ANTI-ULCER ACTIVITY OF ETHANOL EXTRACT OF PARKIA SPECIOSA AGAINST INDOMETHACIN INDUCED PEPTIC ULCER IN ALBINO RATS

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Keywords: Anti-ulcer, omeprazole, indomethacin, Parkia speciosa

ABSTRACT: This study was performed to determine the anti-ulcer effects of ethanol extract of Parkia speciosa on Indomethacin induced peptic ulcer in Albino rats. Rats of female sex weighing between 150 g - 200 g were used. Rats were divided into six groups with six rats in each group. Omeprazole (20 mg/kg) were used as a standard drug for this study. Parkia speciosa extracts were given in three different doses comprised of 100 mg/kg, 200 mg/kg and 400 mg/kg. Pre-treatment were done for 14 days and at the end of the 14th day, rats were kept fasted for 24 hours before administration of Indomethacin at 30 mg/kg. Administration of drugs was done orally. At the end of the study, rats were sacrificed and stomachs were opened and stored in 10% formalin solution. The acidity of gastric juice, gastric mucosal lesion and histological changes were studied. Extracts of Parkia speciosa showed a significant (P<0.05) reduction in acidity of gastric juice as compared to the ulcer control group. Two doses of Parkia speciosa ethanol extract that is 200 mg/kg and 400 mg/kg showed significant (P<0.05) reduction in lesion length as compared to ulcer control group. Histological studies showed lesser collagen and fibrosis were present in tissue from rats treated with Parkia speciosa extract compared to tissues of rats from ulcer control group.

INDRODUCTION: The ulcer is the open sore in the lining of the stomach or intestine, similar to mouth ulcers (stomatitis) and skin ulcer. Gastric ulcer is the ulcer which occurs in the stomach. Duodenal ulcer is the ulcer that occurs in the first part of the intestine. Peptic ulcer is the term used for either of the ulcers or both ulcers. The most common gastrointestinal disease is peptic ulcer disease (PUD). Peptic ulcer occurs due to the reaction of acid and pepsin which is the digestive enzyme present in the stomach towards the mucosal lining of the stomach which leads to the excoriation of it.

PUD is caused by infection, stress, smoking, nutritional deficiencies and frequent use of non-steroidal anti-inflammatory drugs (NSAIDs). The disequilibrium of gastric aggressive factor and mucosal defensive factor results in ulcer. The aggressive factors are acid, pepsin, free radicals, infectious agent such as Helicobacter pylori and chemicals, bile salts and pancreatic enzyme, whereas the defensive factors are adherent mucin, bicarbonate, prostaglandin (PG) and mucosal blood flow.

The main way to heal peptic ulcer would be to reduce gastric acid production and to increase the gastric mucosal protection. H2 receptor blockers and proton pump inhibitors reduce acid secretion and aid the healing of ulcer but it does not prevent the re-occurrence of it where as antacid only gives relief and does not inhibit gastric secretion or aids in healing. Synthetic drugs have side effects such as arrhythmias, impotence, gynaecomastia,
enterochromaffin-like cell, hyperplasia, and haemopoietic changes. Therefore, alternative ways are being explored to produce drug from natural product derived from plants to treat peptic ulcer. *Parkia speciosa* is better known as ‘petai’ among Malaysians. It is from the family Fabaceae – Mimosoideae. *Parkia speciosa* seed are encapsulated in the pods. This seeds are also reported to treat diseases such as diabetes, cholera and kidney pain. Studies showed that *Parkia speciosa* had a hypoglycaemic effect of chloroform extract from empty pods. It is also reported to have anti-bacterial activity and anti-oxidative effect. The current finding showed that *Parkia speciosa* seed has hemagglutinating activity of proteins.

**MATERIALS AND METHODS:**

**Plant collection**
The commercially available fresh pods of *Parkia speciosa* were obtained from local markets in Ipoh, Perak, Malaysia and were authenticated by the Institute of Bioscience, UPM, Serdang, Selangor. A voucher specimen No: SK 2150/13 was deposited in the herbarium.

**Ethanol extraction**
The seeds of *Parkia speciosa* were separated from the pods and cleaned. The seeds were dried for ten days and were powdered using an electric blender. The powder was soaked for 48 hours in ethanol. Then the powder was filtered and the filtrate was evaporated in a rotary evaporator. The crude material *Parkia speciosa* was obtained and it was stored in an airtight container until the study was conducted.

**Phytochemical screenings**
The ethanol extract of *Parkia speciosa* (EEPS) were tested for flavonoids, tannins and phenolic compound and saponins and terpenoids.

