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ORIGINAL ARTICLE

# Synthesis, spectral characterization and biological activity of Zn(II) complex with 2′-[1-(2-hydroxyphenyl)ethylidene]benzenesulfanohydrazide

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**KEYWORDS**

Benzenesulfanohydrazone;
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**Abstract** Zinc(II) complex with 2′-[1-(2-hydroxyphenyl) ethylidene] benzenesulfanohydrazide, was synthesized, and structurally characterized by elemental analysis CHN, TGA, UV, FT-IR, $^{1}$H NMR and $^{13}$C NMR spectroscopy. Its preventive effect on ulcer activity induced by absolute ethanol in Sprague–Dawley rats was studied *in vivo*. Gastric ulcers were induced in rats by administrating absolute ethanol. Twenty four Spragu–Dawley rats (12 males and 12 females) were assigned equally into the following 4 groups ($n$ = 6): negative control; positive control; low dose, which received low oral doses of the compound; and high dose, which received high oral doses of the compounds . These animals were subjected to overnight fasting (food but not water) prior to dosing. Gastric wall mucus, ulcer areas and histology of gastric walls were assessed in addition gastric homogenate was determined for superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA). Pre-treatment of rats with compound significantly prevented ethanol-induced gastric wall depletion and restored non-sulfhydryl (NP-SH) content in glandular stomachs. The Zinc(II) complex also exhibits high levels of SOD, and GSH enzymes as well as a reduced amount of lipid peroxidation enzyme, malondialdehyde. This study describes a model to produce extensive gastric necrosis in rats by induction with absolute ethanol. The administration of zinc(II) complex with

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benzensulfanohydrazone at 30 min before induction with absolute ethanol reduced or totally eliminated lesions.



## 1. Introduction

A Schiff base of benzenesulfanohydrazones that was synthesized from a zinc(II) acetate metal ion and benzenesulfanohydrazide with hydroxyacetophenone was found to possess a significant preventive effect on ulcer activity induced by absolute ethanol in Sprague–Dawley rats. After the synthesis and chemical study of this compound to confirm the structure, a biological study was run. Oral administration of benzenesulfanohydrazone (low dose 50 mg/kg and high dose 100 mg/kg) before absolute ethanol administration inhibited gastric lesions formation (93–100% reduction) without increasing the mucus weight compared to the control group. Based on its structure and potentials to generate hydroxyl radicals, the compound can be described as an oxidant and appears to increase the load of free radicals. The carbonyl group in the compound appears to achieve the antioxidant balance and to protect the gastric mucosa from injurious agents. Therefore, this group can play a role as an electrophilic acceptor in the structure. The mechanism of cytoprotective activity has been suggested to be mediated through a reaction between the electrophilic acceptor and the sulfhydryl-containing groups of the mucosa. Hydroxyl group-containing compounds can form hydroxyl radicals, which are known to play a major role in the pathogenesis of gastric mucosal injury. These results suggested that benzenesulfanohydrazones prevented ethanol induced gastric mucosal lesions by reducing the damaging effects of free radicals. There are literature reports on the antimicrobial activities for benzenesulfanohydrazones and their complexes (Tawfik et al., 1989).

Previous work on the synthesis and characterization of Schiff base ligands has revealed that these compounds display antibacterial (Ali et al., 2006), antiproliferative and antiviral activities (Paola et al., 2003), as well as more potent antioxidative activities than vitamin E (Ali et al., 2006). The present study extends these previous works by using different compounds with varying biological activities. Very few studies have been reported on Schiff bases of benzosulfanohydrazones and their biological activity especially as anti-ulcerogenic agents. An ulcer is an open sore, or lesion, usually found on the skin or mucous membrane areas of the body. A peptic ulcer is a lesion occurring at the lining of the stomach or duodenum, where hydrochloric acid and pepsin are present. In the past, it was believed that lifestyle factors, such as stress and diet, could cause ulcers. Later, researchers determined that stomach acids such as hydrochloric acid and pepsin contribute to ulcer infection. Today, research indicates that the most ulcers (80% of gastric ulcers and 90% of duodenal ulcers) develop as a result of infection with a bacterium called *Helicobacter pylori (H. pylori)*. Although all 3 factors – lifestyle, stomach acid, and *H. pylori* – play a role in ulcer development, *H. pylori* is thought to be the primary cause.

Many of the past successes in medicinal chemistry have involved the fortuitous discovery of useful pharmaceutical agents from natural sources, such as plants or microorganisms. Analogues of these structures were then made in an effort to improve activity and/or to reduce side-effects, but often these variations were identified on a trial-and-error basis. While this approach yielded a large range of medicinal compounds, it was wasteful with respect to the time and effort involved. In the last 20–30 years, greater emphasis has been placed on rational drug design whereby drugs are designed to interact with a known biological system. An early example of this rational approach to drug design was the development of the anti-ulcer drug cimetidine by scientists at Smith, Kline, & French (SK&F) (Patrick, 2001). As part of an ongoing study of Schiff base complexes, we aimed to synthesis and study the bioactivity of 2′-[1-(2-hydroxyphenyl)ethylidene]benzenesulfanohydrazide complex with Zn(II) ion. UV–Vis, IR, $^{1}$H NMR, $^{13}$C NMR and thermo gravimetric analyses (TGA) spectroscopy were used to examine the coordination site of the ligand and geometry of the complex (Fig. 1).

## 2. Experimental

### 2.1. Materials and measurements

All chemicals used in this study were obtained from Fluka and Aldrich. They were used as received without further purification. UV spectra were obtained with a Shimadzu UV-1650 pc UV–Vis spectrophotometer. Infrared spectra were obtained by using KBr discs (400–4000 cm$^{-1}$) on a Perkin–Elmer FT–IR spectrometer. $^{1}$H and $^{13}$C NMR spectra were recorded on a Jeol JNM-LA400 FT–NMR system. Tetramethylsilane (TMS) was used as internal standard, and deuterated DMSO was used as a solvent. Elemental analysis (C, H, N) were performed on a Flash EA 1112 Series elemental analyzer at the University of Technology Malaysia. Thermal gravimetric analysis was recorded with a Perkin Elmer TGA 4000 Thermogravimetric Analyzer.

