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Assessing the Protective Effects of PC-1 Formulation on Ethanol-Induced Gastric Ulcer Model in Wistar Rats: A Comprehensive Study

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ABSTRACT

The PC-1 formulation, renowned for its diverse biological properties, has yet to be thoroughly examined for its effects on stomach ulceration. Acute toxicity assessment of a lower dose (1000 mg/kg) of PC-1 formulation did not exhibit any observable signs of toxicity, suggesting its potential as protective mediator in contrast to stomach epithelial mutilation. The omeprazole group received 30 mg/kg omeprazole orally. The investigational groups were orally gavage with PC-1 extract formulation at dosage 50 mg/kg, 100 mg/kg, and 200 mg/kg in (1%) CMC. Subsequently, after an hour, the normal group received normal water via gavage, while groups 2–6 were administered absolute ethanol via gavage. Treatment with PC-1 extract formulation significantly mitigated ethanol-induced gastric injuries, as demonstrated through improved gastric mucus secretion, pH levels, reduced ulceration size, and decreased infiltration of leukocytes in submucosal layer. Analysis of stomach epithelial homogenate revealed a significant upsurge in the superoxide dismutase, catalase, and glutathione, along with a notable decrease in malondialdehyde (MDA) levels upon treatment with PC-1 formulation. The observed gastroprotective effects of PC-1 formulation could be attributed to its capacity to improve pH and mucus secretion, elevate SOD, GSH, and CAT levels, while reducing MDA levels.

Keywords: Curcumin, Ethanol, Gastric mucosal injury, Piperine, Omeprazole.

INTRODUCTION

Peptic ulcer disease (PUD) is characterized by the presence of lesions in the gastrointestinal system, which mostly affects the proximal duodenum and the stomach wall. These lesions, which are sometimes referred to as stomach or peptic ulcers, are erosions that have penetrated the epithelium and reached deeper tissues. Rarely, PUD might also have an impact on the lower esophagus[1]. Based on epidemiological research, PUD appears to be one of the most common gastrointestinal illnesses globally. Although improvements in diagnosis and treatment have lessened its overall effect, there are still worries

about its considerable morbidity and in particular circumstances, possibly excessive mortality[2]. Although the causes of gastric ulcers (GUs) and duodenal ulcers (DUs) are exactly unknown, it is believed that an inequality between stomach mucosal invasive and protective elements primes to the collapse of the protective barrier of mucosa and the expansion of ulcers[3,4]. The animal model of ethanol-induced gastric ulcer proves to be a valuable instrument in exploring the pathophysiology and potential therapeutic approaches for acute gastritis in research investigations[5]. The build-up levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to a state of oxidative stress and tenderness within the gastric stomach tissue, ultimately leading to detrimental effects on the gastric mucosa[6]. Elevated reactive oxygen and nitrogen species (ROS/RNS) levels contribute to oxidative stress, inducing lipid and protein oxidation within the intestinal mucosa. This compromises barrier function, leading to increased permeability. Additionally, stimulated macrophages amplify the inflammatory response by releasing interleukin-6, Tumor necrosis factor-alpha, further activating NF-kB signaling trail[7]. In resource-limited settings, a significant proportion of the population relies on herbal remedies as a primary source of healthcare to address diverse health concerns[8]. According to World Health Organization, an estimated 80% of the African population utilizes natural products for primary healthcare needs. This widespread reliance is attributed to several factors, including affordability compared to conventional medicines, accessibility within local communities, and perceived lower prevalence of adverse effects[9,10]. Approximately 25% of modern drugs listed in pharmacopeias and numerous synthetic analogs trace their origins to natural products, including herbal remedies[11].

Curcumin, the primary curcuminoid found in turmeric *(Curcuma longa)*, is a bioactive dietary polyphenol with the chemical name diferuloylmethane. Widely used as a spice in Indian cuisine and other Middle Eastern and South Asian countries, turmeric possesses curcumin as its major bioactive component[12,13]. Due to its diverse molecular targets, curcumin demonstrates promising therapeutic potential in clinical studies for a multitude of enduring sicknesses, encompassing respiratory syndromes, numerous malignances, autoimmune conditions[14]. curcumin's potential antidepressant effects, recent meta-analysis identified overexpression of inflammatory biomarkers, like cytokines key contributors to major depression development. Additionally, studies have demonstrated curcumin's potential to alleviate these depressive symptoms[15]. The potent antioxidant activity of curcumin is hypothesized to be a key component of its beneficial effects in various disease states[16].

Piper nigrum has a long-standing history of use in traditional Chinese therapy for addressing diverse ailments, including gastrointestinal distress, chills, muscle aches, and earaches[17,18]. *Piper nigrum* (black pepper) possesses a diverse biochemical profile, containing volatile oils, alkaloids (including the major bioactive component piperine), polyphenols, starch, and terpenes. Notably, piperine displays extensive band of biological events such as antioxidant, immunomodulatory, anticonvulsant, anti-tumor, as well as hypolipidemic properties[19,20,21,22,23,24].

MATERIALS AND METHODS

Preparation of PC-1 formulation

As previously studied disclosed that Piperine (20 mg/kg p.o.) administered alongside through curcumin (2000 mg/kg p.o.) which significantly boost bioavailability of concluding by upto 20 times, then the Piperine extract, and Curcumin extract are mixed in the ratio 1:10[37,38].

Drugs, Chemicals, and Equipment

In this study, All the chemicals and drugs were utilized: Piperine extract, Curcumin extract, absolute ethanol, phenolphthalein, omeprazole, ketamine hydrochloride, normal saline, Wagner's reagent, lead acetate 1%, ferric chloride, NaOH, sulfuric acid, hand lens (10x). All the chemicals and drugs supplies were of pure in form and of analytical grade.

