EVALUATION OF THE ANTI-ULCEROGENIC PROPERTIES OF ETHANOLIC EXTRACT OF *Pterocarpus erinaceus* AND HOMOPTEROCARPIN AGAINST ASPIRIN-INDUCED ULCER IN ALBINO RATS

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**ABSTRACT:** *Pterocarpus erinaceus* is a medicinal plant with many therapeutic properties. This study evaluated anti-ulcerogenic activity of stem bark extract of *P. erinaceus* and homopterocarpin against aspirin-induced gastric mucosal injury in albino rats. The albino rats were divided into five groups. Group I received olive oil, groups III-V were pre-treated with 100mg/kg cimetidine, 25mg/kg homopterocarpin and 100mg/kg test extract forty-five minutes before ulcer induction. The stomachs of the sacrificed animals were evaluated for ulcer indices, pH, volume and acid concentration of the gastric juice, mucus concentration, malondialdehyde level, reduced glutathione level, superoxide dismutase activity and total protein. Aspirin produced significant alterations in ulcer index, mucus concentration, and gastric juice parameters, compared to the control. The results show that test extract and homopterocarpin were able to exert protective effects against aspirin induced gastric ulcers in albino rats. The protective effects might be via anti-secretory, cytoprotective and antioxidative mechanisms. **Keywords:** *P. erinaceus*; antioxidative; ulcer; aspirin

**INTRODUCTION**

Peptic ulcer disease (PUD) is a lesion of gastric or duodenal mucosa. Peptic ulceration occurs only in areas which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of duodenum (Dey and Dey, 2002). Peptic ulcers also occur at the lower end of the esophagus, on the jejunum side of a gastroenterostomy and in Meckel’s diverticulum (Boyd, 1970). The type of ulcer that occurs in the stomach is termed gastric. Gastric ulceration is as result of persistent erosions and damage of the stomach wall that is usually perforated and normally developed into peritonitis and massive hemorrhage as a result of inhibition in the synthesis of mucus, bicarbonate and prostaglandins (Wallace, 2008). The aetiology of gastric ulceration is usually multifactorial and not clearly defined, but some predisposing factors have been implicated. This usually include duration of starvation, nature of food ingested, bile reflux, lessened mucosal resistance, alteration of gastric mucosal blood flow, disruption of gastric mucosal barrier by stress, decrease in alkaline mucosal bicarbonate and mucus secretion, over dosage and prolonged administration of non-steroidal anti-inflammatory drugs, persistent
infection with *Helicobacter pylori*, Zollinger-Ellison syndrome and genetic factors as suggested by a higher incidence in patients with positive family history of this disorder or blood type O (Suerbaum and Michetti, 2002; Berardi and Welase, 2005). These ulcers are produced when there is an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach (Del Valle et al, 2003; Ojewole, 2004).

The treatment of ulcer condition is usually overshadowed by various side effects arising from the drugs used. For example *H*-receptor antagonists (e.g. cimetidine) may cause gynecomasia in men and galactorrhea in women (Reilly, 1999). Due to these side effects, there is a need to find new anti-ulcerogenic compounds with potentially less or no side effects and medicinal plants have always been the main source of new drugs for the treatment of gastric ulcers. The high degree of efficacy and safety with herbal medicines makes them more acceptable when compared to other therapeutics intervention (Chaturvedi et al, 2007). *P. erinaceus* is a small to medium size deciduous legume tree that is 12-15m tall and 1.2m in diameter. The bark of the trunk is dark grey and rough with scales that curl up at the ends. The leaves are compound and imparipinnate. It is distributed throughout the West and Central Africans Savanna and dry forest. It is popularly known as African Rosewood. A decoction of the stem bark is used as astringent for severe diarrhea and dysentery and as an ingredient in abortifacient prescriptions. It is also used traditionally as a dressing on ring worm of the scalp and treatment of chronic ulcers (Dalziel, 1948). Previous study by Aliyu et al (2005) showed that the ethanolic stem bark extract of the plant possesses significant and dose-dependent analgesic and anti-inflammatory activities in laboratory rats and mice. Reports by Aliyu and Chedi (2010) show the phytochemical constituent of *P. erinaceus* which include steroids, flavonoids, tannins, carbohydrate, proteins and amino acids. This study was undertaken in order to give a scientific basis and corroborate the folkloric claim that the *P. erinaceus* possess active constituents with anti-ulcerogenic properties.