### 2.2. Synthesis of Zn(BzSO–HAP)-complex

A solution of zinc(II) acetate (0.15 g, 0.0006 mole) in basified ethanol (50 ml) was mixed with a (50 ml) ethanolic solution

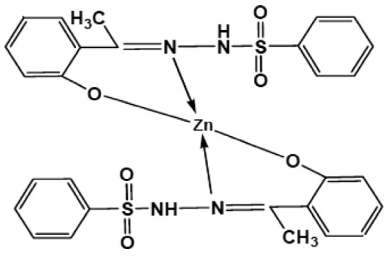



**Figure 1** Molecular structure of Zn (BzSO–HAP)-complex.

of 2′-[1-(2-hydroxyphenyl) ethylidene] benzenesulfanohydrazide (0.35 g, 0.0012 mol) at a 1:2 M ratio. The mixture was stirred and refluxed for 5 h. Dark brown solid was formed. Yield = 65%, m.p. = 160 °C. Anal. Calcd. (found)% for $C_{28}H_{26}N_4S_2O_6$: C, 52.77 (51.36); H, 4.08 (5.07); N, 8.79 (7.25). UV spectra (DMSO): 270, 280 nm ($\pi\rightarrow\pi^*$), 320 nm ($n\rightarrow\pi^*$). IR spectra (KBr): 3172 $cm^{-1}$ ν(N–H), 1600 $cm^{-1}$ ν(C═N), 1353 $cm^{-1}$ ν(S═O), 1164 $cm^{-1}$ ν(S═O), 1087 $cm^{-1}$ ν(CO), 1035 $cm^{-1}$ ν(N–N), 441 $cm^{-1}$ ν(M–N), 503 $cm^{-1}$ ν(M–O), $^1$H NMR (DMSO): 12.93 ppm [δ (N–H)], 6.70–7.74 ppm [δ(aromatic), m], 2.16 ppm [δ(–CH3), 3H, s] and $^{13}$C NMR (DMSO): 157.76 ppm δ(C═N), 116.33–131.35 ppm δ(aromatic), 13.13 ppm δ(–$CH_3$).

### 2.3. Biological experiments

#### 2.3.1. Ethics statement

Animal experiments were performed in accordance with the guidelines for animal experimentation issued by the Animal Care and Use Committee at the University of Malaya (Ethics Number: PM/27/07/2010/MAA (R)).

#### 2.3.2. Chemical derivatives

Tween 20 was manufactured by Merck Schuchardt OHG. 85662 Hohen brunn, Germany and Cimetidine was the reference antiulcer drug, which was obtained from the University Malaya Medical Centre. Each tablet contained 200 mg of Cimetidine; the tablets were ground to powder and suspended in the vehicle (tween 20, 10%v/v) and administered to the rats in a concentration of 50 mg/kg orally as described by (Tan et al., 2000).

#### 2.3.3. Experimental animals

Sprague–Dawley healthy adult male rats were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya. The rats were divided randomly into 6 groups of 6 rats each. Each rat 200–225 g was placed individually in a separate cage (1 rat per cage) with wide-mesh wire bottoms to prevent coprophagia during the experiment. The animals were maintained on standard pellet diet and tap water.

#### 2.3.4. Acute toxicity studies

An acute toxicity study was used to determine a safe dose for Zn(BzSO–HAP). Forty eight healthy Sprague–Dawley rats (24 males and 24 females) were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya. The rats were assigned equally to 4 groups, which were labeled as vehicle (gum acacia in normal saline), 1 g/kg, 2 g/kg, or 5 g/kg of Zn(BzSO–HAP) in vehicle. The animals were fasted overnight (food but not water) prior to dosing. Food was withheld for a further 3–4 h after dosing. The animals were observed for 30 min and at 2, 4, 24 and 48 h after administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The animals were sacrificed on day 15. Hematological, serum biochemical and histological (liver and kidney) parameters were determined with standard methods (Bergmeyer, 1980; Tietz et al., 1983). Throughout the experiments, all animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health.

#### 2.3.5. Gastric ulcer-induction by ethanol

Gastric ulcers were induced in rats by administrating absolute ethanol according to the recommendation of (Mahmood et al., 2010). The experimental animals were divided into 4 groups ($n$ = 6) and the negative control animals received 5 ml $kg^{-1}$ of 10% Tween 20 orally by metal or gastric intubations (per os); positive control animals received oral doses of 50 mg $kg^{-1}$ cimetidine in (5 ml $kg^{-1}$) suspended in 10% Tween 20; high dose animals received oral doses of 100 mg $kg^{-1}$ of the compound (5 ml $kg^{-1}$) suspended in 10% Tween 20; and low dose animals received oral doses of 50 mg $kg^{-1}$ of the compound (5 ml $kg^{-1}$) suspended in 10% Tween 20. After 30 min of the pre-treatment, 5 ml $kg^{-1}$ of absolute ethanol was administered orally to the animals. The animals were killed 30 min later by overdoses of xylazin and ketamine anesthesia (Mahmood et al., 2010) and their stomachs were excised. Each stomach was opened along the greater curvature, washed with distilled water and fixed in 10% buffered formalin (Morimoto et al., 1991).

#### 2.3.6. Preparation of homogenate

Gastric tissue was washed thoroughly with ice cold saline. 10% (w/v) homogenate was prepared tephlon homogenizer (Polytron, Heidolph RZR 1, Germany) in ice-cold 50 mM phosphate buffer pH 7.4 containing mammalian protease inhibitor cocktail. The homogenate was centrifuged at 10,000$g$ for 30 min at 4 °C. The supernatant was used for the assay of antioxidant activities/levels and lipid peroxidation.

#### 2.3.7. Measurement superoxide dismutase (SOD) activity

SOD activity was measured according to Sun et al. (1988). The activity of the enzyme was evaluated by measuring its capacity to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT). In this assay, the photochemical reduction of riboflavin generates $O^{2-}$ that reduces the NBT to produce formazan salt, which absorbs at a wavelength of 560 nm. In the presence of SOD, the reduction of the NBT is inhibited because the enzyme converts the superoxide radical to peroxide. The results are expressed as the quantity of SOD necessary to inhibit the rate of reduction of the NBT by 50% in units of the enzyme per gram of protein. Homogenates (10% of tissue in buffer phosphate) were centrifuged (10 min, 3600 rpm, 4 °C), and the supernatant was removed and centrifuged a second time (20 min, 12,000 rpm, 4 °C). The resulting supernatant was assayed. In a dark chamber, 1 mL of the reactant (50 mM phosphate buffer, 100 nM EDTA and 13 mM L-methionine, pH 7.8) was mixed with 30 μL of the sample, 150 μL of 75 μM NBT and 300 μL of 2 μM riboflavin. The tubes containing the resulting solution were exposed to fluorescent light bulbs (15 W) for 15 min and then read using a spectrophotometer at 560 nm.

