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Study on *Ocimum Sanctum, Abutilon Indicum* and *Triumfetta Rhomboidea* Plants for its Combined Anti-Ulcer Activity

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ABSTRACT

The cause of ulceration in patients is mainly due to excess secretion of gastric juice and pepsin. As most of the prescribed synthetic drugs effective in treating ulcer were found to cause some potential side effects, the usage of natural drugs were found to be the safer alternatives to cure ulcers. Hence a number of herbal preparations have been used for the treatment of peptic ulcers. In view of this in the present study Poly Herbal Formulation (PHF), containing the extracts of *Ocimum sanctum, Abutilon indicum and Triumfetta rhomboidea* were evaluated for its combined Anti-Ulcer activity over Ethanol and Indomethacin induced ulcers in rats. Our study shows that PHF has the potential anti-ulcer activity at the dose level of 200mg/kg and 400 mg/kg which is comparable with that of standard drugs like Misoprostol (0.012mg/kg) and Omeprazole (10mg/kg) in Indomethacin and Ethanol induced ulcers respectively. The anti ulcer activity of formulation was found to be dose significant.

Keywords: Poly Herbal Formulation, Anti-ulcer, Anti-Oxidant, Cytoprotective.

INTRODUCTION

Ulcer is the one of the most common disorder that people suffer from. It is a wound inside the stomach or duodenum. It occurs due to localized destruction of the inner wall (mucosa) of the stomach (gastric ulcer) or the upper part of the small intestine (duodenal ulcer). It is usually associated with the hyperacidity. The causes of peptic ulcer are complex and generally caused due to chemicals, physiological stress, improper diet habits or prolonged disturbances in the daily routine. Most of the prescribed synthetic drugs effective in treating ulcer were found to cause some potential side effects (Barocelli *et al*., 1997). The natural drugs were found to be the safer alternatives to cure ulcers. In this study we evaluated the anti-ulcer efficacy of a Polyherbal formulation containing the extracts of *Ocimum sanctum, Abutilon indicum and Triumfetta rhomboidea* with potent antioxidant activity in Ethanol and Indomethacin induced gastric ulcers in rats **(**Madhava Chetty *et al.,* 2008).

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MATERIALS AND METHODS

Plant Material

Fresh leaves of *Ocimum sanctum, Abutilon indicum and* roots of *Triumfetta rhomboidea* were collected from Sri Venkateswara University region in Tirupati, Andhra Pradesh. The taxonomical identification of the plant was done by Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu **(**Madhava Chetty *et al.,* 2008). The voucher specimen for the plants *Ocimum sanctum* (Ref. No. **PARC/2011/833),** *Abutilon indicum* (Ref. No. **PARC/2011/834)** and *Triumfetta rhomboidea* (Ref. No. **PARC/2011/835)** was preserved in laboratory for future reference.

Extraction

The collected leaves and roots of the plants were shade dried and powdered in a grinder mixture to get coarse powder and then passed through 40 mesh sieve. The powdered leaves and roots (120g) were later extracted with methanol using Soxhlet extractor separately. The extract was evaporated to dryness, gave a residue 12% W/W.

Poly Herbal Formulation (PHF)

The Poly Herbal Formulation was prepared by dissolving the extracts of *Ocimum sanctum, Abutilon indicum and Triumfetta rhomboidea* in suitable vehicle at a ratio of 1:2:1 respectively.

Phytochemical screening

A Preliminary phytochemical screening of Methanolic extract of *Ocimum sanctum, Abutilon indicum and* roots of *Triumfetta rhomboidea* was carried by using standard procedures described by Khandelwal (Khandelwal, 2003).

Animals

Inbred adult wistar rats (150-200g) of male/female sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The rats were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water was provided *ad libitum* throughout experimentation period. Rats were acclimatized to laboratory conditions one week prior to initiation of various experiments. A group of eight animals were used for all the experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). **(Reg. No. IAEC/ 930/a/06/ CPCSEA).**

TOXICITY STUDIES

Acute Toxicity Studies

An acute toxicity study was carried out to determination of LD_{50} values by using different doses of the poly herbal formulation as per the method described by Ghosh *et al*., 1984 from the toxicity study, it was indicated that the extract is safe up to dose 2.0g/kg body weight. It is very safe for further studies at different doses.

