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ANTI-ULCER EFFECT OF FOUR INDIAN MEDICINAL HERBAL PLANT EXTRACTS IN ETHANOL INDUCED ULCER AND LEVELS OF ANTIOXIDANT ENZYMES IN WISTER RATS

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ABSTRACT

This study was aimed to evaluate the efficacy of methanolic extracts of four Indian medicinal plant extracts *Nyctanthes arbor-tristis, Moringa Olefera, Catharanthus roseus* and *Jetropha curcas* for their Antiulcer and Anti-oxidant activities. Seven groups of male wister rats were used for the study, each group contains six rats. The ulcer was induced in test groups of rats by the administration of 1ml of absolute ethanol after 24 hours of fasting. The animals were sacrificed and ulcer intensity and ulcer index were measured and compared after treatment with methanolic extract of these four medicinal plants. The Anti-oxidant levels (Superoxide dismutase and Catalase) were also evaluated and compared. The comparative evaluation shows that *Catharanthus roseus* and *Moringa olefera* causes marked reduction in ulcer severity, and also increases the levels of Antioxidant enzymes.

KEYWORDS: *Nyctanthes arbor-tristis, Moringa olefera, Catharanthus roseus Jetropha curcas*, Anti- oxidant enzymes.

INTRODUCTION

Peptic ulcer or Gastric ulcer is the most common gastrointestinal disorder. It is responsible for mortality and morbidity of people worldwide. Various risk factors are involved for the development of this disorder, including *Helicobacter pylori* infection, imbalance between aggressive factors (increased gastric secretion and acid pepsin secretion), and defensive (mucus layer and bicarbonate secretion).increased levels of reactive oxygen species and decreased levels of Anti-oxidant enzymes like Superoxide dismutase and catalase may also lead to the development of disorder. Many types of drugs like Proton pump inhibitor, prostaglandin analogs Histamine receptor antagonist and cytoprotective agents are used for the effective management of this disorder. But these drugs produce severe adverse side effects and contraindications and are costly. Ayurvedic, Unani and Homeopathic systems of medicine are using herbal plant extracts for the effective management and curing of this disorder. Plants extract shows less contraindications and toxicities and can be easily prepared and are also less costly. The selected four herbal plants produce certain phytochemicals which may be helpful for the reduction of ulcer severity and ulcer index. Some of the phytochemicals produced by these plants are as follows-

1- ***Nyctantyhes arbor-tristis***-: the leave extract of N. arbor-tristis containsalkaloids, glucosides, saponins tannins, flavanoids and steroids.
2- ***Moringa olefera***-: the leaves extracts of this plant shows the presence of flavanoids tannins, glycosides, and terpenoids.
3- ***Catharanthus roseus***-: The *Catharanthus roseus* contains two classes of active compounds Alkaloids and tannins. More than 100 alkaloids are reported in this plant in which Vincristine and Vinblastin are more notable (Neeraj et al, Madhvi et al, 2011).
4- ***Jetropha curcas***-: This plant is resistant to drought and pests and produces seeds that contain 27 to 40% oil. Extracts of this plant shows significant Anti-microbial and Antioxidant activities.

METHOD

Grouping of Animals- The male wister rats weighing between 120 to 180g were divided into following groups-

Groups of ethanol induced rats.

Group1	Control, untreated
Group 2	Ethanol induced ulcer, control
Group 3	Ethanol induced ulcer treated with standared drug
Group 4	Ethanol induced ulcer treated with *N. arbor-tristis* leaf extract
Group 5	Ethanol induced ulcer treated with *J. curcas* leaf extract
Group 6	Ethanol induced ulcer treated with *Catharanthus roseus* leaf extract
Group 7	Ethanol induced ulcer treated with *Moringa olefera* leaf extract

EXTRACTION OF PLANT MATERIAL

The extraction of plant material was carried out in Soxhlet extractor. The extraction vessel was made up of borosil glass (J.SIL) which includes round bottom flask, extractor and condenser. The plant material to be extracted was weighed and packed in the extractor and heated under reflux. The aqueous extraction with distilled water was carried out. The average time period of extraction was 48 hrs. Heat was supplied through heating mantle. The extract was collected directly from round bottom flask and solvent was evaporated using Rota flask evaporator. The individual extract was filtered, concentrated in vacuum followed by freeze storage for further use.

ACUTE TOXICITY

The acute oral toxicity study was carried out by Panchal *et al.*, (2010) as per the guidelines set by Organization for Economic Co-operation and development (OECD) 423. The median lethal doses of methanol and extract were determined by orally administering the extracts in increasing dose levels of 50, 100, 200, 500 and 1000 mg/kg body weight to healthy adult Wistar albino rats of either sex. All the extracts were found to be safe. On the basis of literature reviews, the study was carried out at dose level of 100 mg/kg body weight for the present study. So, it was considered as safe drug.

3.2.2. Parameter Assessed

A. Antiulcer activity

Physical Parameters

- Ulcer score.
- Ulcer index.

B. Antioxidants activity

- Catalase level.
- Super oxide dismutase level.

Measurement of Various Parameters

A- Antiulcer activity

a) Ulcer score

The following arbitrary scoring system was used to grade the incidence and severity of lesion, Kulkarni (2002).

Normal stomach.....0
Red coloration.....0.5
Spot ulcer.....1.0
Hemorrhagic streak.....1.5
Ulcers.....2.0

b) Anti-oxidant enzyme level score

Negligible level……0
Mild level……1
Moderate level……2
High level……3

Biochemical Parameters

a) Catalase

CAT is a ubiquitous heme protein that reduces H_2O_2 to water. Hence catalase activity was determined by measuring decreasing absorbance of hydrogen peroxide.

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Procedure

100 µL of supernatant was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2. Decrease in absorbance was read at 240 nm for 3min at interval of 30 sec. The activity was calculated using extinction coefficient of H_2O_2 0.041µM/cm^2. Results were expressed as micromole of H_2O_2 utilized/min/gm tissue. The rate of Decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm mentioned by Sinha *et al.*, (1972).

b) Superoxide dismutase Assay

This assay relies on the ability of enzyme to inhibit the phenazine methosulphate (PMS) mediated reduction of NBT dye. Reaction is initiated by the addition of PMS, and the increase in absorbance at 560 nm due to formation of reduced NBT recorded on spectrophotometer. SOD shows to inhibit the initial rate of PMS induced reduction of NBT hence less absorbance reported by Misra *et al.*, (1972).

Procedure

Prepare 10 % w/v tissue homogenate in 0.15 M Tris HCl or, 0.1 M phosphate buffer Centrifuge at 1500 rpm for 15 min at 4°C. Take supernatant (0.1 mL), consider it as sample. Take 0.1 mL sample add 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL phenazine methosulphate (186 µM), 0.3 mL of 300 µM Nitroblutetrazolium and 0.2 mL NADH (750 µM) and incubate at 30°C for 90s. Add 0.1 mL glacial acetic acid stir with 4.0 mL n-butanol, allow to stand for 10 min, centrifuge and separate butanol layer and take OD at 560 nm (take butanol as blank).

RESULT AND DISCUSSION

The methanolic leaf extracts of *Catharanthus roseus* and *N. arbor-tristis* showed marked reduction in ulcer severity. Treatment with these plant extracts also increases Anti-oxidant enzyme levels. *J. curcas* leaf extract showed marked antibacterial activity therefore it can be used for the management of gastric ulcer caused by *H. pylori.*

Proper use of these plant extracts can be helpful in the management of Gastric ulcer, Peptic ulcer and other types of cancers, because these plant extracts also increases the levels of Antioxidant enzymes.

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