#### 2.3.8. Measurement of the amount of reduced glutathione

To assess the changes in the gastric mucosal amount of GSH (a non-protein sulfhydryl compound), its content was measured according to the method described by Sedlak and Lindsay (1968). The glandular segment from each stomach was homogenized in ice-cold 0.02 M EDTA solution (at 10%). Aliquots (400 μL) of tissue homogenate were mixed with 320 μL of distilled water and 80 μL of 50% (w/v) trichloroacetic acid in glass tubes and centrifuged at 3000 rpm for 15 min. Subsequently, supernatants (400 μL) were mixed with 800 μL Tris buffer (0.4 M, pH 8.9) and 5,5-dithio-bis(2-nitrobenzoic acid)

(DTNB; 0.01 M) was added. After shaking the reaction mixture for 3 min, its absorbance was measured at 412 nm within 5 min of the addition of DTNB against a blank with no homogenate. The absorbance values were extrapolated from a glutathione standard curve and the GSH content was expressed in microgram GSH/g of protein. The concentration of proteins was measured using the method described by Bradford (1976).

*2.3.9. Measurement of the membrane lipids peroxidation*

The rate of lipoperoxidation in the gastric mucous membrane was estimated by determination of malondialdehyd (MDA) using the Thiobarbituric Acid Reactive Substances (TBARS) test. The stomachs were washed with saline to minimize the interference of hemoglobin with free radicals and to remove blood adhered to the mucous membrane. The stomachs were homogenized to 10% of tissue with potassium phosphate buffer. Then, 250 μL was removed and stored at 37 °C for 1 h, after which 400 μL of 35% perchloric acid was added, and the mixture was centrifuged at 14,000 rpm for 20 min at 4 °C. The supernatant was removed, mixed with 400 μL of 0.6% thiobarbituric acid and incubated at 95–100 °C for 1 h. After cooling, the absorbance at 532 nm was measured. A standard curve was generated using 1,1,3,3-tetrametoxypropane. The results were expressed as nmol of MDA/mg of protein. The concentration of proteins was measured using the method described by Bradford (1976). The method is based on the interaction of the Coomassie Blue G250 dye with proteins. At the pH of the reaction, the interaction between proteins of high molecular weight and the dye causes a shift in the dye to the anionic form, which absorbs strongly at 595 nm. Solutions of albumin standard, distilled water, buffer and samples were added to the wells. For sample preparation, 2 μL of sample and 38 μL of buffer were added to each well. Then, 200 μL Bradford's solution (diluted 5×) was added to each well. After 5 min, a reading was taken at the wavelength of 595 nm (Bradford, 1976).

*2.3.10. Biochemical assays of SOD, GSH and MDA in gastric tissue homogenate*

In gastric tissue, both SOD and GSH activities in ulcer control group were significantly lower compared with normal group (Table 5). Administration of cimetidine or compounds before absolute alcohol significantly rose the SOD and GSH compared with ulcer control group (Table 5). Administration of absolute alcohol significantly increased the MDA level of gastric homogenate compared with normal control. Administration of cimetidine or compound decreased the MDA level in gastric tissue compared with ulcer control group (Table 5). The results indicated that GSH levels in tissue homogenate in animals-treated with cimetidine or compound increased if compared to the ulcer control group, demonstrating a possible antioxidant capacity of compound (Table 5).

*2.3.11. Macroscopic examination*

The abdomens of the rats were opened and the pylorus of each was ligated. The stomachs were removed and the gastric juice was obtained from each stomach. The surface area ($mm^2$) covered by each lesion was measured (Mahmood et al., 2010) and the sum of the erosion areas per rat stomach was calculated by using a dissecting microscope at a magnification of ×1.8. The percentage of ulcerated surfaces (US), was calculated as follows: US ($mm^2$) = (total area covered by ulcers/total corpus mucosal surface) × 100. The ulcer index (UI) for each animal was calculated as the mean ulcer score. Percentage inhibition (%I) was determined as follows: [(UI in control−UI in test group)/UI in control group] × 100 (Mahmood et al., 2010). The stomach contents were collected and centrifuged. The gastric juice was separated from the mucus. The mucus content was weighed and expressed in terms of grams (Varley et al., 1980).

*2.3.12. Microscopic examination (histology examination)*

The stomach was cut into small pieces of tissues and was fixed in 10% buffered formalin overnight. The tissues were processed in an automated tissue processor through dehydration, cleaning and impregnation steps. Next, the tissues were embedded in paraffin wax and sectioned into 5 μm thick section by votary microtome. The sections were stained with hematoxylin and eosin. Each specimen was analyzed under a light microscope at ×10, ×40 and ×100 magnification to observe changes in the tissues structures compared to the control group (Mahmood et al., 2010; Holander et al., 1984).

*2.3.13. Statistical analysis*

The results are expressed as the standard error of the mean (S.E.M.). The statistical differences of each group of rats were calculated by using Student's *t*-test with SPSS for Windows Evaluation Version 14. Statistically significant differences were accepted at $P < 0.05$ level.

## 3. Results

### 3.1. Thermal studies

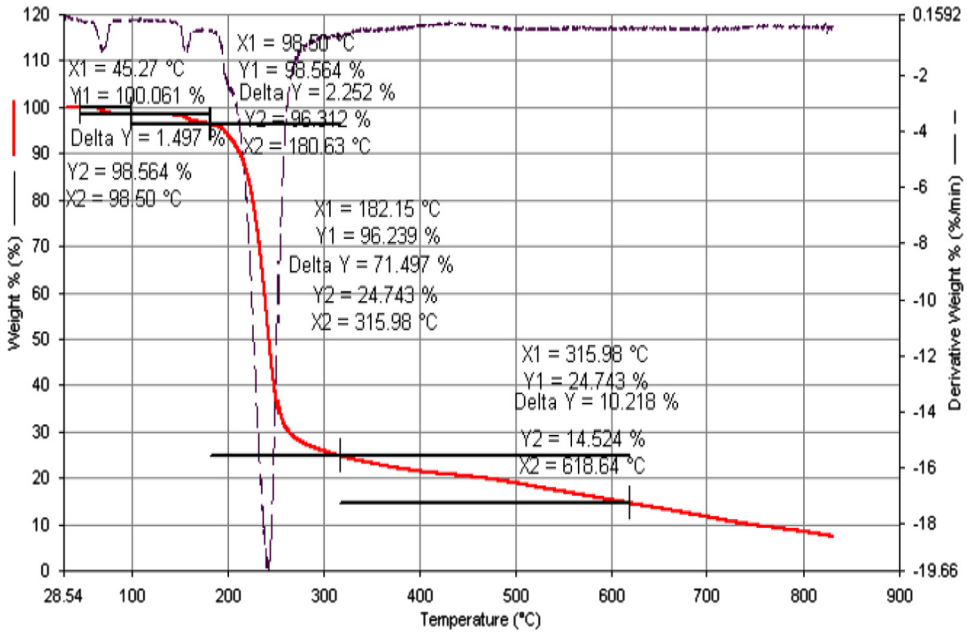
The thermal decomposition of the Zn(II) complex was investigated by (TGA). The results showed good agreement with the formula suggested from the analytical data. A general decomposition pattern was obtained, in which the complex decomposed in 4 steps as shown in (Spectrum 1). The first and second stages involved the loss of water molecules at 45.27–98.50 °C and 98.50–180.63 °C respectively. The third and fourth stages involved the loss of part of the organic ligand. Moreover, the Zn(II) complex decomposed and produced $ZnO_2$ as a residue (Gouda et al., 2009; Chen et al., 2010; Elbehery and El-Twigry, 2007). The experimental determination of the complex by TGA is presented in Table 1.

