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EFFECTS OF THE METHANOLIC EXTRACT OF OCIMUM BASILICUM (GREEN PEAS) LEAVES ON ETHANOL-INDUCED GASTRIC MUCOSAL INJURY IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: There is a great interest towards the medicinal herbs as a new line of treatment with less side effects.

Objective: Evaluation and standardization of the protective effect of Ocimum basilicum extract which is used as traditional medicine against ethanol-induced gastric mucosal damage.

Material and Methods: Fifty adult male albino rats of local strain weighing between 150 and 185 grams were used for this study. The rats were divided into four groups, control, ethanol-treated, Ocimum basilicum plus ethanol-treated, and cimetidine plus ethanol-treated. The rats received the extract in two different dose concentrations (100 and 200 mg/kg b.w. orally), and cimetidine (100 mg/kg b.w. subcutaneously) for seven successive days. One hour later after the seventh day dose, the rats of the third and fourth groups received a single dose of 70% ethanol (1 ml/rat) orally.

Results: The tested two concentrations of the extract showed a dose-dependent preventive index of 54.3% and 84.3% for the small and large dose respectively. It was noticed that the larger dose of the Ocimum basilicum extract showed a gastric mucosal protection nearly similar to that produced by cimetidine.

Conclusion: Ocimum basilicum leaves extract was effective in protection of gastric mucosa against ethanol-induced gastric mucosal injury.

Key words: Ocimum basilicum, antiulcerogenic, ulcer index and peptic ulcer.

INTRODUCTION

Peptic ulcer is defined as an excoriated area of resistant origin in gastric or intestinal mucosa ***(Guyton and Hall, 2016).*** It is caused by high acid and peptic content, irritation, poor blood supply, poor secretion of mucus, infection (H. pylori) ***(Hajrezaie et al., 2012).***

Gastroduodenal ulcers are common but frequently has declined over the past decades, with the decrease being more marked for gastric than duodenal ulcers. Duodenal ulcers are four times more common in men than in women and 4 to 5 times more common than gastric ulcers. Both are becoming common in the elderly ***(Thorsen et al., 2013).***

For treatment of gastric and duodenal ulcers, considerable effort has gone into finding compounds that prevent ulcer formation. The following pharmaceutical products were developed to heal ulcers: antacids, H_2 receptor antagonists, gastric

acid pump inhibitors and barrier agents. These drugs enhance the mucosa resistance by various mechanisms. They are also used in combination with antibiotic treatment for H. pylori-induced ulcers (***Sarhan and Orooba, 2015).*** This management is often effective in alleviating ulceration symptoms but cannot be used for protection because of their numerous side effects ***(Kuer et al., 2012).***

Medicinal plants have contributed immensely to health care. Members of the genus Ocimum, especially known as Basil that comes from the Greek word 'basileus', meaning "king". Recently there has been much research into the health benefits conferred by the essential oils found in basil. ***(Nawfor and Okoye, 2005).***

Most of the anti-secretory drugs such as proton pump inhibitors (omeprazole) and histamine H_2 receptor blocker (Ranitidine) are extensively used to control increased acid secretion and acid related disorders caused by stress, non-steroidal anti-inflammatory drugs (NSAIDs) and Helicobacter pylori but there are reports of adverse effects and relapse in the long run ***(Gisbert et al., 2004).*** Most of the herbal drugs reduce the offensive factors and proved to be safe, clinically effective, better patient tolerance, relatively less expensive and globally competitive ***(Goel et al., 2005)***.

The aim of the present study was to detect the effect of *Ocimum basilicum* leaves extract on ethanol-induced gastric mucosal injury and gastric secretion.

MATERIALS AND METHODS

Chemicals and drugs: Absolute ethanol, Sodium hydroxide (Sigma Chemical Co.); Cimetidine (Smith Kline and Beecham), Phenolphthalein (E. Merck Darmstadt) and Chloroform (Aldrich Chemical Co.) were used.

Preparation of the plant extract: Fresh leaves of basil were obtained from a farm in Kalubiya state. These fresh basil leaves were smashed using a pestle and a mortar then blended. The extraction was carried out using 70% methanol. The mixture was agitated over a mechanical shaker for 12 hours. The resulting mixture was filtered, and the filtrate was concentrated into a residue over water bath ***(Brian and Turner, 1975).*** Ocimum basilicum extract was dissolved in distilled water and used in the study.

Animals: A total number of fifty adult male albino rats of local strain weighing between 150 and 185 grams were used for this study. The animals were housed under similar standard environmental conditions in cages (35×30×35cm for 5 rats) with wide meshed raised floors to prevent coprophagia. The rats were kept on ad libitum food and tap water at room temperature and natural light/dark cycle. The animals were fasted for 36 hours before the commencement of the experiment but allowed free access to water. The animals were randomly distributed into four groups as follows:

(1) Control group (10 rats) received 1ml of oral distilled water per rat.

