EVALUATION OF ANTI-ULCER ACTIVITY IN ETHANOLIC EXTRACT OF *WEDELIA TRILOBATA (L.)A.HITCHC*.

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ABSTRACT

Aim of the study: The aim of this research was to investigate the phytochemical profile and evaluation of Anti-ulcer activity of ethanolic extract of Indian Medicinal plant of *Wedelia trilobata (L.)A.Hitchc*. **Materials and methods:** Qualitative phytochemical analysis for their phytoconstituents. Ethanol was used to extract the crude bio active compound from whole *Wedelia trilobata (L.)A.Hitchc* plant. Phenolic derivative were isolated from the ethanolic extract of *Wedelia trilobata (L.)A.Hitchc* by using suitable solvent system. The Anti-ulcer activity of *Wedelia trilobata (L.)A.Hitchc* extract were measured by Pylorus ligated induced ulcer model in rats. **Result:** Qualitative phytochemical analysis showed phenolic compounds, Flavonoids, Glycosides, carbohydrates and tannins.*In-vivo* anti-ulcer activity in rats were studied using 100 and 200mg/kg, p.odoses of*Wedelia trilobata (L.)A.Hitchc*. The percentage of protection were significantly found to be increased, with EEWT at dose of 100mg/kg and 200mg/kg (ip). **Conclusion:** Overall results the study proved that *Wedelia trilobata (L.)A.Hitchc* possessed potential components involving in anti -ulcer treatment significantly attenuated the various types of ulcer and also increased healing of wound and protection level.

KEYWORDS
Wedelia trilobata (L.)A.Hitchc, Anti-Ulcer activity, Ethanol extract,Pylorus- Ligated (Pl)- Induced Ulcer method.

INTRODUCTION

India has 2.4% of world's area with 8% of global biodiversity. It is one of the 12 mega diversity hotspot region of the world. Other countries being Brazil, Colombia, China, South Africa, Mexico, Venezuela, Indonesia, Peru, USA, Bolivia, etc.

Across the country, the forest of India are estimated to harbour 90% of India's medicinal plants diversity. Only about 10% of known medicinal plants of India are restricted to non-forest habitats. The estimated number of plant species and those used for medicinal purpose vary. According to (Schippmann, 2002),one fifth of all the plants found in India are used for medicinal purposes. The world average stands at 12.5% while India has 20% plant species of medicinal value.

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side-effects (arrhythmias, impotence, gynacomastia and haematopoeitic changes) of modern medicinal, indigenous drugs possessing fever side-effects should be looked for as a better alternative for the treatment of peptic ulcer.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer.

The genesis of gastro duodenal ulcers requires acid peptic activity and breakdown of mucosal defense mechanism. The exact cause of ulceration is not known. Although several factors are reported to be involved in ulcer genesis, that is aggressive (acid pepsin and pylori) and offensive (gastric mucous and bicarbonate secretion, prostaglandins) mucosal factors. Peptic ulceration occurs only in area which is bathed by acid juice and it is true to say no acid no ulcers. Several of the drugs used for the treatment of ulcers are those, which can reduce or neutralize the offensive acid secretion like antacids. Some other drugs like carbinoxolon, Sucralfate, and prostaglandins analog increases the defensive factors without effecting the offensive secretion they are termed as cytoprotective drugs and are proved useful in ulcer therapy.

Wedelia trilobata is an Indian medicinal plant belonging to the family Asteraceae. Wedlia trilobata is widely found in India, Australia, Bermuda, United States, Indonesia, South Africa, Bangladesh. Especially in India, Kerala, Tamilnadu, Karnataka, and Maharastra The stem, flower and leaves of *Wedelia trilobata*are used to traditionally used as a cough syrup, wound healing and also used for the symptoms of cold and flu, fever and inflammation. There have been very few scientific studies conducted on *Wedelia trilobata,* an anthelmintic, antipyretic, CNS depressant, antibacterial, antifungal, analgesic and anti-diabetic properties of substances in its aerial parts. However, a comprehensive and exclusive study of the medicinal properties of *Wedelia trilobata* is still lacking. Hence, the present study aims to analyse the major phytoconstituent in ethanolic extract of the leaves and stems and smoothening effect of anti-ulcer activity of *Wedelia trilobata.*

MATERIAL AND METHODS

Collection of Plant Material

Fresh leaves and stems of *Wedelia trilobata* were collected in the month of February 2019. The plant material was taxonomically identified, confirmed and authenticated by ***ABS Herbal Gardens***, Vidhya nagar, Salem, Tamilnadu., India. (AUT/VMCP/141). The collected plant parts were dried under shadow. The dried material was crushed with mechanical

grinder. The resulting plant material was used for the further studies.

Animals

Wistar rats (100-125g) of either sex were obtained from Srinivasa Enterprises, Bengaluru, India, The rats were housed in the polypropylene cages with paddy husk as bedding and with stainless steel top grill having facilities for providing food and drinking water in polypropylene bottles with stainless steel sipper tube. The animals were housed at temperature 22+2'C and relative humidity of 30-70%. A 12:12 hour light and dark cycles were followed. The animals were fed with standard rodent pellet diet and water ad libitum. The Institutional Animal Ethics Committee, Chennai, India, approved (IAEC,Col/11/2017/IAEC/VMCP) experimental protocols and guidelines were followed in conducting the experiments on animals for the purpose of control and supervision of experiments onanimals.