*3.1.1. $^1H$ and $^{13}C$ NMR spectroscopy*

$^1H$ and $^{13}C$ NMR spectra Zn(BzSO–HAP)-complex is recorded in $d_6$-dimethylsulfoxide (DMSO) solution with chemical shifts expressed in ppm using tetramethylsilane (TMS) as internal standard. The important data are given in the experimental part.

$^1H$ NMR spectra displayed singlet signals in the 12.93 ppm which assigned to N–H group. The other singlet signals appeared in the region of 2.16 ppm which is assigned to protons of methyl group for acetophenone. The aromatic ring protons of Zn(BzSO–HAP) are observed as multiplets in the region of 0.70–7.74 ppm.

The $^{13}C$ NMR spectra of the compound confirm the 1H spectral results. The azomethine group C═N was observed downfield in the 157.76 ppm chemical shift, while the aromatic




**Spectrum 1** TGA spectrum of Zn(BzSO–HAP)-complex.

**Table 1** TGA data of the Zn(BzSO–HAP)-complex.

| Complex | Step | Temp. (°C) | Weight loss (%) found (calculated) | Assignment | Residue (%) found (calculated) |
|---|---|---|---|---|---|
| Zn(II) | 1 | 45.27–98.50 | 1.49(2.61) | $H_2O$ | ZnO 16.03(14.12) |
| | 2 | 98.50–180.63 | 2.25(2.61) | $H_2O$ | |
| | 3 | 182.15–315.98 | 71.49(72.38) | $(Ph–SO_2–NH–N{=}C–CH_3)_2{\cdot}Ph–CH_3{\cdot}CH_3$ | |
| | 4 | 315.98–618.64 | 10.21(10.87) | Ph– | |

ring signals were observed in the region of 116.33–131.35 ppm. The peak up field in the region of 13.13 ppm is assigned to methyl group, $CH_3$ of the zinc complex.

*3.1.2. FT-IR spectroscopy*

The IR spectra shifts of Zn(BzSO–HAP)-complex is summarized in the experimental part. The major functional groups focused on the vibration of *v*(NH), *v*(C=N), *v*(C–O), *v*as($SO_2$), and *v* s($SO_2$) which provide conclusive structural evidence for the coordination of the ligand to the central metal atoms.

The complex displays the *v*(C=N) band at 1600 $cm^{-1}$ at a lower wave number indicating a decrease in the bond order due to the coordination of the metal atom to the azomethine nitrogen lone pair. The higher wave number of *v*(N–N)at 1035 $cm^{-1}$ is an evidence of this coordination.

There are two bands at the wave number of 1353 $cm^{-1}$ and 1164 $cm^{-1}$ assigned to two (S=O) groups. The new bands in 441 and 503 $cm^{-1}$ were assigned as *v*(M–N) and *v*(M–O) bonds, respectively.

*3.1.3. UV–Visible spectroscopy*

The electronic absorption data of the compound are given in the experimental part. In the spectrum the shoulder band in the 320 nm was assigned to the $n\rightarrow\pi^*$ transition of the azomethine group. The intense bands in the 270, 280 nm are attributed to the $\pi\rightarrow\pi^*$ transition of the azomethine group and benzene ring, the high energy transition observed is suggesting that the nitrogen atom of the azomethine group is coordinated to the metal ion.

*3.2. Biological activity*

*3.2.1. Acute toxicity study*

Animals treated with Zn(BzSO–HAP) at a dose of 2 g/kg or 5 g/kg were kept under observation for 14 days. All of the animals remained alive and no animal manifested any significant sign of toxicity at these doses. There were no abnormal signs, behavioral changes, body weight changes, or macroscopic finding at any time of observation. There was no mortality in the above-mentioned doses at the end of 14 days of observation. Histological examination of the liver and kidney, hematology, and serum biochemistry revealed no significant differences between the different groups as shown in Fig. 2. All of the data of the acute toxicity tests are listed in Tables 2 and 3.

*3.2.2. Cytoprotective activity*

The metal compound in Table 4 showed an important cytoprotective activity against ulcers induced by absolute ethanol. The values of ulcer area, percentage of ulcer inhibition and pH compared with those of the cimetidine group showed that the compound is bioactive.

The ulcer areas in the benzenesulfanohydrazone treated groups were significantly smaller than the control group. As can be observed in Table 4 the oral administration of the metal

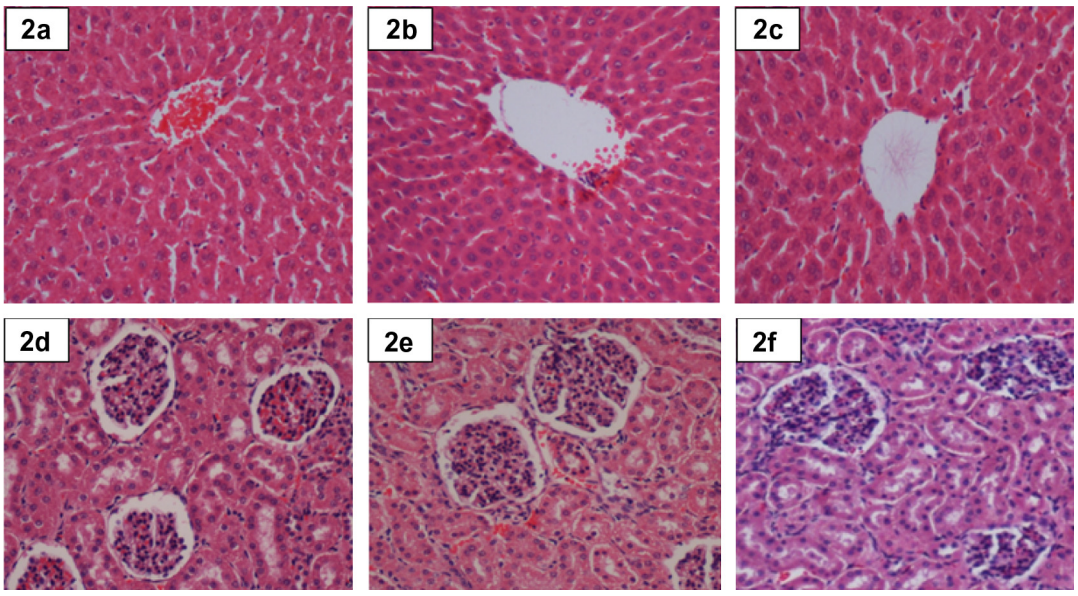



**Figure 2** Histological sections of acute toxicity test. a, b and c (20×) liver section in rats treated with 5 g/kg vehicle, LD and HD compound, respectively. (d, e (20×) and f (10×)) Kidney section in rats treated with 5 ml/kg vehicle. There is no significant differences in structures of liver and kidney between treated (LD, HD) and control groups.