(2) Ethanol – treated group (10 rats): received 1ml each of 70% ethanol orally per rat ***(Hollander et al., 1985).***

(3) Cimetidine plus ethanol-treated group (10 rats) received 100 mg/kg b.w. cimetidine subcutaneously per rat ***(Satoh et al., 1983)*** for 7 days. One hour after the last dose, 1ml of 70% ethanol was administered orally.

(4) ***Ocimum basilicum*** **extract plus ethanol-treated group (20 rats)** each rat received the extract in two different dose concentrations (100 and 200 mg/kg b.w. orally) for 7 days. One hour after the last dose, 1ml of 70% ethanol was administered orally.

Each group was subdivided equally into two groups, i.e. one for studying of the effect on gastric mucosal injury, and the other for studying of the effect on gastric secretion

Acute Toxicity Study: It was conducted according to the method of ***Lorke (1983).***

Study of Gastric Ulceration: In the day of the experiment and after 36 hour fasting but allowing water, each rat was given 1ml of 70% ethanol orally one hour after the last dose of the extract or cimetidine. One hour after ethanol administration, the animals were sacrificed by cervical dislocation ***(Al-Harbi et al., 1997)***. Then, the stomachs were removed and opened along the greater curvature, rinsed slowly with normal saline, then stretched out as much as possible on No. 1 Whatman's filter paper. The ulcerated surfaces in each stomach were measured with a transparent millimeter scale rule. The result for each group was expressed in mm of mean ulcer ± S.E. (ulcer index = U.I.) ***(Scepovic and Radmanovic, 1984).*** Preventive index (P.I. %) was later calculated according to the method of ***Hano et al. (1976)*** as follows:

$$\text{P.I. \%} = \frac{\text{U. I. ethanol} - \text{U. I. extract (or cimetidine) plus ethanol}}{\text{U. I. ethanol}} \times 100$$

Study of Gastric Secretion: After 36 hours fasting with free access to water, the rats were administered 1 ml of 70% ethanol one hour after the last dose of the extract or cimetidine. Then, under light anaesthesia, the abdomen was opened by midline incision and the stomach was identified. A pyloric ligation was made to collect gastric juice according to the technique of ***Shay et al. (1954***) as modified by ***Levine (1965)*** where esophageal ligation was avoided. The abdomen was closed by sutures and cleaned thoroughly with saline. The anaesthesia was discontinued and animals were allowed to recover for a period of three hours. After the three hours, the animals were sacrificed and the abdomen of each animal was opened and the esophagus was ligated, then the stomach was removed and washed with saline. An opening along the greater curvature was made and the gastric contents were drained into a graduated centrifuge tube and then centrifuged at 3000 rpm for 10 minutes.

Analysis of the Gastric Juice

1. Volume of Gastric Juice: After centrifugation, the supernatant was measured as volume of the gastric juice.

2. Determination of the Titritable Acidity: A given volume of the gastric secretion (0.2 ml) was titrated against 0.01N NaOH using an end point of pH 7.0 as determined colorimetrically with phenol red as an indicator ***(Grossman, 1963).*** It was calculated as the milliequivalents per liter (mEq/L) which is equal to the number of milliliters of 0.1N NaOH required to neutralize 100 ml of the gastric juice**.**

$$= \frac{\text{Volume of 0.01N NaOH which neutralize 1 ml of gastric juice}}{10} \times 100$$

(Davenport, 1977).

3. Determination of Acid Output: This was calculated as µEq/h by multiplying the volume of gastric secretion per hour (ml/h) of an animal by titritable acidity (acid concentration) of gastric secretion (mEq/L) in that animal ***(Okabe et al., 1975).***

Statistical Analysis: Results were presented as mean ± SEM. (Standard Error of Mean). The data was statistically analyzed using the one way ANOVA followed by Scheffe and Duncan post-hoc tests. Results were considered to be significant when P values are less than 0.05 (P<0.05).

RESULTS

The LD_{50} of the Ocimum basilicum extract was found to be 1264.9 mg/kg. Preliminary phytochemical screening of the Methanolic extract of Ocimum basilicum revealed the presence of tannins, resins, steroids, glycosides, reducing sugars, flavonoids and saponins.

Table (1): Ulcer index (mm) and the preventive index (%) in the different rat groups.

Groups / Parameters	Control group (n= 5)	Ethanol treated group (n= 5)	Cimetidine + ethanol - treated group (n= 5)	*Ocimum basilicum* + ethanol – treated group	
				100mg/kg (n= 5)	200mg/kg (n= 5)
Ulcer index (mean ± S.E.M.)	0.00 ± 0.00	26.70 ± 5.62	2.50 ± 1.76*	12.20 ± 5.28*	4.20 ± 1.83*
Preventive index (%)	100	-	90.6	54.3	84.3

- n: Number of rats in each group
- Values were statistically significant = * compared to ethanol – treated group when p < 0.05.