**MATERIALS AND METHODS.**

**Chemicals and drugs.**

Glutathione, thioarbituric acid, trichloroacetic acid, sodium dodecyl sulfate, epinephrine and 5’, 5’-dithiobis-2-nitrobenzoate (DTNB, Elman’s reagent) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

**Plant material.**

African Rosewood (*P. erinaceus*) was purchased from Erekesan Market in Akure, Ondo State. It was identified in the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure.

**Methods of extraction.**

The stem-bark of *P. erinaceus* were cleaned, air-dried at room temperature (27°C) for three days, ground using a hand operated warring grinder into fine powder. The powdered sample was soaked in ethanol (1g to 3ml (w/v)) for three days. The overall grams of the plant soaked were 522.5g in 1567.5ml of ethanol (96%). It was filtered and concentrated to a small volume to remove the entire ethanol using rotary evaporator. The extract was later kept in the freezer at 4°C and used for various analyses and assays. Homopterocarpin was extracted according to the method described by Adetuyi et al (2012).

**Phytochemical screening of extract.**

Qualitative testing of the extract for alkaloids, terpenes, flavonoids, cardiac glycosides, saponin, tannin and anthraquinone was carried out according to the methods described by Sofowora, (1993).

**Determination of total polyphenolic content.**

The total polyphenolic content was estimated according to the Folin-Ciocalteu method described by Spanos and Worlstad (1990). Known concentrations of the extract (0.1ml) was diluted with distilled water (0.9ml) and mixed with 5ml of 10-fold diluted solution of Folin-Ciocalteu reagent. Four milliliters of 7.5% standard sodium carbonate solution was added to the above mixture and shaken. The absorbance of the
reaction mixture was measured at 765nm after two hours. Total phenolic content was expressed as gallic acid equivalent (mg/g gallic equivalent).

**Determination of total flavonoid.**
The total flavonoid content was determined using the Dowd method described by (Mede et al., 2005). In this experiment, 5ml of 2% AlCl₃ in methanol was mixed with 2ml of the plant extract. After 10 minutes the absorbance of the reaction mixture was measured at 415nm. Total flavonoid content was expressed as quercetin equivalent (mg/g quercetin equivalent).

**Animal handling.**
Wistar rats (150-200g) were bred in our colony. They were maintained in standard laboratory conditions under natural light/dark cycle and fed with standard rat chow and water ad libitum. Animals were handled and used in accordance with the international guide for the care and use of laboratory animals (Institute of Laboratory Animal Resources Commission in Life Sciences, 1996).

**Ulceration process.**
Aspirin induced ulcers were produced in the albino rats as previously described (Debnath and Guha, 2007). Wistar albino rats were fasted for 48 hours being completely deprived of water and food. In all ulcer models, animals were divided into five groups with six animals in each group. Group 1(control) received olive oil (1ml), Group 2 (negative control) was treated with 200mg of aspirin/kg (orally) in the aspirin induced ulceration models. Group 3-5 were subjected to the same treatment as group 2 after pretreatment with 100mg/kg of cimetidine, 25mg/kg homopterocarpin, 100mg/kg of P. erinaceus extract respectively. Four hours later, animals were killed under ether anesthesia. The stomach was removed, opened along the greater curvature, and washed in physiologic saline and the gastric juice was also collected for further analysis.

**Evaluation of extent of ulcerogenesis.**
Measurement of the gross gastric mucosal lesions was carried out on freshly excised stomachs according to the method of Gamberini et al., (1991). In all ulcer model groups, ulcer index was determined by adding the values of all the scores for the evaluated indices.

**Evaluation of volume, pH and total acidity of stomach gastric juice.**
The gastric juice of the stomach previously kept in serum bottles were centrifuged at 3500 x g for 15 minutes to determine the gastric volume (ml) and pH using a digital pH meter. The acidity in the gastric secretions was determined by back titration with 0.01N NaOH according to the method described by Reitman (1970). This is done by putting the base in the biuret while the stomach juice was in the conical flask and methyl orange as indicator according to Reitman (1970).