**Acute toxicity study**
According to literature survey, doses of *Parkia speciosa* were used from 50-400 mg/kg. On this basis, the dose level of *Parkia speciosa* that were selected for evaluation of anti-ulcer activity was 100 mg/kg (PS 100), 200 mg/kg (PS 200) and 400 mg/kg (PS 400) dose level were selected for the evaluation of anti-ulcer activity.

**Experimental animal**
Albino rats of female sex weighing between 150 g to 200 g were used for the study because female rats are sensitive to changes compared to male rats. They were maintained on the synthetic pellet feed and clean water ad libitum. Animals were housed in controlled conditions with a temperature of 25 ± 2°C, 55 ± 10% relative humidity and 12/12 hrs light-dark cycle environment. Animals were kept in cages at least 5 days before dosing to allow for acclimatisation to laboratory condition. The experimental protocol was approved by the University Animal Ethics Committee with its letter No- AMU/AEC/HS-FBH/2013/7 dated 24th July 2013.

**Experimental design**
Six groups of rats with six rats per group were selected for the present study. The first group was the normal control while the second group was the standard group where omeprazole were administered at 20 mg/kg. The third group was the test group (EEPS – 100 mg/kg). Forth group was a test group (EEPS – 200 mg/kg). Fifth group was a test group (EEPS – 400 mg/kg). Sixth group was the Indomethacin (30 mg/kg) induced ulcer group. The pre-treatment were continued for 14 days, at the end of the 14th day, rats from group two to group 6 were kept fasting for 24 hrs and Indomethacin were administered orally. After 6 hours of Indomethacin administration, rats were sacrificed, stomach were opened and washed with normal saline and stored in 10% formalin solution.

**Analysis of gastric juice**
The stomachs of rats were cut to collect the gastric contents. It were then measured and centrifuged at 1109 RCF for ten minutes. This was done to remove solid debris that was present in the gastric contents. 1 ml of gastric juice that appears to be the supernatant was pipette and diluted in 10 ml of distilled water in 100 ml conical flask. Two drops of phenolphthalein were added and titrated with 0.1M sodium hydroxide until a permanent pink colour appears. The amount of alkali added was recorded and the acidity was calculated using the formula below.

\[
\text{Acidity} = \frac{\text{volume of NaOH} \times \text{normality of NaOH} \times 100}{0.1} \text{ mEq/l}
\]
Evaluation of Gastric Mucosal Lesion

The removed stomachs were opened along the greater curvature. It was then washed with normal saline and observed using microscope for perforation and size and number of ulcers. The lesions were expressed in the terms of ulcer index (U.I).

The score that were assigned was 0-3 scoring system referring to the severity of the lesion. Severity factor 0 indicates no lesion, severity factor 1 indicates a lesion less than 1mm length, severity factor 2 indicates lesion 2 mm to 4 mm length and severity factor 3 indicates a lesion more than 4 mm in length. The number of lesions that appeared in the stomach were calculated and multiplied by its respective severity factor. The U.I for each group was calculated by finding the mean score for all rats in the group. Preventive index (P.I) was calculated using this equation.

\[
P.I = \frac{U.I \text{ of indomethacin group} - U.I \text{ of pretreated group}}{U.I \text{ of indomethacin group}} \times 100
\]

TABLE 1: CHART OF EVALUATION OF GASTRIC LESION

<table>
<thead>
<tr>
<th>Ulcer score</th>
<th>Size of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No lesion</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 1mm</td>
</tr>
<tr>
<td>2</td>
<td>2mm - 4mm</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 4mm</td>
</tr>
</tbody>
</table>

Analysis of gastric juice

TABLE 2: AVERAGE ACIDITY OF GASTRIC JUICE

<table>
<thead>
<tr>
<th>Group</th>
<th>Average acidity (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ± 0 *</td>
</tr>
<tr>
<td>Omeperazole</td>
<td>10 ± 0 *</td>
</tr>
<tr>
<td>P.S - 100</td>
<td>26.66 ± 5.78 * * *</td>
</tr>
<tr>
<td>P.S - 200</td>
<td>23.33 ± 5.78 *</td>
</tr>
<tr>
<td>P.S - 400</td>
<td>16.67 ± 5.78 *</td>
</tr>
<tr>
<td>Ulcer Control</td>
<td>50 ± 0 *</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.D of observation from 6 rats.