Ethanol (70%) produced gastric mucosal ulceration in the corpus with an ulcer index of 26.70 ± 5.62. Administration of cimetidine (100mg/S.C.) produced a significant reduction in the ulcer index (2.50 ± 1.76) as compared to ethanol-treated group with a preventive index of 90.6%.

Table (2): Changes in gastric secretion in the different groups (mean ± SE) for control, ethanol – treated, cimetidine + ethanol – treated and *Ocimum basilicum (O.b.)* extract + ethanol – treated groups..

Groups / Parameters	Control (n= 5)	Ethanol (n= 5)	Cimetidine + + ethanol (n= 5)	*O.b.* extract + ethanol	
				100mg/kg (n= 5)	200mg/kg (n= 5)
Volume (ml/3h)	3.68 ± 0.4	1.90 ± 0.4*	2.76 ± 0.3	2.90 ± 0.6	2.52 ± 0.2
Titritable acidity (mEq/L)	30.00 ± 5.2	17.00 ± 2.0*	25.00 ± 1.6	10.00 ± 1.6*	18.00 ± 3.7*
Acid output (µEq/h)	30.44 ± 8.3	12.66 ± 2.1*	22.52 ± 1.4	8.55 ± 1.2*	14.52 ± 2.5*

- n: Number of rats in each group.
- Values were statistically significant = * compared to control group when p< 0.05.
- Values given mean ± S.E.M.
- O.b: Ocimum basilicum.

As regards the Ocimum basilicum extract plus ethanol-treated group, it showed ulcer indices of 12.20 ± 5.28 and 4.20 ± 1.83 for the dose concentrations of 100 and 200 mg/kg respectively, which are significantly lower than that of ethanol-treated group. The preventive indices of the smaller and larger doses are 54.3 and 84.3% respectively. Besides, the larger dose of the extract (200 mg/kg) showed a preventive index (84.3%) which similar to that of cimetidine 90.6%.

Ethanol-treated group produced marked decrease in volume, titritable acidity and acid output as compared to control group. Administration of cimetidine showed significant reduction in titritable acidity and acid output, while the volume did not report any change as compared to the control group. When compared with the ethanol-treated group, the cimetidine-treated group did not report any change in all parameters of gastric secretion. In terms of the two doses of Ocimum basilicum (100 and 200 mg/kg) plus ethanol-treated rats, both doses reported decrease in titritable acidity and acid output when compared with the control group. It was observed that the decrease was more appreciable with the smaller dose. The decrease in volume was insignificant in both doses as compared to the control group. However, there was no significant change recorded when the extract group was compared to the ethanol-treated group or when compared to each other (Table, 2).

DISCUSSION

Different studies reported that ethanol-induced damage to the gastric mucosa is associated with a significant production of free radicals leading to an increased lipid peroxidation and damage to the cell and cell membrane. Also, accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals ***(Maryam et al., 2015).*** Besides, disturbances in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effect of ethanol. Also, ethanol-induced gastric lesion may be due to stasis in gastric blood flow, hence contributing to the development of the haemorrhage and necrotic aspects of tissue injury ***(Mofleh et al., 2010).***

The results of the present study revealed that the methanolic extract of *Ocimum basilicum* produced a significant reduction of the ulcer index. The preventive index of the larger dose of the extract (200 mg/kg) showed protection nearly similar to that of the reference drug cimetidine (84.3% and 90.6% respectively).

Since ethanol-induced gastric mucosal damage is associated with reduction in all parameters of gastric secretion, so the acid output has no (or minimal) role in pathogenesis of the ethanol-induced gastric mucosal damage but it can worsen it when it is developed ***(Edy, 2002).*** In addition, O. basilicum extract did not produce changes in the different parameters of gastric secretion as compared to the ethanol-treated group. Hence, the significant protection offered against ethanol-induced gastric mucosal injury by Ocimum basilicum can be attributed to other factors than the affection of gastric secretion ***(Chalchat and Ozcan, 2008).***

The high amount of essential fatty acids especially linalool and eugenol in Ocimum basilicum is likely to be responsible for the higher antioxidant activity of the basil oil ***(EL-Moselhy et al., 2008).*** These acids can block the activity of an enzyme in the body called cyclooxygenase 2 (COX 2). Another study by ***Vats and his co-workers (2004)*** who reported that the basil also has an inflammatory-cascade normalizing action that promotes healthy metabolism and activity of arachadonic acid, prostaglandins, leukotrienes and platelets. Basil is a natural COX-2 enzyme modulator, by stopping the cascading effect caused by COX-2 enzyme. It is also an antioxidant that helps to support the body's functions and maintain them in a normal range by neutralizing free radicals ***(Haithem et al., 2014).***