**Determination of mucus content of stomach.**
Alcian blue binding to gastric wall mucus was determined by a modified method of (Corne et al., 1974). The stomach of the rats to be used was rinsed with 0.25M sucrose solution. These stomachs were incubated in 10ml aliquots of 0.1% alcian blue solution for two hours at room temperature (30°C). After two hours stomachs were removed, washed with 0.25M sucrose solution and separately incubated in 10ml aliquots of 0.5M MgCl₂ solution for two hours at room temperature while shaking at 30 minutes interval to elute the alcian blue bound to the mucosa of the stomachs. Two hours later, the stomach were removed and 5ml of each aliquot of MgCl₂ solution containing the alcian blue eluted from each stomach was shaken with 5ml of diethyl ether. The aqueous phase was separated out, centrifuged at 3200 x g for 5 minutes and the absorbance of the supernatant was measured at 605nm. The amount of alcian blue bound per stomach in micrograms was determined using a standard calibration curve. Alcian blue was the standard compound used.

**Biochemical estimation.**

**Homogenization.**
The stomach tissue from the glandular portion of stomach was excised and washed in ice cold 1.15% potassium chloride solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in four volumes of the homogenizing buffer (pH 7.4) using mortar and pestle. The resulting homogenate was
centrifuged at 10000 x g at 4°C for 10 minutes to obtain the supernatant which is then stored in a freezer at 4°C.

**Determination of the invivo lipid peroxidation (malonaldehyde level).**

The clear supernatant obtained as described during the homogenization process was placed on ice as described by (Ohkawa et al, 1979). The supernatant (300µL) of various groups were pipette into test tubes already containing 300µL of 8.1% SDS (sodium duodecyl sulphate), 600µL of 1.34M acetate buffer (pH 3.4) and 600µL of 0.8% thiobarbituric acid. The reaction mixtures were incubated at 100°C for one hour and allowed to cool. The absorbance was taken against the blank at 532nm.

**Determination of reduced glutathione (GSH) level.**

Reduced glutathione was determined according to the method described by Sedlak and Lindsay (1968). 0.2ml of sample was added to 1.8ml of distilled water and 3ml of the precipitating solution (sulphosalicylic acid) was mixed with sample. The rate of addition was not critical. The mixture was then allowed to stand for approximately five minutes and then filtered. At the end of the fifth minute, 1ml of filtrate was added to 4ml of 0.1M phosphate buffer. Finally 0.5ml of the Elman reagent was added. A blank was prepared with 4ml of the 0.1M phosphate buffer, 1ml of diluted precipitating solution (3 parts to 2 parts of distilled water) and 0.5ml of the Elman’s reagent. The optical density was measured at 412nm. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard curve.

**Determination of superoxide dismutase (SOD) activity.**

The activity of SOD in the test samples were determined according to the method describe by Sun et al, (1988). 1ml of the sample of the various groups was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml of buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30seconds for 150seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1minute. The total activity was then divided by the amount of total protein to get the specific activity of SOD in the homogenate.

**Determination of total protein.**

This was carried out by means of the Biuret method as described below. 0.1ml of sample was dissolved in 3.9ml of 0.9% saline to give a 1 in 40 dilution. 3ml of Biuret reagent was added to 2ml of diluted sample. The mixture was incubated at room temperature for 30 minutes after which the absorbance was read at 540nm. The values of the protein concentration of the various samples were extrapolated from a total protein standard curve.

**Statistical analysis.**

Data are given as means ± S.E.M. Statistical comparisons were made using one-way ANOVA followed by Duncan’s multiple range test. A P value < 0.05 was considered as significant.

**RESULTS.**

**Phytochemical screening of extract.**

The result of preliminary phytochemical screening of the plant extracts showed presence of phlobatannins, flavonoids, terpenoids, steroids.