Animal used

This study adhered to the ethical guidelines established by Institutional Animal Ethics Committee, with approval granted for protocol IAEC/KSOP/2023-24/11. Male albino Wistar rats (10-12 weeks, 200-250 g) were obtained from the Animal House at KSOP, Ghaziabad. Housing occurred in polypropylene cages with a maximum of four rats per cage. Standard laboratory conditions were maintained (temperature: 25±2°C, humidity:30-70%, light/dark cycle:12 hours). Animals must ad libitum

access to standard rodent chow, tap water. Cage card labels and tail markings facilitated individual identification. Prior to experimentation, rats underwent a 7-day acclimatization period to the animal house environment.

Studies on acute oral toxicity.

In accordance with OECD guidelines 423 (2001), a study on acute oral toxicity was conducted, involving each group of three animals. Oral administration was employed for the formulations exhibiting the greatest stability at doses of 2000 mg/kg body weight (BW). Animal behavior was monitored at 3, 24, and 48-h post-administration, with observations focused on alterations in behavior, skin, fur, eyes, and presence of tremors.

Animal grouping and dosing

36 male Albino Wistar rats of certain weighing between 200-250 g were randomly allocated to six groups set each composed of 6 animals as depicted in Table 1. Doses for the test extract were selected according to the findings of acute toxicity study, doses were administered daily for one week.

Table 1: Design of the experiment

Groups	Schedule of treatment
Group No. I (6 animals))	Sham Control group (10 mL/kg, DW)
Group No. II (6 animals)	Ethanol (5 mL/kg)
Group No. III (6 animal)	Omeprazole (30 mg/kg,p.o. in 1% w/v CMC)
Group No. IV (6 animals)	PC1 (50 mg/kg p.o. in 1% w/v CMC) + Ethanol (5 mL/kg)
Group No. V (6 animals)	PC1 (100 mg/kg p.o. in 1% w/v CMC) + Ethanol (5 mL/kg)
Group No. VI (6 animals)	PC1 (200 mg/kg p.o. in 1% w/v CMC) + Ethanol(5 mL/kg)

Ethanol-induced gastric ulceration

In each experimental group, rats underwent a seven-day pre-treatment regimen, as detailed earlier, followed by a 24-h fasting period on the eighth day. Ulceration was provoked on the eighth day by administering absolute ethanol (1 mL/250 g) to the animals[25,26]. One hour after following the induction of ulcer, animals were euthanized through the cervical dislocation in a humane manner. In order to inspect their stomachs for ulcers, the animals underwent a macroscopic dissection. Ulcer evaluation was performed following a methodology described in a prior research study.

Assessment of Volume of gastric juice, overall acidity, pH level, and content of gastric mucus

Following the Examination and analysis, extraction of tissue stomach, gastric juice was obtained. Subsequently, the gastric juice was subjected to centrifugation at 3000×g for 5 minutes, resulting in separation of the liquid above the sediment. The liquid remaining after centrifugation (supernatant) was tested for its acidity, volume, and how much acid it contained (total acidity). The determination of total acidity involved titration with (0.01N) NaOH solution using Topfer's reagent, a pH indicator, and results were articulated in mEq/l[27]. Gastric mucus composition were evaluated employing a technique modified from the methodology described in Corne *et al.,*[28,29], appropriate modifications. The stomach were extract out, dissected along the greater curvature, flushed tissue with chilled saline solution.

Histological evaluation

The specimens of gastric mucosa wall preserved in 10% formalin solution., managed routinely additionally, the specimens were entrenched in alkane series paraffin wax. Subsequently, segments through a thickness of 5 µm were organized and stained using hematoxylin and eosin (H&E) evaluation of histlogy[30]. Additionally, periodic acid-Schiff staining were performed to recognize glycoprotein localization (magenta staining). Pathological changes associated with the ethanol-induced injury encompassed inflammatory intrusion, erosive lesions, hemorrhage, and ulceration, were assessed histological evaluation was performed, utilizing predefined injury index[31].

The degree of inflammatory cell infiltration was categorized into four categories: 0 (none), 1 (limited to below the lamina propria), 2 (invasion of half the mucosa), 3 (Infiltrating the epithelial glandular layer of the mucosa). The percentage of the damaged mucosa was used to determine the erosion score: 0 indicates no erosion, 1 indicates loss of the epithelial glandular layer of the mucosa, 2 indicates abrasion impacting two-thirds of the mucosa epithelium, and 3 Indicates abrasion that impacts the entirety of the mucosal lining.

Biochemical investigation

Measurement of antioxidant parameters

To explore the antioxidative defense

strategies in stomach tissues, the activities of SOD and CAT were assessed. Additionally, levels of glutathione and malondialdehyde (MDA) evaluated as symbols of oxidative stress and damage, respectively. Stomach tissue were homogenized in a (10%w/v, pH 7.4) phosphate buffer solution by using Hei Dolph Silent Crusher S homogenizer. The homogenate was then centrifuged at 1500rpm for 10 min at 4°C to isolate the liquid left over(A1). This fraction (A1) was then utilized to quantify the measure of GSH and MDA levels. For further analysis, aliquots of A1 were ultracentrifuged at 10,000rpm for 10 min at 4°C. This results a second liquid left over (A2), which remained subjected to analysis of quantify's SOD and CAT antioxidant capacity. Protein concentration in both fractions (A1 and A2) was determined using the Bradford method with bovine serum albumin (BSA) which taken as the standard[32].