**Table 2** Acute toxicity test.

| Animals group | Renal function test | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sodium (mmol/L) | Pottasium (mmol/L) | Chloride (mmol/L) | $CO_2$ (mmol/L) | Anion gap (mmol/L) | Urea (mmol/L) | Creatinine (μmol/L) |
| Control grp | 138.17 ± 2.64 | 5.12 ± 0.09 | 103.33 ± 1.91 | 23.13 ± 0.88 | 18.17 ± 0.60 | 5.73 ± 0.37 | 50.83 ± 1.79 |
| L. D. (2 g/kg) | 137.67 ± 1.65 | 5.30 ± 0.05 | 102.67 ± 1.60 | 20.42 ± 0.61 | 19.17 ± 0.40 | 4.85 ± 0.08 | 47.67 ± 1.22 |
| H. D. (5 g/kg) | 138.50 ± 1.59 | 4.63 ± 0.04 | 102.83 ± 1.69 | 22.9 ± 0.36 | 17.33 ± 0.42 | 5.98 ± 0.21 | 48.00 ± 1.16 |

All values expressed as mean and standards error mean. There is no significant difference between groups.

complex (50 and 100 mg/kg) at 30 min before ethanol administration markedly reduced the mucosal damage by up to 100% in animals treated with a high dose of the compound. At the same time the compound did not induce any significant changes in the normal gastric mucosa of the rats of any tested group. The results of the present study confirm the known ulcerogenic effects of ethanol. The chemical structure (Fig. 1) shows that the benzenesulfanohydrazones complexes have hydroxyl coordinated groups. Based on its structure and potentials to generate hydroxyl radicals, benzenesulfanohydrazone complexes can be described as oxidants and appear to increase the load of free radicals. The carbonyl group in the compounds appears to achieve the antioxidant balance and to protect the gastric mucosa from injurious agents. Therefore, this group may play a role as an electrophilic acceptor in the structure. The mechanism of cytoprotective activity has been suggested to be mediated through a reaction between the electrophilic acceptor and the sulfhydryl-containing groups of the mucosa. Also, compounds with the hydroxyl group can form hydroxyl radicals, which are known to mediate in the pathogenesis of gastric mucosal injury. This result has been reported in a study (Al-shabanah et al, 2000) about the effect of ninhydrin on the biochemical and histopathological changes in the gastric mucosa of rats. Further studies are warranted to identify the detailed mechanism of action of these bioactive compounds.

The administration of ethanol to rats produces gastric mucosal lesions and erosions similar to those occurring in gastric ulcer. These lesions are produced because ethanol can affect the protective defense mechanisms at the mucosal layer (Repetto and Liesuy, 2002). This study describes a model to produce extensive gastric necrosis in rats after induction with absolute ethanol. Once administered to rats, the ethanol rapidly penetrates the mucosa layer and causes extensive damage (Rajeshkumar et al., 2001; Abdulla et al., 2010). This process supports the finding that only 30 min was needed to produce acute gastric ulceration in rats. Administration of absolute ethanol to fasted rats caused severe gastric damage, which was visible from the outside of the stomach as thick reddish-black lines in the ulcerated control group (Fig. 3). Administration of the zinc(II) metal complex of benzenesulfanohydrazones at 30 min before the administration of absolute ethanol lead to good prevention with reduction or absence of the lesions. This result shown in Fig. 3 is the view of the outside of the stomach and (Fig. 4) is the view of the opened stomach.

**Table 3** Acute toxicity test.

| Animals group | Liver function test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total protein (g/L) | Albumin (g/L) | Globulin (g/L) | Total bilirubin (μmol/L) | Conjugated bilirubin (μmol/L) | AP (IU/L) | ALT (IU/L) | AST (IU/L) | G-glutaml. transferase (IU/L) |
| Control grp | 71.17 ± 0.64 | 11.33 ± 0.38 | 59.83 ± 1.10 | 1.81 ± 0.08 | 0.83 ± 0.02 | 134.83 ± 3.00 | 53.5 ± 0.59 | 151.5 ± 2.93 | 4.67 ± 0.13 |
| L.D. (2 g/kg) | 71.50 ± 1.39 | 11.67 ± 0.13 | 59.50 ± 0.70 | 2.17 ± 0.06 | 1.00 ± 0.09 | 133.17 ± 1.87 | 50.00 ± 1.40 | 163.67 ± 2.13 | 5.00 ± 0.29 |
| H | 71.83 ± 0.50 | 11.83 ± 0.39 | 60.17 ± 0.42 | 1.50 ± 0.06 | 1.00 ± 0.04 | 135.33 ± 0.09 | 52.17 ± 1.28 | 155.00 ± 2.27 | 5.33 ± 0.10 |

*3.2.3. Histological evaluation of gastric lesions*

The histological analysis of ethanol treated and control rat stomachs revealed the presence of necrotic debris in the lamina propria of the mucosa. The lesions extended down the mucosal layer involving the surface epithelium (Fig. 5). The treated groups had less damage along the surface of the epithelium. Mucosal blood flow has been observed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin (Holander et al., 1984). The effectiveness of benzenesulfanohydrazones complex in prevention against mucosal damage caused by ethanol may be an indication of its effect on prostaglandin as shown in Fig. 6.

## 4. Discussion

### *4.1. Chemistry*

To determine the safety of drugs and products for human use, toxicological evaluations are carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in humans. The Hematological, gastrointestinal and cardiovascular adverse effects have the highest overall concordance between animals and humans (Olson et al., 2000). The liver and kidney of the treated rats showed no significant change compared to the control group. Hematology and clinical biochemistry values were within the range of the control animals tested and were similar to some of the control reference values published by other researchers (Ringler and Dabich, 1979; Witthawaskul et al., 2003). In acute toxicity studies, a product is considered nontoxic if no deaths are registered after 14 days of observation and no clinical signs of toxicity are observed at doses $\leqslant$5 g/kg. Because the highest dose of zinc(II) complex that did not cause any toxicity was 5 g/kg body weight, this finding suggests that the compound is relatively nontoxic (Brock et al., 1995).