**Total Polyphenol and Flavonoids Content of Extract.**

The total polyphenolic and flavonoid content in the ethanolic stem bark extract of *P. ernaceus* were 0.12mg gallic acid equivalents/ g extract and 0.046mg quercetin equivalents / g extract respectively.

**Macroscopic observations**

Figure 1 shows the representative stomachs of rats after aspirin induction of gastric ulcer. Administration of aspirin (200mg/kg) produced superficial or deep erosions, bleeding, and antral ulcers. However, pretreatment with homopterocarpin and *P.
erinaceus reduced the severity of aspirin-induced gastric ulcer.

**Acid secretion studies.**

In Table 1, the effect of homopterocarpin and *P. erinaceus* pre-treatment on the various gastroprotective parameters of the aspirin-induced ulcer in albino rats is presented. There was significant difference (P < 0.05) between the groups treated with aspirin and *P. erinaceus* as compared with the control group for ulcer indices. However there is no significant (P < 0.05) difference in the group treated with aspirin and cimetidine as compared with the control for total acidity, while *P. erinaceus* and homopterocarpin were able to bring an ameliorative effect on total acidity as presented in Table 1.

Table 1 shows the effect of homopterocarpin and *P. erinaceus* on pH of rats of the aspirin-induced ulcer. Aspirin caused a significant reduction in the pH of albino rats as compared with the control. Pre-treatment with homopterocarpin or *P. erinaceus* ameliorated the effects of aspirin on stomach pH of ulcerated albino rats by causing a statistically significant increase in pH as compared with the group treated with aspirin. Volume of gastric juice (ml) in rats treated with aspirin and oral administration of homopterocarpin and *P. erinaceus* at different doses is shown in Table 1. It was obvious from the figure that volume of gastric juice (ml) as mean±SE of group administered aspirin alone was significantly higher (0.52±0.03ml) at p < 0.05 as compared to control group (0.17±0.02ml). Rats pre-treated with homopterocarpin and *P. erinaceus* had their volume of gastric juice significantly reduced compared to aspirin treated rats.

![Figure 1](image-url)  
**Figure 1** Macroscopic observation of ulcers in ulcer-induced/treated stomachs in aspirin-induced ulcer models.  
(a) Control; (b) Aspirin-induced ulcer; (c) 100mg/kg cimetidine + aspirin-induced ulcer; (d) 25mg/kg homopterocarpin + aspirin-induced ulcer; (e) 100mg/kg *P. erinaceus* + aspirin-induced ulcer.

<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>Ulcer indices</th>
<th>Total acidity $\times 10^{-2}$</th>
<th>pH</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.00 ± 1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.19 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aspirin</td>
<td>19.50 ± 2.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.80 ± 0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.23 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.52 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>7.50 ± 0.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.78 ± 0.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.96 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homopterocarpin</td>
<td>3.00 ± 0.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.61 ± 1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.99 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. erinaceus</em></td>
<td>1.33 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.24 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are presented as mean ± S.E.M., n = 6. Values of ulcer indices, gastric juice pH, volume and total acidity for aspirin-induced ulcer groups are significantly different from the respective control values (P < 0.05).
Gastric mucus studies.

The gastric mucus of aspirin induced group was statistically significantly reduced compared to the control. Treatment with homopterocarpin and *P. erinaceus* restored it to normalcy, while there was significant difference in the mucus content of the cimetidine group as compared to the induced group (Figure 2).

Studies on the biochemical parameters.