Ethanol induced gastric ulceration

Grouping

Group 1: Negative control (Saline water)

Group 2: Positive control (Standard drug, Omeprazole (10mg/kg, p.o) for Ethanol induced method and Misoprostol (0.012mg/kg, p.o) for Indomethacin induced method)

Group 3: PHF 200mg/kg, p.o

Group 4: PHF 400mg/kg, p.o

Procedure

The experiment was performed according to the method of Morimoto et al. (Morimoto *et al.,* 2006). After 12 hour of fasting; the rats were randomly divided into four groups of eight animals each. First group was given 1ml of vehicle (Distilled water), and the second group was treated with Omeprazole 10mg/kg, p.o. The third group receives 200mg/kg of PHF and fourth group receives 400mg/kg of PHF respectively. All the treatments were administered orally. One hour after treatment, all the rats received 1ml of 99.5% ethanol to induce gastric ulcer. One hour later, the animals were sacrificed by cervical dislocation, and the stomachs removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots, for subsequent scanning.

Indomethacin-induced gastric ulcers

Indomethacin (30mg/kg b.w.) suspended in 0.5% carboxymethyl cellulose was given as a single oral dose to induce gastric ulcers after 30 min of test or standard drug treatment. Misoprostol was given as reference drug (0.012mg/kg, p.o). After 5hrs, the animals were killed and lesions in the gastric mucosa were scored. The tissues were subjected to histopathological studies. The numbers of ulcers per stomach are noted and severity of the ulcers scored microscopically with the help of hand lens (10x) and scoring was done.

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcers
1.5 = Hemorrhagic streaks
2 = Ulcer > 3 mm but < 5 mm
3 = Ulcers > 5 mm.

Ulcer index=$U_A+U_S+U_P/10$.

Where, U_A=Average number of ulcers per animal, U_S=Ulcer severity score, U_P=Percentage of animals with ulcers.

This can be calculated as, U_P=Total ulcers in a group/total number of animals in a group x 100.

Percentage ulcer inhibition was calculated by the formula,

Percentage inhibition=UIC-UIT/UIC X 100

Where UIC=Ulcer index of control group, UIT= Ulcer index of test group.

Statistical Analysis

All values were expressed as mean ± SEM. The data were statistically analyzed by ANOVA Followed by Dunnets't test (Eixeira CC *et al*., 1990).

RESULTS

Phytochemical screening

The Preliminary Phytochemical studies of Poly herbal formulation revealed the presence of alkaloids. Tannins, proteins, glycosides, flavonoids and carbohydrates saponins, steroids and glycosides as shown in Table.1.

Acute toxicity Studies

From the toxicity study it was observed that PHF is non-toxic and caused no death up to 2 g/kg orally. The results presented in Table.2.

EFFECT OF PHF ON INDOMETHACIN INDUCED GASTRIC ULCER

The Polyherbal formulation (PHF) showed the antiulcer activity dose dependently in Indomethacin induced gastric ulceration, but it was found lesser compared to the standard that is Mesoprostal. Before the results been discussed the actual ulcerative mechanism of Indomethacin should be prior studied. Indomethacin is an anti-inflammatory drug which decreases the synthesis of pain mediators, prostaglandins that are main reasons for inflammatory responses. This lowering in the production of prostaglandins will result in the increase of gastric acid secretion and reduction in the mucosal secretion thereby causing peptic ulcers. Concerning this mechanism Mesoprostal, a prostaglandin analogue was used a standard. Observation of the results, the antiulcer activity of PHF was lesser than that of standard in the Indomethacin induced ulceration suggests the actual antiulcer mechanism of the extract is certainly not the Prostaglandin mechanism. But there might be a wound healing activity and antioxidant activity of the Polyphenols that was responsible for the antiulcer activity. Overall, PHF 400 mg/kg was much higher

Table.1 Phytochemical screening of Poly Herbal Formulation (PHF)

S.No	COMPOUNDS	MEOS	MEAI	METR
1	Carbohydrates	+	+	+
2	Alkaloids	+	+	-
3	Glycosides	+	+	+
4	Phytosteroids and sterols	+	+	+
5	Flavonoids	+	+	+
6	Triterpinoids	+	-	+
7	Phenolic compounds and Tannins	+	+	+
8	Fixed oils	+	-	-

MEOS –METHANOLIC EXTRACT OF *Ocimum sanctum*
MEAI– METHANOLIC EXTRACT OF *Abutilon indicum*
METR – METHANOLIC EXTRACT OF *Triumfetta rhomboidea*
\+ indicates presence
– indicates absence

Table.2 Acute Toxicity test studies of Poly Herbal Formulation (PHF)

S.No	Groups	Dose/kg (body weight)*p.o*	Weight of Rats		Signs of Toxicity	Onset of Toxicity	Duration of Study
			Before Test	After Test			
1	PHF	2000 mg	160 g	160 g	No signs of Toxicity	Nil	14 days
2	PHF	2000 mg	180 g	180 g	No signs of Toxicity	Nil	14 days
3	PHF	2000 mg	200 g	200 g	No signs of Toxicity	Nil	14 days

