED GASTRIC INJURY IN RATS. MDA: MALONDIALDEHYDE, SOD: SUPEROXIDE DISMUTASE, CAT: CATALASE, GSH: REDUCED GLUTATHIONE. (n = 10, X ± SEM). BARS WITH DIFFERENT SUPERSCRIPTS [A, B, C], FOR THE PARAMETER ARE SIGNIFICANTLY DIFFERENT (p<0.05). HP-LPS: *HELICOBACTER PYLORI* LIPOPOLYSACCHARIDE: (50 µg/kg b.w.), RANITIDINE (20 mg/kg b.w.), *L. SATIVUM*: *LIPEDIUM SATIVUM* EXTRACT (400 mg/kg b.w)

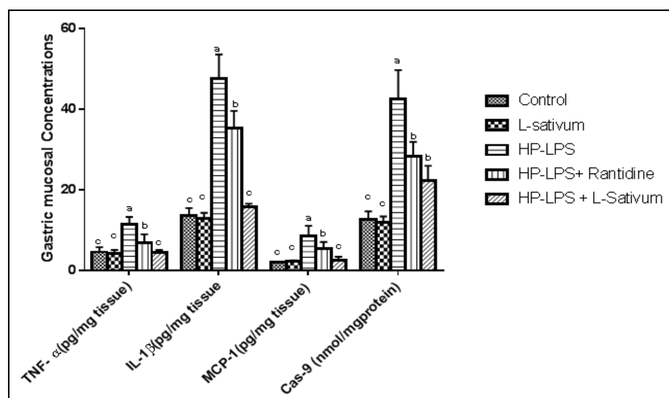

FIG. 7: EFFECT OF *L. SATIVUM* EXTRACT ON INFLAMMATORY CYTOKINES AND APOPTOTIC MARKER IN HP-LPSs INDUCED GASTRIC INJURY IN RATS. TNF-α: TUMOR NECROSIS FACTOR ALPHA, IL-1B: INTERLEUKIN 1-BETA, MCP-1: MONOCYTE CHEMO-ATTRACTANT PROTEIN, Cas-9: CASPASE 9. (n = 10, X ± SEM). BARS WITH DIFFERENT SUPERSCRIPTS [a, b, c], FOR THE PARAMETER ARE SIGNIFICANTLY DIFFERENT (p<0.05). HP-LPS: *HELICOBACTER PYLORI* LIPO-POLYSACCHARIDE: (50 µg/kg b.w.), RANITIDINE (20 mg/kg b.w.), *L. SATIVUM: LIPEDIUM SATIVUM* EXTRACT (400 mg/kg b.w)

Antiinflammatory and Antiapoptotic Effect of the *L. sativum* Extract: An upsurge of 153, 250, 347 and 240% in the TNF-α, IL-1β, MCP-1, and caspase-9 gastric mucosal levels, respectively, was observed after HP-LPS treatment, as compared to the control group. Rats treated with the *L. sativum* extract showed a significant reduction in the aforementioned markers by 62, 67, 71 and 48% in the same respect, as compared to HP-LPS-treated rats **Fig. 7**.

Histopathological Investigation: The gastric mucosa of normal control rats and *L. sativum*-treated rats showed normal histological structures **Fig. 8A** and **8B**. However, those of HP-LPS-treated rats indicated areas of ulcer represented by a necrotic surface (arrow). The lamina propria infiltrated by extravagated erythrocytes and inflammatory cells **Fig. 8C**. Rats treated with ranitidine showed regenerative attempts of enterocytes with activated enterocytes (arrow), whereas their muscular layers exhibited hyalinisation (star) **Fig. 8D**. The gastric mucosa of *L. sativum* treated rats showed amelioration of epithelial erosion, restoration of enterocytes and an intact mucosal layer **Fig. 8E**.

H. pylori infect about 50% of the world's population and induce various pathologic alterations in the gut, including atrophic gastritis, peptic ulcer and gastric adenocarcinoma [53-54].

H. pylori weakens the mucosal barrier of the stomach by releasing the toxin VacA, cytokines and gastrin, thereby loosening the protective mucous layer, disrupting the mucus layer and altering the mucous glycoproteins [39]. The ulceration induced by *H. pylori* may be attributed primarily to the release of cytotoxins coupled with free radical formation, in addition to inflammatory and apoptotic responses.

These observations are consistent with the results that were obtained in the present study, where the intragastric administration of *H. pylori* triggered the ulcer index, oxidative stress and inflammatory and apoptotic responses, as indicated by the elevated levels of lipid peroxides and mucosal TNF-α, IL-1β, MCP-1 and caspase-9 associated with depletion in mucosal anti-oxidants SOD, CAT and GSH.