It has been reported that leukotriene antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol-induced gastric ulceration in rats. So the protection afforded by the Ocimum basilicum extract against alcohol-induced gastric mucosal damage could also be due to inhibition of the 5-lipoxygenase pathway or leukotriene antagonistic activity ***(Sarhan and Orooba, 2015).*** The lipoxygenase inhibiting, histamine antagonistic and antisecretory effects of Ocimum sutave (same family of Ocimum basilicum) extract could probably contribute towards anti-ulcer activity ***(Tan et al., 2002; Goel et al., 2005).*** Thus, Ocimum basilicum extract could be considered to be a drug of natural origin which possesses both anti-inflammatory and anti-ulcer activity.

The results of our study are consistent with the study of ***Mahmood et al. (2007)*** who reported that the seed extracts of *Ocimum basilicum* possesses antiulcerogenic activity. Furthermore, other studies carried out on other family members of *Ocimum basilicum* have revealed their use in gastroprotection.

Kath and Gupta. (2012) reported that gastric ulceration and secretion have been inhibited by Ocimum sanctum (Tulsi) in albino rats. This curative effect of Tulsi in the treatment of gastric ulcer has been attributed to antiulcerogenic action of eugenol and essential oil extracted from Tulsi leaves.

Essential oils extracted from the leaves of Ocimum sanctum L. have also been found to inhibit in-vitro growth of *E. coli, B. anthracis* and *P. aeruginosa* showing its antibacterial activity. However, further investigations are required to isolate the active components of this extracts from Ocimum family to elucidate their exact mechanism of anti-ulcerogenic activity ***(Mahboobeh et al., 2006).***

In conclusion, it appears that methanolic extract of Ocimum basilicum leaves possess antiulcerogenic activity, however, further investigations are required to isolate the active components of this extract to elucidate the exact mechanism of its anti-ulcerogenic activity.

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تأثير مستخلص الميثانول لأوراق البازلاء على تلف بطانة المعدة المحدث بمادة الإيثانول في ذكور الجرذان البيضاء البالغة

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خلفية البحث: يتجه العالم الآن إلى العلاج بالأعشاب الطبيعية كبديل للطب الدوائي حيث يتميز بقلة الأعراض الجانبية.

الهدف من البحث: تقييم ومعايرة قدرة مستخلص الميثانول لأوراق البازلاء على حماية بطانة المعدة المتلفة بمادة الإيثانول في الجرذان.

طرق ومواد البحث: إستخدم في هذا البحث خمسون جرذا ذكراً أبيضاً بالغا من سلالة محلية كنموذج للدراسة. ويتراوح وزن كل جرذ ما بين 150-185 جراما وقت بداية الدراسة. وقد قسمت الفئران إلى أربع مجموعات كالآتي: مجموعة ضابطة ومجموعة تم إعطاؤها الإيثانول ومجموعة تم إعطاؤها مستخلص الميثانول لأوراق البازلاء مع الإيثانول والمجموعة الأخيرة تم إعطاؤها عقار السيميتيدين مع الإيثانول. وقد أعطيت الجرذان مستخلص الميثانول لأوراق البازلاء بجرعتين مختلفتين 100 و200 ملجم/كجم من وزن الجرذ عن طريق الفم، أما عقار السيميتيدين فقد تم إعطاؤه بجرعة 100 ملجم /كجم من وزن الجرذ تحت الجلد لمدة سبعة أيام، ثم بعد ساعة من نهاية السبعة ايام أعطيت المجموعتين الثالثة والرابعة جرعة واحدة من الإيثانول تركيزه 70% بجرعة (1 ملى/ جرذ) عن طريق الفم.

وتم سحب عينات الدم في آخر التجربة وذلك لقياس:

- مؤشر حدوث القرحة.
- مؤشر منع حدوث القرحة.
- حجم إفراز العصارة المعدية .
- الحموضة المعيارية.
- الشق الحامضي المنتج من المعدة.

نتائج البحث: تأثير مستخلص الميثانول لأوراق البازلاء يعتمد على التركيز حيث وجد أن مؤشر حدوث تلف بطانة المعدة هو54.3 % و84.3 % للجرعة الكبيرة والصغيرة على الترتيب. كما لوحظ ان مؤشر حماية بطانة المعدة لخلاصة أوراق البازلاء بجرعته الكبيرة مشابها لعقار السيميتيدين.

الإستنتاج: إستخدام مستخلص الميثانول لأوراق البازلاء يمكن أن يكون ذا فائدة تطبيقية وعاملا مساعداً في حماية بطانة المعدة من التلف المحدث بمادة الإيثانول.