Malondialdehyde levels (MDA) of rats stomach are shown in Figure 3. The administration of aspirin significantly (P<0.05) increased MDA levels compared with the control. In contrast pre-treatment with cimetidine (100mg/kg), *P. erinaceus* (100mg/kg) and homopterocarpin (25mg/kg) significantly decreased the MDA levels in all models compared with the control group. *P. erinaceus* and homopterocarpin at doses of 100 and 25 caused a reversal of the increased MDA levels in the aspirin induced model to levels comparable with control values. Reduced glutathione (GSH) level in rat stomach was decreased in the aspirin ulcer induced group. In contrast, the reduced GSH level was significantly increased in the *P. erinaceus* and homopterocarpin treated groups model compared with the control group. *P. erinaceus* (100mg/kg) showed greater overall efficiency than homopterocarpin in restoring GSH level to normal (figure 4). The ulcerogen (aspirin) caused statistically significant decrease in activity of superoxide dismutase (SOD) compared with the control. Pre-treatment with cimetidine significantly increased SOD activity in the aspirin induced model compared with the ulcer-induced groups. Similarly, all doses of *P. erinaceus* and homopterocarpin significantly increased SOD activity in the aspirin induced ulcer model compared with the ulcer induced group (Figure 5). Compared to the control group, the total protein content of the ulcerated rat was significantly (P<0.05) reduced (1.6284±0.0181 mg/ml) after four hours of ulceration. *P. erinaceus* and homopterocarpin significantly increased the total protein at a value of (2.2014±0.0038 mg/ml) and (2.0686±0.0074 mg/ml) respectively, while cimetidine increased the total protein at a value of (1.7345±0.01 mg/ml), compared to control group. All the test samples increased the total protein significantly, compared to that of the control group. The results are presented in Figure 6.

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**Figure 2** Effects of *P. erinaceus* and homopterocarpin on the mucus concentration of the stomach tissue of the experimental animals. Values are expressed as Mean ± S.E.M; Values with different letters are statistically different (P<0.05).
Figure 3 Effects of *P. erinaceus* and homopterocarpin on the MDA level of the stomach tissue of the experimental animals. Values are expressed as Mean ± S.E.M; Values with different letters are statistically different (P<0.05).

Figure 4 Effects of *P. erinaceus* and homopterocarpin on the GSH concentration of the stomach tissue of the experimental animals. Values are expressed as Mean ± S.E.M; Values with different letters are statistically different (P<0.05).
Figure 5 Effects of *P. erinaceus* and homopterocarpin on the SOD activity of the stomach tissue of the experimental animals. Values are expressed as Mean ± S.E.M; Values with different letters are statistically different (P<0.05).

Figure 6 Effects of *P. erinaceus* and homopterocarpin on the total protein of the stomach tissue of the experimental animals. Values are expressed as Mean ± S.E.M; Values with different letters are statistically different (P<0.05).
DISCUSSION.

Aspirin induced gastric ulcer model is a useful model to induce severe ulceration in experimental animals (Sanmugapriya and Venkataraman, 2007). As shown in figure 1, the macroscopic observation depicting the aspirin induced stomach of the albino rat shows the evidence of mucosa lesion, haemorrhage streaks, perforations while in the stomach tissue treated with *P. erinaceus* and homopterocarpin, the effect were reduced. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of $H^+$ ions (Rao et al., 2000). The inhibition of mucosa prostaglandin production occurs rapidly following oral administration of aspirin. This is correlated with the rapid absorption of these drugs through mucosa (Parmer and Desai, 1993).

Previous studies have also shown that deficiency of prostaglandins plays a key role in aspirin induced gastrointestinal side effects (Fiorucci., 2009). Table 1 shows the various ulcer indices of the aspirin induced ulceration. It is observed that *P. erinaceus* and homopterocarpin were able to bring about a significant reduction in ulcer indices compared to the aspirin induced group which caused a significant increase in the ulcer indices. The decrease in ulcer indices is in conformity with those of Goel et al., (2000) who reported the decrease in ulcer indices on treatment with vegetable plantain banana in aspirin induced rats and Agarwal et al., (2000) who also reported the decrease in ulcer indices on treatment with *Piper longum* Linn, *Zingiber officianalis* Linn and *Ferula species* in aspirin induced gastric ulcer rats. Scientists have established the relationship between acid production, pH, volume of gastric juice and gastric ulcers (Levine and Senayo, 1970; Dai and Ogle, 2005; Brodie et al., 2006). As shown in table 1 there was a significant increase in the gastric juice volume, total acid concentration and significant decrease in the pH of the gastric juice as compared to the control. While *P. erinaceus* and homopterocarpin and cimetidine were able to bring about a positive ameliorative to these parameters. The results corroborate the findings of Surender, (1999), who reported a decrease in total acidity and increase in pH on treatment with fixed oil of *Ocimum basilicum* Linn in aspirin induced rats. It has been studied that inhibition of gastric secretion protects the gastroduodenal mucosa against the injuries caused by aspirin. Sanmugapriya and Venkataraman (2007) have shown that decrease in gastric juice volume reduced the total acidity and thus ulcer indices. These studies support the results that the enhancement of production of gastric wall mucus, decrease in gastric juice volume reduces the total acidity and thus ulcer indices elicted by *P. erinaceus* and homopterocarpin. The result of this present study demonstrates that *P. erinaceus* and homopterocarpin treatment has anti-secretory activity, as observed by the decrease in total acidity, volume of gastric juice and increase in pH.