Determination of superoxide dismutase activity

The antioxidant superoxide dismutase (SOD) activity in the supernatant fractions was assessed using a quick and easy technique that employed pyrogallol auto-oxidation inhibition. The assay mixture comprised 100 µL of 15 mM pyrogallol solution {dissolved in (10 mM) HCl), 0.01 mL supernatant aliquots was taken, and 1.4 mL (50mM) Tris-HCl buffer containing (1mM, pH 8.2) EDTA, resulting in total volume of 1.5 mL. Superoxide dismutase SOD Activity Measurement by Monitoring Absorbance at 420nm for 3 min (Shimadzu UV-1800 spectrophotometer). SOD activity was defined as the enzyme amount causing a 50% reduction in reaction rate compared to an enzyme-free control. Precise activity were then expressed as units per minute per milligram of protein (U/min/mg protein)[33].

Catalase Measurement

Catalase was determined spectrophotometrically through monitoring the consumption of hydrogen peroxide (H_2O_2) absorbance at 240nm over a one minute at 25°C. The total volume of blend delimited 0.37 mL of (0.1M, pH 6.4) potassium phosphate buffer, 1.5 mL (36Mm) H_2O_2, and 15 µL of the A2 fraction (supernatant after ultracentrifugation). One unit (U) catalase was described as the amount of enzyme which catalyzing the decomposition of 1 µmol.H_2O_2 U/mg protein/min[34].

Measurement of Glutathione Content

Stomach tissue samples were blended in 6 mL of ice-cold solution 0.02 M EDTA. An aliquot (2 mL) of blended tissue assorted to 2 mL in deionized water, add 1.5mL (50%w/v) TCA to precipitate proteins. The mixture was shaken intermittently for 12 min at room temperature, also centrifugated at 3,000rpm for 12-15 minute. To collected supernatant (1 mL), 3 mL (0.4 M) (pH 8.4) Tris-HCl buffer, 0.2 mL (0.01M) DTNB were added. The reaction mixture were incubated for 5 minute. at room temperature and absorbance were noted at 410nm against blank[35].

Determination of malondialdehyde content

Peroxidation of lipid within the gastric mucosa might contribute to evaluated employing the TBARS assay. Briefly, 0.1 mL gastric homogenate blended with 0.75 mL sodium dodecyl sulfate (8.1%), 0.75 mL of acetic acid (20%), and 1 mL TBA (0.8%) solution. This mixture remained incubated at 95°C, 1 h in water bath. Cooling after, 0.5 mL DW and 3.2 mL n-butanol-pyridine, followed by dynamic shaking for 60 seconds then centrifugation 4,000rpm to 10-12 minute. TBARS levels were concluded from a standard curve[36].

Statistical data analysis

The outcomes displayed the average value with standard error mean (SEM). Statistical evaluations remained conducted employing GraphPad software version 5.0. Mean comparisons were executed through analysis of one way, the significance flanked by groups were assessed using Dunnett's test. Implication remained established at $p<0.05$.

RESULT

Acute toxicity observations

Assessment of acute toxicity test demonstrated that administering a dosage of 2000 mg/kg of PC-1 extract preparation orally to three Wistar rats resulted in observable indication of toxicity, also mortality perceived within the period of 14-day observation. The rats were administered PC-1 extract at dosage of 500 mg/kg,1000 mg/kg and observed for duration period-14 days. Thus, we see that two animals are dead as depicted in Table 2. So, the estimated LD_{50} is said to be 1000 mg/

kg. At dose of 500 mg/kg and observed for period of 14 days, throughout this period rats exhibited normal activity levels, and there were no apparent signs of hepatotoxicity, behavioral toxicity or nephrotoxicity. Furthermore, there were no clear indications of toxicity observed in any of the experimental groups.

Table 2: Mortality Data of Acute Oral Toxicity of rats treated with different dose levels of PC-1

Group	No. of animal Dosed	Dosing (mg/Kg)	Mortality Rates (%)	Deaths (N)	Survivals (N)
1	3	500	0%	0	3
2	3	1000	66.66%	2	1
3	3	2000	100%	3	0

Impact of PC-1 Extract Formulation on the macroscopic assessment of gastric stomach

The findings of study indicate the histopathological changes at 500 mg/kg and 1000 mg/kg in various organ kidney, liver and heart, as depicted in the below Figure 1.