\* Significantly different from control group

\* a Significantly different from ulcer control group

\* b Significantly different from omeprazole group

Histopathological study

The stomachs were removed and fixed in neutral buffer formalin for 24 hrs. The tissues were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and five μm thick sections were cut using a rotary microtome. The sections were stained using Haematoxylin and Eosin (H & E) Stain and Trichrome Stain using routine procedure. The slides were examined for any changes using a microscope.

RESULT:

Phytochemical Screening

The preliminary phytochemical screening on the seed of Parkia speciosa revealed the presence of phytoconstituents such as flavanoid, terpenoid and phenolic acid.

TABLE 1: PHYTOCHEMICAL SCREENING OF EEPS

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>+</td>
</tr>
</tbody>
</table>

+: presence of active compounds
-: absence of active compounds

TABLE 3: EFFECT OF EEPS AND OMEPRAZOLE PRE-TREATMENT ON ULCER INDEX (U.I) AND PREVENTIVE INDEX (P.I) IN INDOMETHACIN INDUCED PEPTIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>U.I</th>
<th>P.I (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 ± 0 *</td>
<td>-</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>0.33 ± 0 *</td>
<td>85.84</td>
</tr>
<tr>
<td>P.S 100</td>
<td>1.33 ± 0.58</td>
<td>42.92</td>
</tr>
<tr>
<td>P.S 200</td>
<td>1.0 ± 0</td>
<td>57.08</td>
</tr>
<tr>
<td>P.S 400</td>
<td>0.67 ± 0.58 *</td>
<td>71.24</td>
</tr>
<tr>
<td>Ulcer control</td>
<td>2.33 ± 0.58 * *</td>
<td>-</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.D of observation from 6 rats.

\* a Significantly different from control group

\* b Significantly different from ulcer control group

\* Significantly different from omeprazole group
**Evaluation of Gastric Mucosal Lesion**

**Histopathological study**

**Haematoxylin and Eosin Stain**

*Figure 3* shows the image of stomach tissue stained using haematoxylin and eosin. Figure A shows control group and Figure B shows the omeprazole group with normal stomach. Figure C, D and E were rats pre-treated with EEPS 100, 200 and 400 mg/kg respectively where the severity of damage decreases. Figure F was the ulcer control group which were pre-treated with Indomethacin where severe damage was seen.

**Trichrome Stain**

*Figure 4* shows the image of stomach tissue stained using Trichrome Stain. Figure A shows control group and Figure B show omeprazole group. Figure C, D and E were rats pre-treated with EEPS 100, 200 and 400 mg/kg respectively, and F was the rats.
pre-treated with Indomethacin. The blue-greenish colour indicates the presence of collagen and higher amount of collagen indicates more damage to the stomach.

FIGURE 4: A AND B SHOWED THE PRESENCE OF COLLAGEN IN NORMAL STOMACH. C SHOWED THE MOST COLLAGEN AMONG THE TEST GROUPS FOLLOWED BY D AND E. F SHOWED PRESENCE OF COLLAGEN AND DEGENERATIVE CELLS.

DISCUSSIONS: Usage of NSAID reported to be the major risk factor in the development of gastric ulcer. The mechanism that is suggested for the mucosal damage that is caused by NSAID is the inhibition of the proliferation of epithelial cell at the ulcer margin and inhibition of PG synthesis. PG plays a role in increasing mucus and bicarbonate secretion in the stomach.

Parkia speciosa have been reported to have anti-diabetic, anti-bacterial, and anti-oxidative properties. This study is conducted to determine if Parkia speciosa have anti-ulcer properties.

Parkia speciosa have been also reported to contain terpenoids, alkaloid and flavanoid. Flavanoid have been reported to show anti-ulcer properties by reducing gastric secretion and peptic activity which prevents the formation of gastric ulcer.

Rats that were used in this study consist of female rats because female rats are sensitive to changes compared to male rats. The rats used are between 150-200 g because it is the standard weight in order to conduct the studies using rats. Albino rats are used as it is the most similar to the metabolism of Asian humans. Three parameters were used in this study that is the acidity of gastric juice, mucosal
lesion and histopathological study. One way analysis of variance (ANOVA) was done using Bonferroni’s Multiple Comparison Test.

**Figure 1** shows the acidity of all the six groups of rats that were used in this study. Comparison between the control group and the ulcer control group which were treated with Indomethacin shows a significant reduction (P < 0.05) which indicates that the model used for this study is valid and comparison between Omeprazole group and ulcer control group also shows a significant difference which indicates that the standard drug is working. The entire test group which comprised of PS 100, PS 200 and PS 400 also shows significant difference as compared to the ulcer control group.