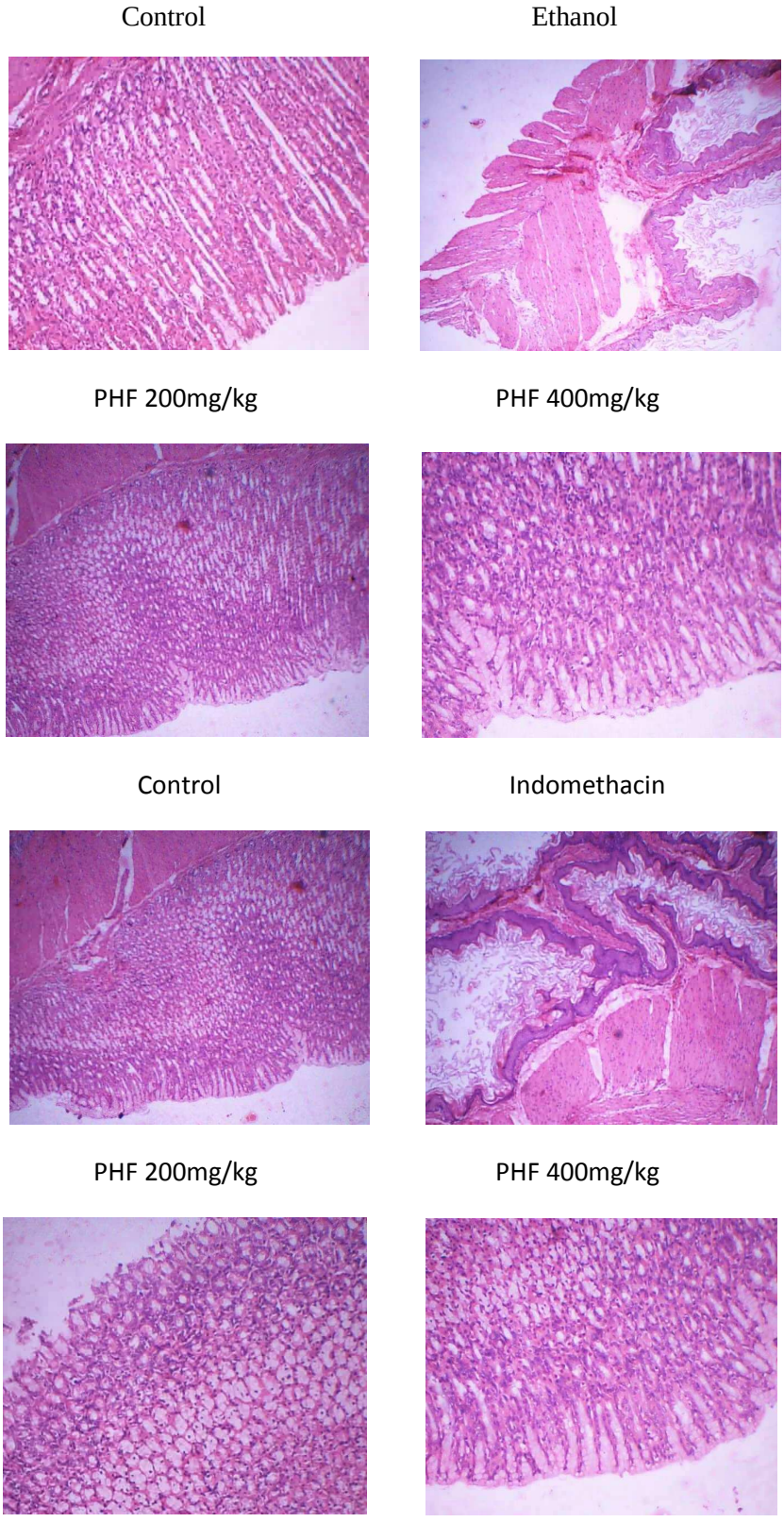
Table.3 Ulcer index and Percentage inhibition in Indomethacin induced model

Group	Treatment	Ulcer index	Percentage ulcer inhibition
1	Negative control (Saline water)	14.27±0.25	-
2	Positive control (Omeprazole (10mg/kg, p.o)	5.47±0.17	61.66%
3	PHF 200mg/kg, p.o	10.19±0.49	28.59%
4	PHF 400mg/kg, p.o	7.47±0.12	47.65%

Table.4 Ulcer index and Percentage inhibition in Ethanol induced model

Group	Treatment	Ulcer index	Percentage ulcer inhibition
1	Negative control (Saline water)	16.27±0.02	-
2	Misoprostol (0.012mg/kg, p.o)	4.56±0.64	71.97%
3	PHF 200mg/kg	12.45±0.76	23.47%
4	PHF 400mg/kg	6.06±0.024	62.70%

Figure.1 Histopathological studies of antiulcer activity in Ethanol and Indomethacin induced model



potent in lowering the ulcers, followed by PHF 200mg/kg. As it was said, the activity is dose dependent PHF 200mg/kg and PHF 400mg/kg follow their higher doses in producing the antiulcer activity. The results are shown in table3.