The effectiveness of the zinc(II) metal complex with 2′-[1-(2-hydroxyphenyl) ethylidene] benzenesulfanohydrazide in preventing mucosal damage caused by ethanol may indicate its effect on prostaglandin. The treatment with the benzenesulfanohydrazones complex reduced the volume of gastric secretion. In the cimetidine group the gastric contents were around ($1.18 \pm 0.04^{b}$) g, while those of the treated groups were in the range of ($1.33 \pm 0.01^{c}$–$1.40 \pm 0.01^{d}$) g. This characteristic indicates that the benzenesulfanohydrazones of the zinc(II) metal complex acts similar to histamine H2 receptor antagonists. Therefore, the cimetidine has been used as standard antiulcer drug in the present study as it can decrease acid output but not decrease the ulcer area. This previous observation supports the result of the present study, in which both doses of zinc(II) complex showed cytoprotective effects, in terms of reducing the ulcerative area, that were similar to the effect of cimetidine, which reduced the gastric secretion. The inhibition activity of the zinc(II) complex with 2′-[1-(2-hydroxyphenyl) ethylidene] benzenesulfanohydrazide against ulcer formation was excellent and was higher than that of the cimetidine group which had a percentage of 89.83% inhibition. As the dosage was increased further, the ulcer inhibition increased to 100%. The reduced gastric acidity measured after pylorus ligation suggests that the cytoprotective mechanism of action of the extract on gastric mucosa may involve direct inhibition of gastric secretion. The reduction of

**Table 4** Observed ulcer area, inhibition (percentage), pH and mucus by $Zn(BzSO–HAP)_2$ in rats.

| Animal Group | Pre-treatment (5 ml/kg dose) | Ulcer area $(mm)^2$ (Mean ± S.E.M) | Inhibition (%) | pH of gastric content | Mucus weight of gastric content |
|---|---|---|---|---|---|
| 1 | Tween 20 (ulcer control) | $840.00 \pm 4.60^{a}$ | - | $3.78 + 0.154^{a}$ | $0.73 + 0.065^{a}$ |
| 2 | Cimetidine (50 mg/kg) | $145.00 \pm 4.91^{b}$ | 82.74% | $6.20 + 0.491^{b}$ | $1.18 + 0\ .069^{b}$ |
| 3 | $Zn(BzSO–HAP)_2$ (50 mg/kg) | $85.40 \pm 4.43^{c}$ | 89.83% | $5.10 + 0.379^{ab}$ | $1.33 + 0.086^{b}$ |
| 4 | $Zn(BzSO–HAP)_2$ (100 mg/kg) | $00.00 \pm 0.00^{d}$ | 100% | $5.54 + 0.415^{b}$ | $1.40 + 0.140^{b}$ |

All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different. The mean difference is significant at the $P < 0.05$ level.

**Table 5** Effects of Zinc(II) complex on gastric tissue homogenate enzymes (SOD, GSH) and level of MDA.

| Pre-treatment | SOD | GSH | MAD |
|---|---|---|---|
| Normal control | $419.33 \pm 11.44^{a}$ | $320.56 \pm 06.85^{a}$ | $33.06 \pm 2.02^{a}$ |
| Ulcer control | $300.22 \pm 15.31^{b}$ | $219.75 \pm 10.05^{b}$ | $89.61 \pm 3.11^{b}$ |
| Cimetidine | $472.88 \pm 17.64^{c}$ | $490.09 \pm 11.12^{c}$ | $50.11 \pm 1.87^{c}$ |
| 50 mg compd | $488.67 \pm 12.28^{c}$ | $435.55 \pm 09.50^{d}$ | $41.28 \pm 2.05^{c}$ |
| 100 mg compd | $513.75 \pm 12.98^{d}$ | $508.48 \pm 07.66^{c}$ | $55.17 \pm 3.82^{c}$ |

All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different. The mean difference is significant at the $P < 0.05$ level.

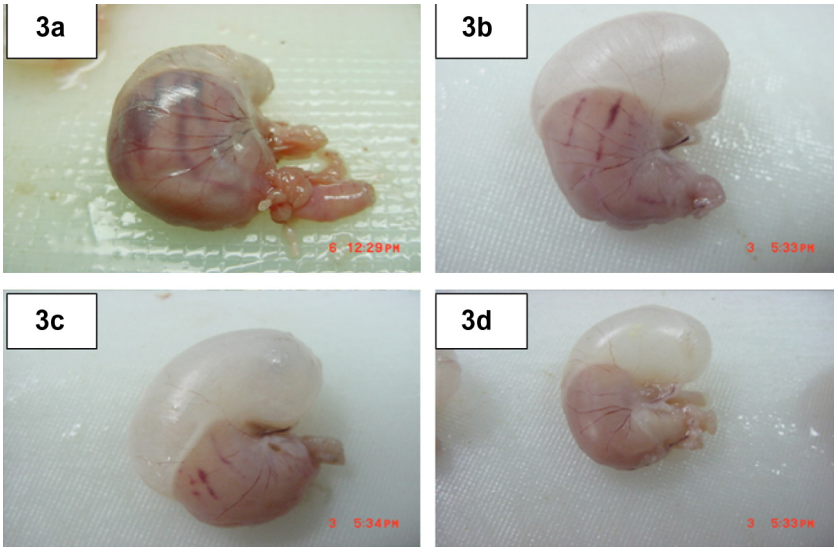



**Figure 3** Macroscopical appearance of gastric mucosa in rats. (a) Rats pre-treated with 10% Tween 20. Severe macroscopic necrosis of gastric mucosa. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (b) Rats pre-treated with cimetidine 50 mg $kg^{-1}$). Mild macroscopic necrosis of gastric mucosa. The drug reduces the formation of gastric lesions induced by absolute ethanol. (c) Rats pre-treated with Zn(BzSO–HAP) (50 mg $kg^{-1}$). Mild macroscopic necrosis of gastric mucosa. The compound reduces the formation of gastric lesions induced by absolute ethanol. (d) Rats pre-treated with Zn(BzSO–HAP) (100 mg $kg^{-1}$). The compound prevents the formation of gastric lesions induced by absolute ethanol.

acidity observed after incubation of the gastric juice with the extract suggests that the cytoprotective mechanism may involve the simple neutralization of the acid secreted in the stomach. Fig. 5b shows the effect of the benzenesulfanohydrazone complex on the acidity of the gastric juice in pH range from $5.1 \pm 0.04^{c}$ to $5.54 \pm 0.03^{d}$ compared to the cimetidine group ($pH = 6.2 \pm 0.06^{b}$). These results agree with previous studies (Mahmood et al., 2010; Murakamu et al., 1990). In this study zn(II) benzenesulfanohydrazone complex was found to reduce gastric lesions induced by ethanol *in vivo*.