At dosage of 500 mg/kg

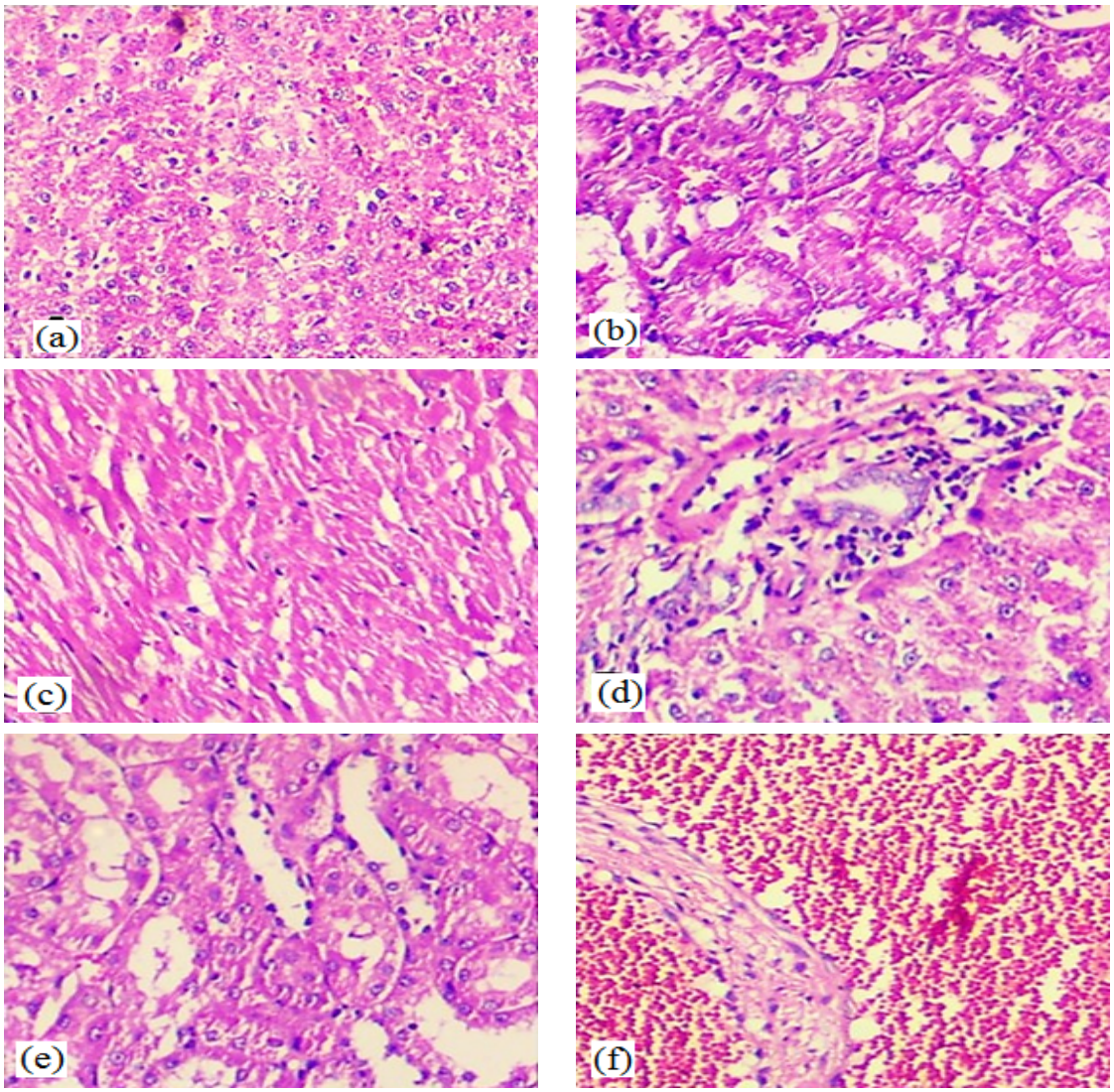

Fig. 1. Histological sections of rats' kidney, liver, and heart were scrutinized for acute toxicity. Slides A and D depict liver tissue, while slides B and E display kidney tissues, and slides C and F present heart tissues. No structural changes variation will be pragmatic among treated clusters and the control set administered 500 mg/kg