The mucus concentration is graphically represented in figure 2. It is observed that there is significant increase in the mucus concentration of the albino rat treated prior with *P. erinaceus* before inducing with aspirin as compared with the control group. Whereas the aspirin induced group significantly reduce the mucus concentration. This study corroborates the findings of Dhuley (1999) reported an increase in gastric wall mucus thickness (barrier mucus) in rats treated with rhinax, a herbal formulation consisting water extracts of *Withania somnifera* L(root), *Asparagus racemosus* wild(root), *Mucuna prurience* (root), *Phyllanthus emblica* gaertin(fruit), *Myristica fragrans* houtt(seed0, *Glycyrrhiza glabra* L(root), against physical and chemical factors induced gastric and duodenal ulcers in rats. This depletion in mucus concentration in gastric stomach tissue can be attributed to depletion of mucosal prostaglandins by inhibiting COX activity. Increase mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid in gastric juice and by acting as an effective barrier to back diffusion of $H^+$ ion (Venables, 1986).

Administration of aspirin to albino rats is usually accompanied with the generation of
reactive oxygen species (ROS) e.g. superoxide anion, hydrogen peroxide, hydroxyl radicals that cause lipid peroxidation especially in membranes and result in tissue injury. Elevation in the levels of end products of lipid peroxidation in the aspirin induced rat stomach was observed (figure 3). This increase in MDA levels in the stomach suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms to prevent formation of excessive free radicals. Pre-treatment with P. erinaceus and homopterocarpin significantly reversed these changes (figure 3). Hence it is likely that the mechanism of anti-ulceration of P. erinaceus and homopterocarpin is due to its antioxidant effect. Reduced glutathione is one of the most abundant non-enzymatic antioxidant biomolecules present in the tissues (Meister, 1984). Its function are removal of free oxygen species such as hydrogen peroxide, superoxide anions and alkoxy radicals, maintenance of membrane proteins thiols and to act as a substrate for glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (Townsend et al, 2003). In the present study, aspirin enhanced the activities of GSH related enzymes GPx and GST, thereby decreasing the GSH content, whereas treatment with P. erinaceus and homopterocarpin reversed these effects (figure 4). It may be understood that the effect of P. erinaceus and homopterocarpin may be due to increased synthesis of GSH or by stimulation of glutathione reductase activity.

Free radical scavenging enzymes SOD is usually the first line of cellular defense against oxidative damage, disposing O2 and hydrogen peroxide before their interaction to form the more harmful hydroxyl(OH) radical (Lil et al, 1988). In the present study SOD activity decreased significantly in the aspirin induced group of albino rats, which may be due to an excessive formation of superoxide anions (figure 5). These excessive superoxide anions might inactivate SOD and decrease its activity. SOD is an important defense enzyme that catalyzes the dismutation of superoxide anions into O2 and hydrogen peroxide. Conclusively, anti-ulcerative effect of P. erinaceus and homopterocarpin against aspirin induced ulcer in albino rats appear to be related to the inhibition of lipid peroxidation processes and to the prevention of GSH depletion. Furthermore, it may be stated that P. erinaceus and homopterocarpin exerts its antioxidant activity by a dual action by enzymatic as well as non-enzymatic pathways. There was a significant decrease in the level of the total protein in the gastric stomach tissue wall as shown in figure 6 in the aspirin induced group. Prior administration of P. erinaceus and homopterocarpin significantly increase or reversed the level of the damage to the total protein as shown in figure 6.

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REFERENCES.