Absolute ethanol-induced gastric ulcers have been widely used in the evaluation of gastroprotective activity (Al-Mofleh et al., 2008). Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the stomach

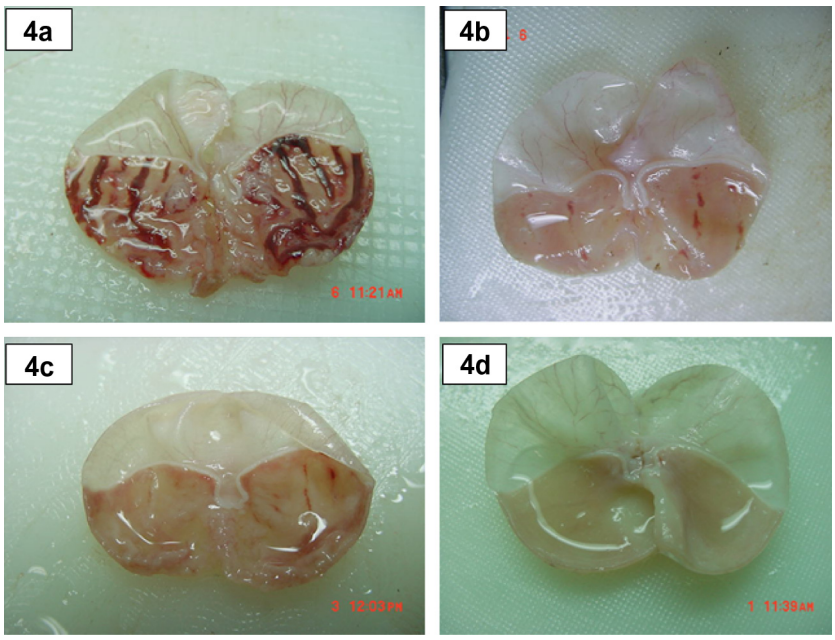



**Figure 4** Macroscopical appearance of gastric mucosa in rats in open stomach. (a) Rats pre-treated with 10% Tween 20. Severe macroscopic necrosis of gastric mucosa. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (b) Rats pre-treated with cimetidine 50 mg $kg^{-1}$). Mild macroscopic necrosis of gastric mucosa. The drug reduces the formation of gastric lesions induced by absolute ethanol. (c) Rats pre-treated with Zn(BzSO–HAP) (50 mg $kg^{-1}$). Mild macroscopic necrosis of gastric mucosa. The compound reduces the formation of gastric lesions induced by absolute ethanol. (d) Rats pre-treated with Zn(BzSO–HAP) (100 mg $kg^{-1}$). The compound prevents the formation of gastric lesions induced by absolute ethanol.

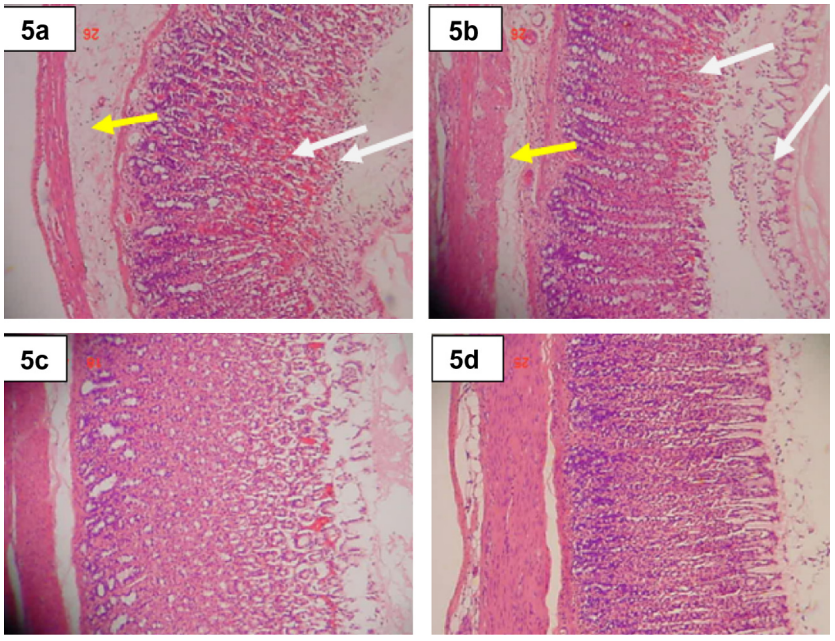



**Figure 5** Histological section of the absolute ethanol-induced gastric mucosal damage in rats. (5a) Rats pre-treated with 10% Tween 20 (ulcer control). Severe disruption of the surface epithelium, deep penetration of necrotic lesions into mucosa (arrow) and edema of the sub mucosa layer (yellow arrow) with leukocyte infiltration of ulcerative tissues. (b) Rats pre-treated with cimetidine (50 mg/kg). Moderate disruption of the surface epithelium and edema of the sub mucosal layer (yellow arrow) with leucocytes infiltration. (c) Rats pre-treated with Zn(BzSO–HAP) (50 mg $kg^{-1}$). There is mild disruption of the surface epithelium. (d). Rats pre-treated with Zn(BzSO–HAP) (100 mg $kg^{-1}$. No disruption to the surface epithelium (arrow) and no edema and leucocytes infiltration of sub mucosal layer (H&E stain, 10×).