At dosage of 1000 mg/kg

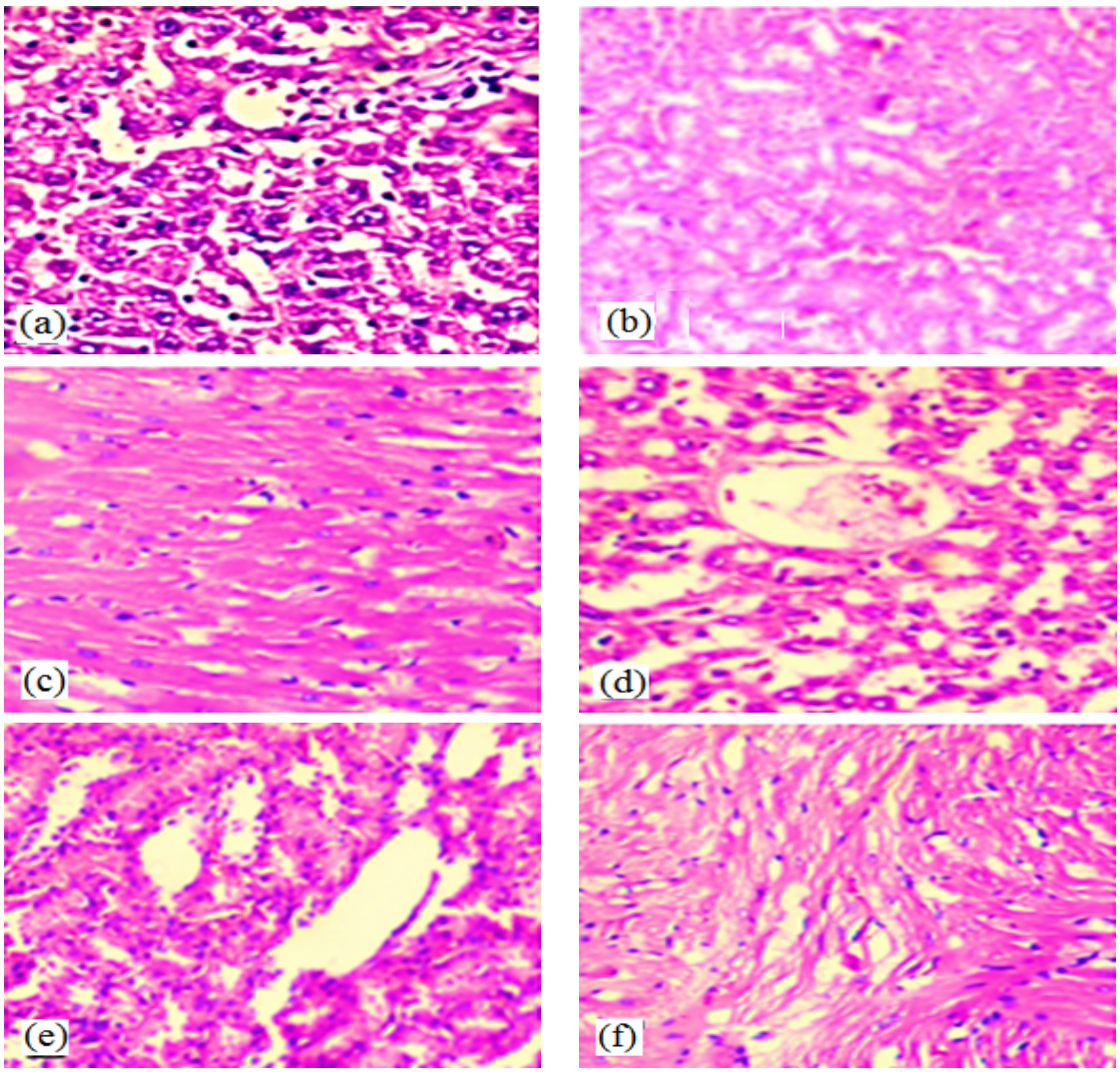

Fig. 2. Histological sections of rats' kidney, liver, and heart were scrutinized for acute toxicity. Slides A and D depict liver tissue which revealed severe hepatocellular necrosis, Congestion of sinusoids and hemorrhagic foci observed. Slides B and E display kidney tissues showed acute tubular necrosis, Loss of brush border in proximal tubule. Slides C and F present heart tissues multifocal myocardial necrosis, Inflammatory cell infiltration

Impact of PC-1 Extract Formulation on the macroscopic assessment of gastric stomach

The findings of study indicate the induction of gastric ulcer using pure ethanol in rats leads to significant stomach damage and severe gastric lesions, as depicted in the below figure. It is apparent, rats treated with Omeprazole and PC-1 extract Formulation exhibit reduced gastric lesions compared to untreated rats, as illustrated in the given below Figure 3.

Impact of PC-1 Formulation on content of gastric mucus

There is substantial boost in mucus content within PC-1 Formulation treatment group associated to diseased group. Notably, ethanol-induced ulceration itself significantly reduced mucus production in diseased control group. Mucus weight in the ulcer Disease control group (0.84 ± 0.03 g) existed statistically lesser compared to the normal, the Omeprazole group, and the groups receiving PC-1 Formulation by 50 mg/kg,100 mg/kg and 200 mg/kg. These latter groups displayed mucus weights of 2.08 ± 0.02 g, 1.12 ± 0.02 g, 1.58 ± 0.03 g, and 1.92 ± 0.05 g, respectively.