Comparison between Omeprazole group and control group shows a significant difference. However, PS 100 and PS 200 do not show any significant difference when it is compared to the control group. The highest dose of plant extracts that is the PS 400 group seemed to show a significant difference when it is compared to the control group. PS 400 does not show a significant difference when it is compared to Omeprazole group, but however it appears to the group among the test group that produced least acidic gastric juice.

The second parameter is mucosal lesion that is used to calculate the preventive index. Preventive index indicated the prevention that is provided by the drug that is administered against ulcer. Control group does not show any ulcer lesion and therefore their preventive index at 100%. Omeprazole is the standard drug used to treat ulcer and it shows a prevention of 85.84%. The preventive index of the test group increases, according to the dose of Parkia speciosa extract. PS 100 shows the lowest preventive index among the entire test groups with the value of 42.92%, followed by PS 200 with the value of 57.08% and PS 400 with the value of 71.24% which is 14.6% lower than the standard drug.

**Figure 2** shows the score for lesion found in the stomach of the rats. There is a significant reduction in control, omeprazole and PS 400 groups in comparison to ulcer control group. However, there was no significant reduction found in the comparison of PS 100 and PS 200 with an ulcer control group. Omeprazole, PS 100, PS 200 and PS 400 groups also does not show any significant difference when it is compared to the control group. There were also no significant difference between Omeprazole and PS 400.

The third parameter is histolopathological study where trichrome staining and H & E staining were done. Trichrome stain is the method used to determine the amount of collagen that was present in the tissues. Collagen appears to be in Blue/greenish colour when it is stained using trichrome stain. Tissue from control group shows small amount of collagen, which are present in normal condition in the stomach. The amount of collagen present in the omeprazole group is very much less compared to ulcer control group and cells in the omeprazole group appear to be columnar cells in intact condition whereas rats from the ulcer group were having degenerative cells with pyknotic nucleus. Collagen amount were also seemed to be less in pre-treated group compared to ulcer control group. A cell from the pre-treated group appears to be intact and normal compared to cells from the ulcer group that were undergoing severe degeneration.

H and E stain is the method used to determine the amount of fibrosis present in tissues. The control group, **Figure 3 (A)** shows normal columnar cells with absence of fibrosis. The cells remain intact with no changes to the size and morphology of the nucleus. Stomach of rat from omeprazole group, **Figure 3 (B)** showed the arrangement of intact cells with a small amount of lymphocyte which indicates inflammation. Tissues that were obtained from rats that were treated with low dose of Parkia speciosa extract that is 100 mg/kg, **Figure 3 (C)** showed degenerative tissue and the presence of lymphocyte which indicate presence of inflammation. Tissues that were obtained from rats that were treated with 200 mg/kg of Parkia speciosa, **Figure 3 (D)** showed slight fibrosis with the presence of lymphocyte. Rats that were treated with 400 mg/kg, **Figure 3 (E)** showed normal columnar cells with the absence of nucleus disruption and slight fibrosis. However, tissue from the ulcer control group rat, **Figure 3 (F)** showed normal columnar cells with the absence of nucleus disruption and slight fibrosis. However, tissue from the ulcer control group rat, **Figure 3 (F)** shows the presence of large amounts of lymphocyte which indicates severe inflammation and bleeding ulcer were also present in the tissue.
Mechanism of action of Parkia speciosa in protecting the mucosal layer of stomach has not been evaluated and this can be the future prospective of this study. Further studies can also be conducted to determine the active constituent that gives Parkia speciosa extract the anti-ulcer property.

CONCLUSIONS:
The anti-ulcer activity of Parkia speciosa is evident from the studies conducted based on the evaluation of acidity of gastric juice, gastric mucosal lesion and histolopathological study. The group of rats that were treated with Parkia speciosa extract showed significant reduction in the acidity of gastric juice and the length of lesion present in the stomach of rats. Among the entire test groups, PS 400 shows the least difference when it is compared to the standard drug, omeprazole in the entire test that was conducted.

Histolopathological studies showed the presence of maximum intact cells in the pre-treated group compared to the ulcer control group. Further increases in the dose of Parkia speciosa extract could be beneficial in the reduction in the acidity of gastric juice and the length of the lesion in stomach. Therefore, the results were suggestive of anti-ulcerogenic activity of Parkia speciosa. Future prospective of the study is to identify the active components and mechanism of action of Parkia speciosa which is responsible for this anti-ulcer property.

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REFERENCES:

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