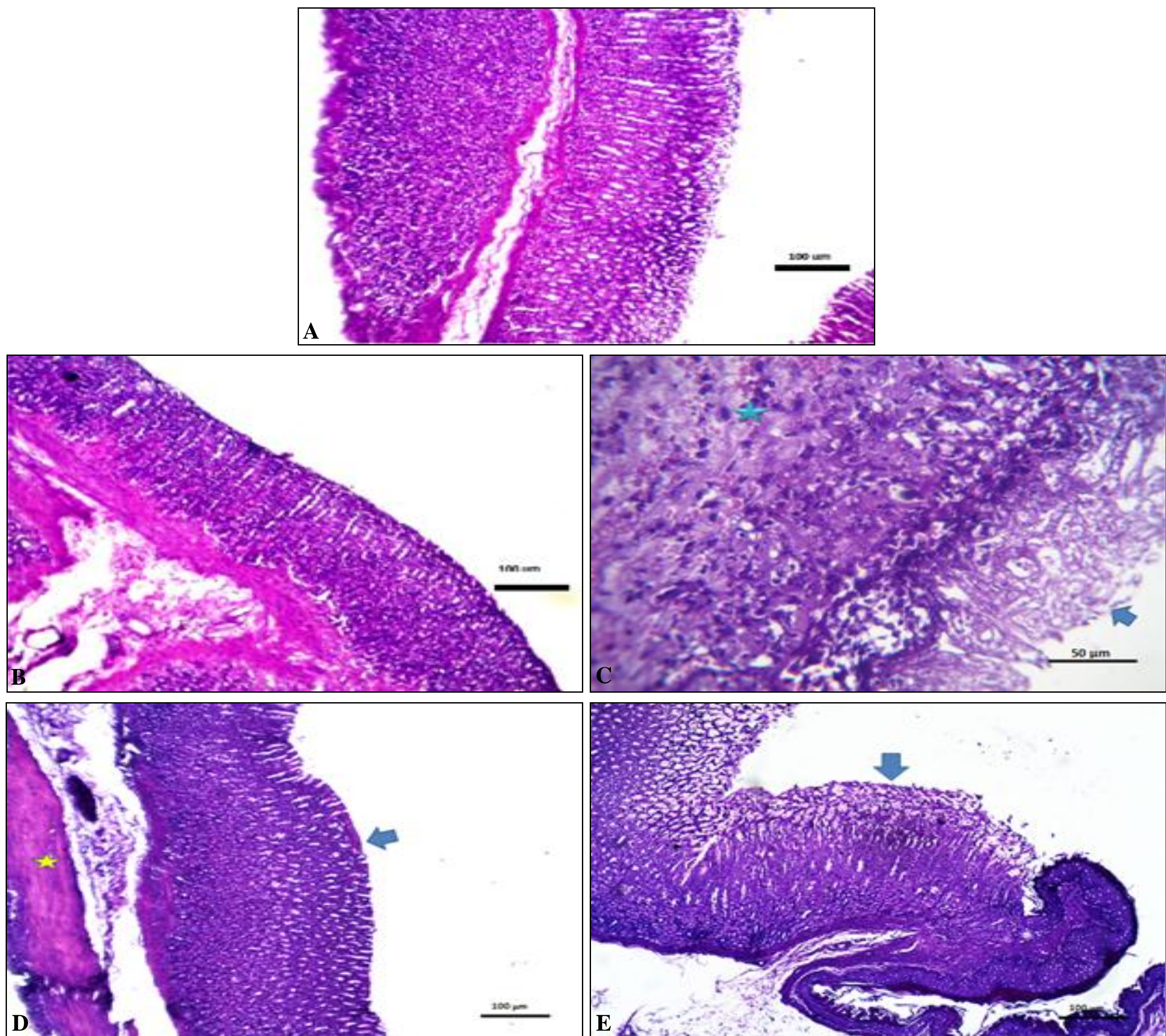

FIG. 8: HISTOPATHOLOGICAL INVESTIGATION OF HEMATOXYLIN AND EOSIN STAINING OF THE GASTRIC MUCOSA SECTIONS OF GASTRIC MUCOSA OF NORMAL CONTROL RATS AND *L. SATIVUM TREATED* RATS (A & B): SHOWING NORMAL HISTOLOGICAL STRUCTURES. SECTIONS OF GASTRIC MUCOSA OF HP-LPS -TREATED RATS (C): INDICATING AREAS OF ULCER REPRESENTED BY NECROTIC SURFACE (ARROW). THE LAMINA PROPRIA INFILTRATED BY EXTRAVASATED ERYTHROCYTES AND INFLAMMATORY CELL. *L. SATIVUM* (STAR). SECTIONS OF GASTRIC MUCOSA OF RANITIDINE - TREATED RATS (D): SHOWING REGENERATIVE ATTEMPTS OF ENTEROCYTES WITH ACTIVATED ENTEROCYTES (ARROW) WHILE THEIR MUSCULAR LAYERS HAVE HYALINIZATION (STAR). SECTIONS OF GASTRIC MUCOSA OF *L. SATIVUM* EXTRACT (E): SHOWING AMELIORATION OF EPITHELIAL EROSION, RESTORATION OF ENTEROCYTES AND INTACT MUCOSAL LAYER