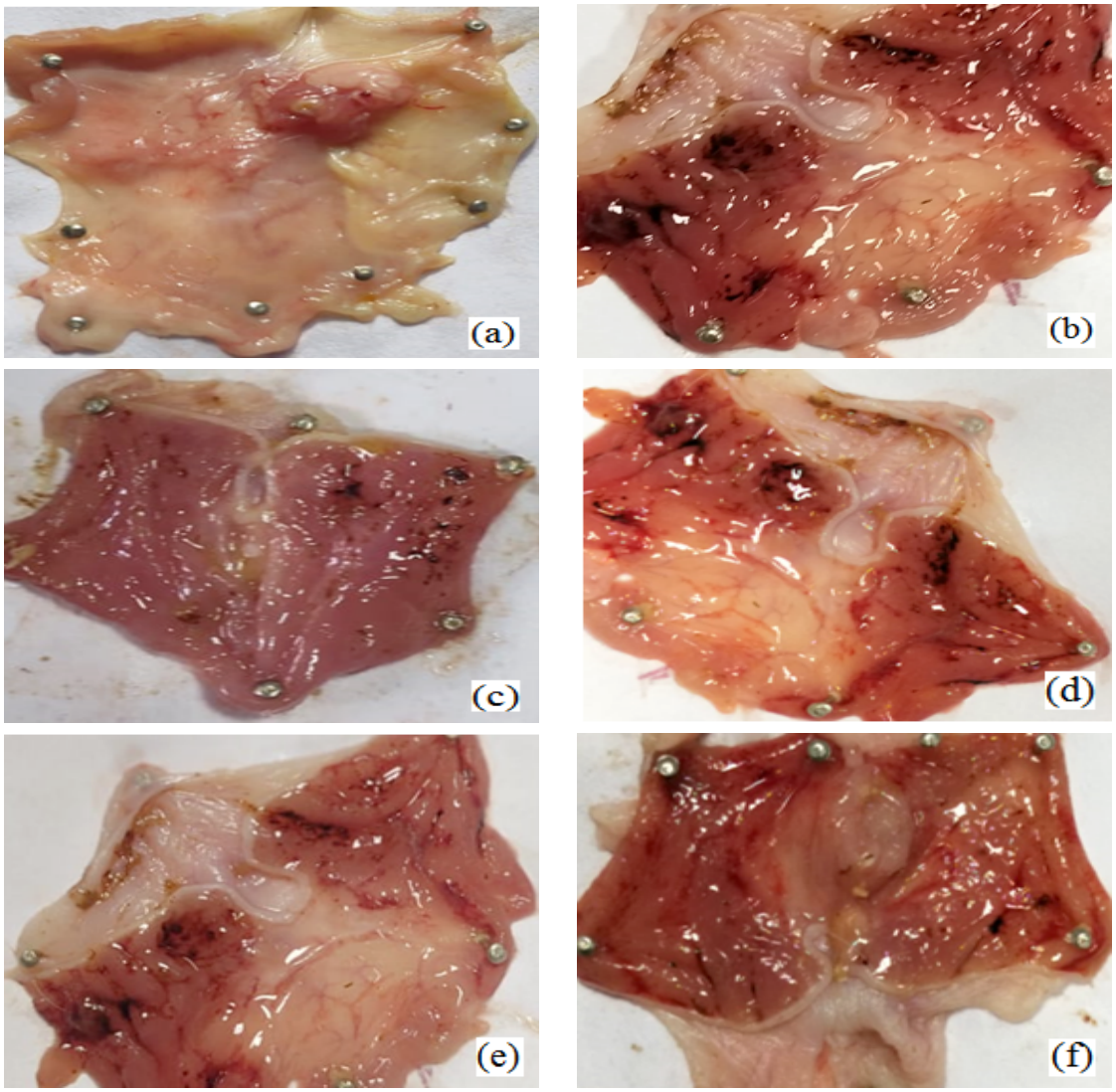

Fig. 3. The impact regarding PC-1 extract formulation on the macroscopic manifestation of ethanol-induced stomach damage was evaluated. Group No. (a) normal control (10 mL/kg, dist. water) represents intact surface of gastric epithelial layer. Group No. (b) Diseased control (5 mL/kg, ethanol) represents hemorrhagic scratches of stomach epithelium. Group No. (c) (30 mg/kg, omeprazole) exhibits minor harm to the mucosal layer of stomach. Group No. (d) (50 mg/kg), Group No. (e) (100 mg/kg), and Group No. (f) (200 mg/kg) of PC-1 formulation illustrate treatment demonstrating a decline in gastric ulcers

Table 3: Analysis of stomach pH, mucus weight and ulcer area inhibition

Group	Treatment	pH (Acidity)	Mucus Content (gram)	%Inhibition
1	Normal Control (distilled water 10 mL/kg)	3.83 ± 0.03***	2.43 ± 0.10***	0
2	Diseased Control	2.10 ± 0.02	0.84 ± 0.03	0
3	Omeprazole (30 mg/kg)	5.27 ± 0.19***	2.08 ± 0.02***	66.92
4	PC 1 (50 mg/kg) + Ethanol (5 mL/kg)	4.18 ± 0.03***	1.12 ± 0.02**	39.23
5	PC 1 (100 mg/kg) + Ethanol (5 mL/kg)	4.65 ± 0.08***	1.58 ± 0.03***	56.43
6	PC 1(200 mg/kg) + Ethanol (5 mL/kg)	7.25 ± 0.26***	1.92 ± 0.05***	76.50

The standards were presented as Mean ± SEM. Means with differing superscripts indicate significant differences. Significance was determined p<0.001***

Impact of PC-1 Formulation on stomach pH

Analysis of stomachic mucus pH in rat rodent model revealed a significant association between pretreatment and gastric acidity. Pretreatment with

omeprazole and in PC-1 Formulation (50 mg/kg, 100 mg/kg, and 200 mg/kg) demonstrably reduced ulceration and gastric acid secretion. Consequently, the pH of gastric mucus in groups G1, G4, G5 and G6, G3 (3.83 ± 0.03, 4.18 ± 0.03, and 4.65 ± 0.08, 7.25 ± 0.26, 5.27 ± 0.19 respectively) drastically superior to the ulcer control (G2), which exhibited a pH of 2.10 ± 0.02.

The effect of PC-1 extracts Formulation on histology of the stomach

Histological analysis of vehicle treated group (Slide A, Fig. 4) revealed an unremarkable gastric architecture with an intact epithelial lining and well-preserved glandular structures. Conversely, the diseased control group (Slide B, Fig. 4) displayed significant disruption of the gastric mucosal integrity, characterized by pronounced epithelial desquamation (loss of surface mucous cells), cellular death (necrosis), vacuole formation, edematous infiltration, and dilated gastric glands. This mucosal injury was accompanied by an influx of inflammatory cells, including neutrophils and eosinophils.