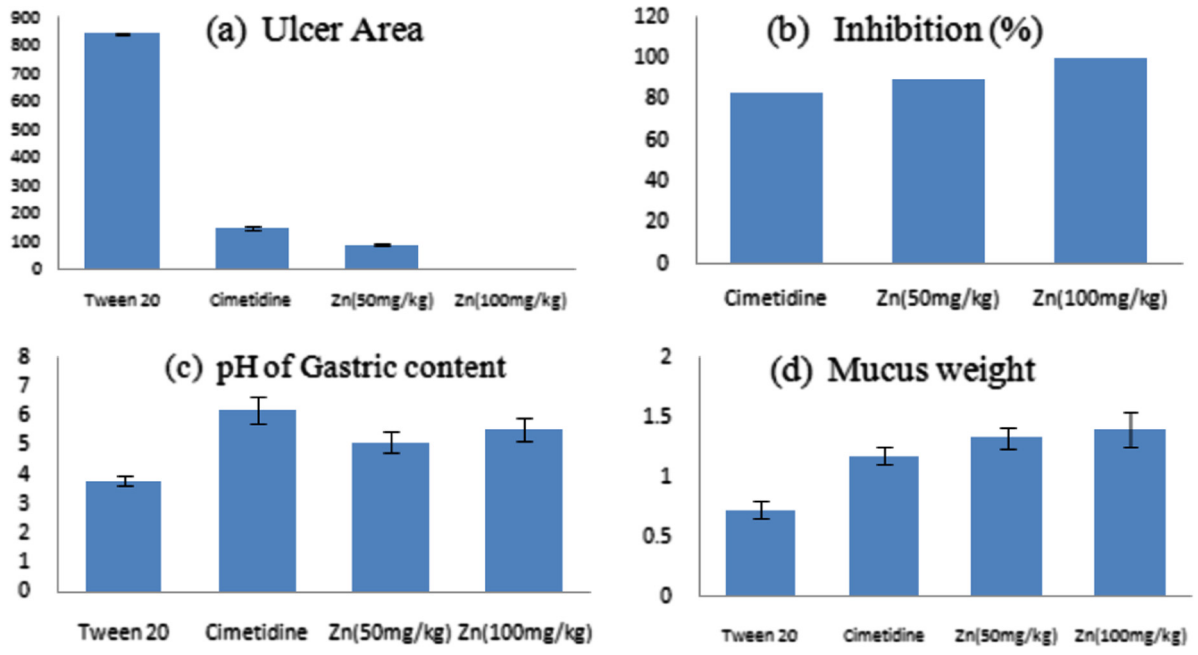



**Figure 6** Shows the effect of compound on ethanol induced gastric mucosal lesions in rats (a) Summarized the effect of compound on mucosal weight. (b) Shows the effect of benzenesulfanohydrazones complex on the acidity of the gastric juice in range of (pH = 5.1 ± 0.04$^{c}$ –5.54 ± 0.03$^{d}$) compared to the cimetidine group (pH = 6.2 ± 0.06$^{b}$). (c) Shows the inhibition of benzenesulfanohydrazones Zinc(II) complex 50 mg kg$^{-1}$ and 100 mg kg$^{-1}$ against the stomach ulcer induced by absolute alcohol and the results are compared to the standard drug, cimetidine (d) this shows the Ulcer area for the compound compared with the control group and standard cimetidine. Cimetidine has been reported to decrease acid output but did not decrease the ulcer area. This supported the present study which both doses had indicated better cytoprotective effects in reducing ulcer area formation compared with cimetidine with less gastric secretion.

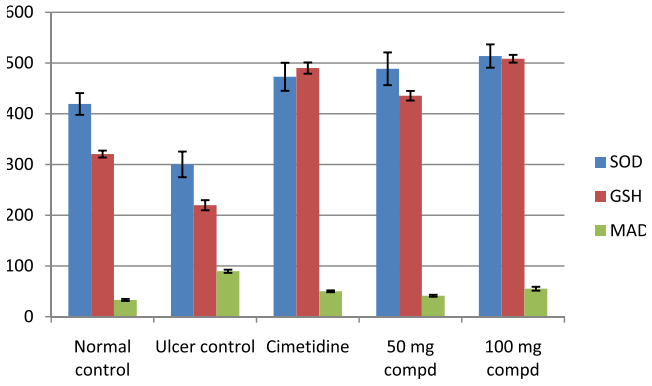



**Figure 7** Histograms of the effects of Zinc(II) complex on gastric tissue homogenate enzymes (SOD, GSH) and level of MDA.

(Umamaheswari et al., 2007). The genesis of ethanol-induced gastric lesions is of multifactorial origin with a decrease in gastric mucus and is associated with the significant production of free radicals leading to increased lipid peroxidation which in turn causes damage to cells and cell membranes (Khazaei and Salehi, 2006). Oral administration of absolute ethanol is noxious to the stomach since it affects the gastric mucosa topically by disrupting its barrier and provoking pronounced micro vascular changes within a few minutes after its application (Moleiro et al., 2009).

In the present study, gastric tissue homogenate (Fig. 7) from animals pre-treated with cimetidine or compound showed significant antioxidant activity by decreasing the levels of MDA and by elevating the levels of GHS and SOD in response to oxidative stress due to absolute ethanol (Table 5). Lipid peroxidation is found to be an important path physiological event in a variety of diseases (Ajitha and Rajnarayana, 2001). Malondialdehyde (MDA) level is a biomarker for oxidative stress (Gutteridge, 1995). Apart from overproduction of free radicals are linked to accumulation of lipid peroxidation by-products (Palanduz et al., 2001). It is well known that MDA from lipid peroxidation reacts with DNA bases and induces mutagenic lesions (Benamira et al., 1995). Free radicals and reactive oxygen species (ROS) are continuously produced in human body. These oxygen species are the cause of cell damage. Therefore, tissues must be protected from oxidative injury through intracellular as well as extracellular antioxidants (Halliwell and Gutteridge, 1999).

Superoxide dismutase (SOD) can catalyze the dismutation of superoxide anions into hydrogen peroxide and dioxygen, to keep the free radical density in the body at a normal level. Superoxide dismutase is one of the key enzymes responsible for radical scavenging activities. It catalyzes superoxide radicals to hydrogen peroxide and oxygen. SOD converts superoxide to hydrogen peroxide ($H_2O_2$) which is then transformed into water by catalase in lysosomes or by glutathione peroxidase (GPx) in mitochondria (Johansen and Sagvolden, 2005).

Glutathione constitutes one of the most important protective mechanisms against lesion formation (Cnubben et al., 2001), while SOD-mediated catalysis of superoxide radical anion ($O_{2+}^{-}$) into less noxious hydrogen peroxide ($H_2O_2$) repre-

sents the first line of antioxidant defense. GSH is known to protect the cellular system against the toxic effects of lipid peroxidation. GSH functions as direct free radical scavenger, as a co-substrate for glutathione peroxidase (GPx) activity and as a cofactor for many enzymes and forms conjugates in endo and xenobiotic reactions (Gregus et al., 1996).

The antiulcer activity may be attributed to the ability of compound to protect gastric mucus against ethanol-induced depletion of the non-protein sulfhydryl (NP-SH) component of the glandular mucosa. Glutathione (GSH) has been resorted to play an important role in the process of cellular injury (Szabo et al., 1981) have reported a prophylactic effect of glutathione against various necrotizing agent. GSH exerts a pivotal role in maintenance of gastric integrity (Dutta et al., 2007). Oxidative damage to the mucosal lining could be minimized by superoxides scavenger like GSH.

## 5. Conclusion

This paper describes a model to produce extensive gastric necrosis in rats by induction with absolute ethanol. It also describes good prevention of gastric necrosis by administration of zinc(II) complex with benzenesulfanohydrazones 30 min before induction with absolute ethanol which reduced or eliminated lesion formation. The compound was tested for acute toxicity. Hematology and clinical biochemistry values were within the range of the control animals tested and were similar to some of the control reference values. The highest dose of zinc(II) complex that did not cause any toxicity was 5 g/kg body weight, which indicates that the compound is relatively non-toxic. Assays of SOD and GSH and MDA levels from gastric tissue homogenate reveal that compound treated group significantly increases the SOD and GSH, and decreased the level of lipid peroxidation compared with the ulcer control group.

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