H. pylori colonize the stomach, inducing inflammation, which triggers the G-cells of the antrum, secreting the gastrin hormone, which reaches the parietal cells of the fundus through the bloodstream. This hormone triggers excess acid secretion from the parietal cells and increases the number of parietal cells. Any upsurge in the acid load damages the epithelial cells of the duodenum, inducing ulcers [40]. Moreover, oxidative stress is implicated in altering epithelial proliferation, increasing apoptosis, and augmenting the DNA damage associated with *H. pylori* infection, as proven by the increased reactive oxygen species (ROS) levels in the patients' mucosae [41]. ROS-releasing phagocytic cells conscripted to the gastric mucosa during infection characterize one clear source of oxidative stress [42]. It has been stated in several studies that *H. pylori* induce ROS, which accumulates in the gastric epithelial cells [43]. Furthermore, proinflammatory cytokines trigger

ROS in different types of cells [44]. The reduced level of ascorbic acid, which is related to *H. pylori* infection [45], contributes to the pro-oxidative environment. *H. pylori* infection increases spermine oxidase expression and activity, which oxidizes the polyamines found in epithelial cells to produce hydrogen peroxide [46]. In addition, *H. pylori* infection induces pro-inflammatory cytokines, thus inducing inflammation at the site of infection. The pathogen first enters the host cell, and its peptidoglycan signals the intra-cellular pathogen receptor Nod1, which activates NF-κβ and AP-1 to produce cytokines (*e.g.* ILs). IL-1β, IL-6, IL-8, TNF-α, and RANTES thus upregulate inflammatory molecules in the host stomach [47]. *H. pylori* infection activates the mechanism of apoptosis *via* mediating the insertion of the VacA toxin into the mitochondrial membrane, which releases cytochrome *c*, which in turn causes caspase-dependent cell death [48].

In the current study, *L. sativum* extract was found to exert significant protection against HP-LPS-induced gastric insults, mainly through suppressing pro-inflammatory cytokines, combating ROS and downregulating apoptotic markers. The gastro-protective effects of the *L. sativum* extract surpassed those exerted by the reference drug ranitidine.

The *L. sativum* extract reduces the value of the lesion index and increases the ulcer inhibition percentage as compared to the control group, suggesting its potent gastroprotective effect against HP-LPS challenge. It has been documented in previous studies that the gastroprotective effect of *L. sativum* is attributed to anti-secretory potency as well as acid-neutralizing effect [49]. Moreover, *L. sativum* stimulates regional blood flow and induces vasodilation [50]. Gastric blood flow has significant importance in the protection and curing of gastric mucosa, as it provides oxygen and bicarbonates to the mucosa and eliminates carbon dioxide, hydrogen ions and toxic agents diffusing from the gastric lumen [51].

The data from the current study demonstrated that the *L. sativum* extract combats oxidative stress and enhances the antioxidant capacity in rats with HP-LPS gastritis, as revealed by the significant reduction in the lipid peroxidation level, in addition to the upregulation of the anti-oxidant concentrations. These results are in accordance with previous studies [52]. *L. sativum* showed a significant antioxidant activity by inhibiting hydroxyl radical, superoxide anion scavenging, nitric oxide, and hydrogen peroxide scavenging activities. The extract has an anti-oxidant activity that may be attributed to the polyphenolic compounds and isoflavonoids that are applied in reducing oxidative stress [53]. These observations indicated that the *L. sativum* extract possesses considerable protective anti-oxidant potential, thereby sparing endogenous anti-oxidants.

The administration of the *L. sativum* extract attenuated the mucosal levels of the pro-inflammatory cytokines TNF-α, IL-1β, MCP-1, and apoptotic marker caspase-9. It has been reported in a previous study [54] that *L. sativum* exhibits potent anti-inflammatory and anti-apoptotic impact manifested by a depression in cytokine (TNF-α, IL-6) levels and downregulation of stress gene (iNOS and HO-1) mRNA expression associated with the upregulation of IL-10 [50]. In addition, the administration of *L. sativum* significantly suppressed the apoptotic protein caspase-9 and upregulated Bcl-2 protein expression, preventing apoptosis. The activity of *L. sativum* may be due to its glucosinolate, flavonoid and natural sterol content. Flavonoids are a potent anti-oxidant, anti-inflammatory, and anti-apoptotic agent that acts by blocking ROS-mediated MAPK stimulation [55] and downregulating cytokines such as TNF-α and IL-6 [53]. Natural sterols act by blocking the expression of MyD88 and VCAM-1 and reducing the production of IL-1β, IL-6, TNF-α and NF-κB [56]. These findings indicate that *L. sativum* ameliorates gastric injuries and pathological damage through combating oxidative stress, inflammation, and apoptosis in HP-LPS-induced mucosal damage.

CONCLUSION: In conclusion, this integrated study using *in-vitro* and *in-vivo* models indicated that the *L. sativum* extract has potent anti-microbial, anti-ulcerogenic and cytoprotective effects by widely affecting several pathways involved in this process. Its pleiotropic effects on oxidative stress, inflammation and apoptosis regulation further suggest that it could represent a potentially effective agent in gastric ulcer therapy.

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