Effect of PHF on Ethanol Induced Gastric Ulcer

Poly herbal formulation (PHF) showed antiulcer activity in Ethanol induced ulceration in a dose dependent manner. The percentage inhibition of the PHF at 400 mg/kg was better than that of the standard and the lesser doses of 200mg/kg were also seemed to possess the similar activity with that of the standard. Omeprazole antiulcer mechanism was found to be inhibition of so called Proton pump by inhibiting the enzyme H-K ATPase of the gastric parietal cells. Results suggest that the wound healing with that of the extracts was much better and the antioxidant capacity of the polyphenols present in the extract helped in the activity. The mechanism still can be supported through the ulcerative mechanism of ethanol. As discussed above Ethanol generates free radicals which decrease the resistance of mucosal barrier and there by destroy the integrity of membranes. This also results in the leakage of proteins into the gastric juice. This disruption of cell membranes might probably be due to lipid peroxidation of the produced free radicals. Finally, this suggests the cytoprotective activity was due to the antioxidant mechanism of the extracts. The results are shown in table.4.

The histopathology of the tissues was performed and the results were shown in figure 1. This explains the mechanism behind healing of ulcer and the tissue structure when treated with drugs. In figure the control group is characterized with a large number of neutrophils and megre fibroblasts which are responsible for ulcer healing. A significant integrated structure of mucosa is clear in the tissue treated with PHF and PHF at doses 400mg/kg. This is due to the potent antiulcer healing activity and cytoprotective activity of Extracts compared to the standard.

DISCUSSION AND CONCLUSION

There are several factors that may induce ulcer in human being such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, among others. (Barocelli *et al.*, 1997). Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. (Piper and Stiel., 1986, Sergio *et al.*, 2007). The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastro duodenal mucosa or by increasing mucosal resistance against them. It has become imperative to scrutinize herbal products for evaluating their acclaimed properties, as recently numbers of herbs are being introduced in the market. Keeping this view, we have attempted to study the PHF for its antiulcer activity by using different experimental models of gastric ulcer, Indomethacin induced and ethanol induced ulcer, which operate by distinct mechanisms of ulcerogenesis.

Oral administration of the damaging agent to the control group clearly produced a mucosal damage characterized by multiple hemorrhage red bands of different sizes along the long axis of the glandular stomach as described in other studies. (Shay *et al.,* 1945, Yassir *et al.,* 1999). Pretreatment with PHF (400 mg/kg) produced significant decrease in the intensity of gastric mucosal damages induced by the necrotizing agent ethanol compared with control group. Cyto-protective action by drugs has been considered to be due to the generation of prostaglandins or blockade of back diffusion of H+ ions (De B *et al.*, 1997) will be the major mechanism which is responsible for anti-ulcer activity. The PHF significantly reduced the gastric acid secretion in the present study.

The cyto-protective action which promotes the generation of prostaglandin and causes decreases in secretion of gastric acid, mixture significantly reduced the gastric ulceration without affecting the gastric secretion or pepsin. But in case of ethanol induced method, the cytoprotective action has been decreases by ethanol, which is due to inhibition of synthesis of endogenous prostaglandin, which promote the formation of ulcer, the protective effect of PHF against ethanol induced ulcer in rats may show the anti-ulcer activity by decreasing the ulcer scores.

The result further point out that, the cyto-protection may be major mechanism responsible for the antiulcer activity of the PHF. PHF may show the anti-ulcer activity against both models by generating the prostaglandin which causes inhibition of secretion of gastric fluid.

Our study shows that PHF containing *Ocimum sanctum*, *Abutilon indicum* and *Triumfetta rhomboidea* has the potential anti-ulcer activity at the dose level of 200mg/kg and 400 mg/kg which is comparable with that of standard drugs like Misoprostol (0.012mg/kg) and Omeprazole (10mg/kg) in Indomethacin and Ethanol induced ulcers respectively. The anti ulcer activity of formulation was found to be dose significant. The mechanism of action responsible for antiulcer activity of PHF may be cytoprotective action or antioxidant property of various herbs it contains.

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