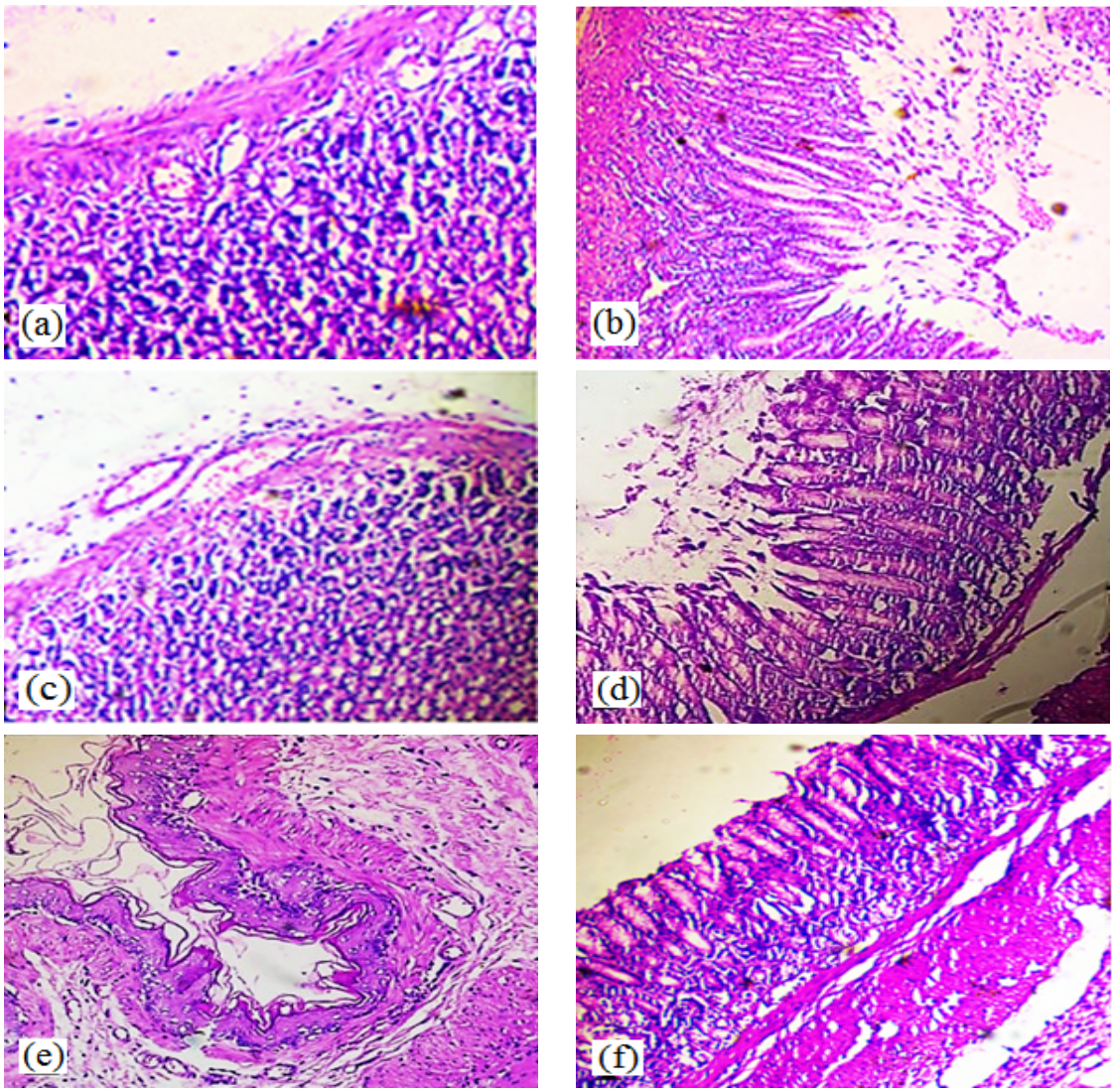

Fig. 4. The impact of the PC-1 Formulation on gastric histological integrity amidst ethanol-induced mucosal insults, assessed via 10 x H & E staining, is elucidated as follows. Slide A (Normal, 10 mL/kg dist. Water) exhibited preserved gastric epithelial architecture. Slide B (Ulcerated Control, 5 mL/kg ethanol) manifested extensive epithelial disruption, edema, and leukocytic infiltration within the submucosal layer. Slide C (Standard, 30 mg/kg omeprazole) demonstrated minor mucosal perturbations. Investigational groups slide D (50 mg/kg), slide E (100 mg/kg), and slide F (200 mg/kg) of PC-1 showcased a mitigated extent of gastric epithelial injury

Omeprazole pretreatment resulted in attenuated gastric gashes associated toward the ulcer diseased group. Gastric mucosa displayed isolated areas of seeming epithelial loss, with a relatively normal glandular appearance, trivial edema, regulated eosinophilic infiltration, and insignificant hemorrhagic activity (Slide C, Fig. 4). In contrast, pretreatment induced gastric abrasions embodied through focal disruptions roughly one-fourth of mucosal surface, lacking gastric layer of mucus in these affected areas. However, the remaining uninvolved mucosa displayed a near-normal glandular architecture, trivial edema, and imperfect eosinophilic infiltration (Fig. 4, Slide D, E and F), demonstrating a significant improvement related to Diseased group (Slide B, Figure 4).

Impact of PC-1 Extract Formulation on intrinsic antioxidants in gastric tissue homogenate

This study investigated the impact of PC-1 formulation on antioxidant enzyme functionality and indicators pertaining to oxidative concern markers in gastric stomach tissue following ethanol administration. SOD activity observed in ulcer control was suggestively lower associated with the vehicle control group (group receiving only the solvent used for the drug). Pre-treatment with both PC-1 formulation (200 mg/kg), also omeprazole (30 mg/kg) substantially reinstated SOD activity compared to ulcer control. Like SOD, CAT activity in ulcer control group stayed drastically minimal than vehicle control. Pre-treatment with PC-1 formulation (200 mg/kg), standard omeprazole group (30 mg/kg) drastically increased CAT activity compared to ulcer control. Glutathione (GSH) Activity the ulcer control group displayed a significant decrease in GSH activity related to the vehicle control. Pre-treatment with both PC-1 formulation (200 mg/kg) and omeprazole extensively reversed this depletion of GSH activity. Gastric MDA levels stayed pointedly privileged in ulcer control group associated to the vehicle control, indicating increased lipid peroxidation (damage to cell membranes). Pre-treatment with PC-1 formulation (200 mg/kg) and omeprazole significantly secure versus this destruction, leading to decreased MDA concentrations.

Table 4: Effect of PC-1 formulation and omeprazole on gastroprotective effects on gastric mucosal lining activity of GSH, SOD, and CAT enzymes

Group No	Groups	SOD(µ/mg)	CAT (µmol/L)	GSH (µmol/L)	MDA (nmol/g gastric tissue)
1	Normal Control (10 mL/kg, dist. Water)	28.77 ± 0.30***	29.47 ± 0.50***	77.51 ± 0.59***	7.28 ± 0.08***
2	Diseased Control (5 mL/kg, ethanol)	11.98 ± 0.28	14.92 ± 0.48	22.06 ± 0.24	21.95 ± 0.10
3	Standard (30 mg/kg, omeprazole)	22.89 ± 0.19***	27.14 ± 0.15***	43.41 ± 0.19**	9.69 ± 0.36***
4	PC-1 (50 mg/kg)	14.84 ± 0.26***	17.74 ± 0.26***	32.33 ± 0.19***	19.29 ± 0.24***
5	PC-1 (100 mg/kg)	17.85 ± 0.19***	21.16 ± 0.23***	45.63 ± 0.19***	13.22 ± 0.24***
6	PC-1 (200 mg/kg)	23.35 ± 0.25***	24.14 ± 0.27***	67.61 ± 0.23***	9.56 ± 0.05***

The standards were presented as Mean ± SEM. Means with differing superscripts indicate significant differences. Significance was determined p<0.001***

Moreover, it led to reduced levels of malondialdehyde (MDA) in comparison to the group with ulcers as the control. Impact of PC-1 formulation concerning about quantities of SOD, MDA, GSH, and CAT into gastric integrate tissue in rats exhibiting peptic ulcers induced by ethanol were assessed. GN.1 represents the normal group; while GN.2 denotes the ulcerated control group; as GN.3 signifies the omeprazole group; also GN.4 corresponds to rats administered with PC-1 extract at a dosage of 50 mg/kg; GN.5 indicates rats administered PC-1 formulation at dosage of 100 mg/kg; and GN.6 represents rats administered with PC-1 at dosage of 200 mg/kg. The presented p values denote statistical significance, with ***indicating p< 0.001.

Discussion

The ongoing study involved an oral acute toxicity test on experimental rats using the PC-1 formulation. The results indicated that there were no negative consequences noticed, including mortality, throughout the entire duration of the research, even at a lower dosage of 500 mg/kg PC-1. Furthermore, multiple investigations by various researchers using different medicinal ingredients also demonstrated no significant adverse effect. Recent studies have definitively shown that administering the PC-1 Formulation via oral gavage can effectively protect against ethanol-induced gastric ulcers. PC-1 shields the stomach lining from the harmful

effects of ethanol, a well-known cause of stomach ulcers that closely resemble in manifestation of keen gastric sores in individuals. Ethanol-induced gastric pustules are characterized by features such as extended hemorrhagic bands, sub-mucosal swelling, white blood cell infiltration, and damage to the mucosal tissue. Traditional herbal and Botanical remedies have been traditionally employed in ethnopharmacology for treating gastric ulcers. Numerous researchers have conducted extensive experiments highlighting the anti-ulcer properties of these natural remedies in rats. Inducing severe ulceration with ethanol through gavage is a common method for evaluating anti-ulcer effects, as pure ethanol directly affects the stomach lining, leading to tissue damage.

Rats administered absolute ethanol (5 mL/kg) displayed pronounced disturbances in gastric mucosa, characterized by evident hemorrhagic abrasions, epithelial extension, and subepithelial edema with infiltration of white blood cells. Remarkably, administration of PC-1 (200 mg/kg) demonstrated potential in mitigating gastric mucosal damage. The defensive efficacy of PC-1 was apparent in both gross observations and histopathological staining, exhibiting substantial lessening in lesions comparable to ulcer disease group. These findings align through previous studies utilizing various therapeutic botanical extracts, suggesting a consistent pattern of protective outcomes.

Stomach mucus secretion has a crucial part in the epithelial defense of the stomach, in conjunction with endogenous factors known for their aggressive properties, including enzymes, acids, and chemicals. The findings of study affirm that oral administration of PC-1 Formulation provides protection to the gastric epithelium by enhancing mucous secretion in response to extensive ethanol-induced damage. Notably, PC-1 has previously demonstrated significant antioxidant activity.

Elevated oxidative stress associated to heightened intracellular echelons of reactive oxygen species are integral particles in cellular homeostasis. Rats administered with PC-1 (200 mg/kg) formulation exhibited elevated pH levels and augmented mucus secretion in gastric contents, demonstrating gastroprotective effects comparable to the ulcer control group. Consistent with our findings, several researchers utilizing various medicinal plants have reported similar increases in gastric pH and mucus secretion.

The experimental outcomes demonstrated that rats administered with the PC-1 extract formulation exhibited notable increases in level of superoxide dismutase & level of glutathione, along with activity of catalase, coupled with a decrease in lipid peroxidation level as indicated by reduced malondialdehyde (MDA) levels. Similarly, previous studies utilizing diverse medicinal plants have reported enhancements in SOD and CAT activities along with reductions in MDA levels. Conversely, the ulcer control group demonstrated extensive hemorrhagic destruction in the histopathological examination of gastric epithelium mucosa layer, characterized by heightened leucocyte infiltration and submucosal edema.

Likewise, rats subjected to omeprazole, or the PC-1 Formulation exhibited gastroprotective effects. Results from this study revealed that rats administered with PC-1 exhibited increased staining intensity in periodic acid-Schiff (PAS) stained gastric tissue slices compared to the ulcerated control group. Consistently, several collaborators employing various therapeutic herbs have reported enhanced PAS staining intensity in experimental rats. These findings align with prior research indicating gastroprotective effects against ethanol-induced gastric ulceration by various therapeutic plants.

PC-1 exerts inhibitory effects leukotrienes, prostaglandins, and tumor necrosis factor-alpha, interleukins are examples of inflammatory mediators. Through the suppression of inflammation, curcumin mitigates tissue damage, facilitating ulcer healing. Additionally, PC-1 modulates cellular proliferation and apoptosis within ulcerated tissue, facilitating the elimination of compromised cells and the regeneration of healthy tissue.

CONCLUSION

In vivo experimentation demonstrated the gastroprotective efficacy of PC-1 formulation efficacy following induction of gastric lesions by absolute ethanol-induced animal models. This effect was substantiated by both macroscopic

and histological evaluations. PC-1 administration resulted in a significant elevation of gastric mucus secretion and gastric luminal pH, while concurrently attenuating submucosal edema and inflammatory cell infiltration within the gastric wall. Furthermore, PC-1 treatment led to a marked significant elevation in activities of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT), also in glutathione (GSH), within gastric homogenates. Conversely, notable reduction in malondialdehyde (MDA) levels were noted. These protective effects are likely attributable to the antioxidant and anti-inflammatory properties of PC-1, which may act by scavenging free radicals, quenching singlet oxygen formation, and consequently mitigating oxidative stress provoked by absolute ethanol in the stomach. This, in turn, could promote gastric mucosal regeneration and dampen inflammatory processes.

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Conflict of Interest

All authors declare that they have no conflict of interest concerning this article.

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