Preparation of Extract

Collected leaves were shade dried and coarsely grounded. 600g of coarsely grounded powder material was weighed and the dried coarse powder of *Wedelia trilobata (L)A.Hitchc* is defatted with 1 liter of petroleum ether (60-80%) in soxhlet apparatus for 72 hrs. After the petroleum ether extract was removed by distillation. The defatted powder material thus obtained was further extracted with ethanol (90%) for 72 hrs in soxhlet apparatus. The petroleum ether and ethanol solvent was removed by distillation under low pressure. The resulting semisolid mass was obtained by vacuum drying using vacuum desiccator. The yield of extracts of *Wedelia trilobata (L)A.Hitchc* were weighed and the percentage yield of the extracts was calculated as follows:

$$\% \text{ of extractive yield(w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried leaves powder}} 100$$

The yield of petroleum ether and ethanol extract was 5.9% and 4.6% (w/w), respectively.

Preliminary Phytochemical Studies

Qualitative chemical tests for establishing the chemical composition profile of given extracts were performed using standard methods/protocols to detect various phyto constituents present.

Detection of Alkaloids

Alkaloids were detected by following in which solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. To few ml of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate formed indicates the test aspositive.

Detection of Carbohydrates

The extract (100mg) was dissolved in 50ml of distilled water, filtered and subjected to the following test

Fehling's test

One ml of filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicated the presence ofsugar.

Benedicts's test

To 0.5ml of filtrate Benedicts reagent was added and heated on a boiling water bath for 2min. a characteristic red precipitate formed indicated the presence ofsugar.

Detection of glycosides

50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2hr on a water bath,

filtered and the hydrolysate was subjected to the following test.

Borntrager's test

2ml of filtrate hydrolysate and 3ml of chloroform was added and mixed together. Chloroform layer was separated and treated with 10% ammonia solution. Pink color indicated the presence of glycosides.

Detection of Saponins (Foam test)

50mg of the extract was diluted with distilled water and made up to 20ml.The suspension was placed in a graduated cylinder and shaken well for 15 min. Foam layer (2cm) indicated the presence of saponins.

Detection of proteins and amino acids

100 mg of the extract was dissolved in 10ml of distilled water and filtered through Whatman No.1 filter paper. The filtrate was then subjected to tests of proteins and amino acids.

Millon's test

To 2ml of filtrate, few drops of millon's reagent were added. A white precipitate indicated the presence of proteins.

Biuret test

An aliquot of 2ml of filtrated was treated with on drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by addition of excess of potassium hydroxide pellets. Pink coloration of the ethanolic layer indicated the presence ofproteins.

Detection of Phenolic Compound Ferric chloridetest

50mg of extract in 5ml of distilled water was treated with few drops of 5% neutral ferric chloride solution. A dark green color formation indicated the presence of phenolic compounds.

Lead acetate test

50 mg of extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate was added. A dense white precipitate indicated the presence phenolic compounds.

Experimental Design: (PYLORUS- LIGATED (PL)- INDUCED ULCERS)

The animals were divided into five groups, Group-I was received only with saline solution i.e., normal control. Group-II was received negative control. Group-III was received ethanolic extract of *Wedelia trilobata* (100mg). Group-IV was received ethanolic extract of *Wedelia trilobata* (200mg). Group-V was received standard Ranitidine (50mg).

Gastric ulcers were produced in rats by the following method. Briefly the rats were fasted for 24hrs before pylorus-ligation but water was allowed ad libitum. At the end of 24hr starvation, rats were anaesthetized with chloroform. Abdomen was opened by a midline incision and a ligature was placed at the pyloric end of the stomach taking care not to exclude any blood vessels. The abdomen was then closed in two layer and rats were left in a cage with a false bottom of wide mesh wire gauze to prevent caprophagy. Water was withheld from one hour before pylorus ligation and till the end of 4 hour period when the rats are sacrificed by overdosing with chloroform. Immediately afterwards abdomen was again opened, cardiac end of the stomach was ligated and the stomach was taken out. The stomach was then cut and open along the greater curvature and the mucosa was washed under slow running tap water. The ulcer index was calculated by adding the total number of ulcers per stomach. The total severity of ulcers was determined by recording the severity of each ulcer after histological confirmation as follows:

0- No ulcer

+- Pin Point ulcer and histological changes limited to superficial layers of mucosa and no congestion,

++ - Ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes,

+++ - **Ulcer size 1-2 mm** with more than two third of mucosal thickness destroyed with marked necrosis and congestion, muscular is remaining unaffected,

+++ ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected.

HistopathologicalStudies

Stomach slices fixed for 12 hrs in 10% formaldehyde solution were processed for paraffin embedding following standard micro technique. Histopathological changes i.e., normal, damaged and recovered stomach were studied.

RESULTS AND DISCUSSION

Phytochemical Screening

The ayurvedic system of medicine includes number of plants and minerals which should be investigated to determine the hidden potential by using the modern methodology. The goal should be searching for drugs of plant origin with minimum side effects and maximum benefits. The plant ***"Wedelia trilobata (L)A.Hitchc."*** is also called as ***Sphagneticola trilobata*** is an indigenous herb which was chosen for this study. The plant belongs to the family *Asteraceae.* The scanty availability of information on this plant facilitates the study on it. Since ages various parts of this plant are being used for their medicinal use. The attempt is made to study the features, phytoconstituents and pharmacological activities of the leaves of the plant.

PHARMACOLOGICAL STUDIES:

Ethanolic extract from the plant possessed anti ulcer activity at the good extent. The extract 200mg was found more potent anti ulcer activity on comparison to the extract 100mg.

Table 1 showed the Phytochemical screening of the ethanolic extract of *Wedelia trilobata*

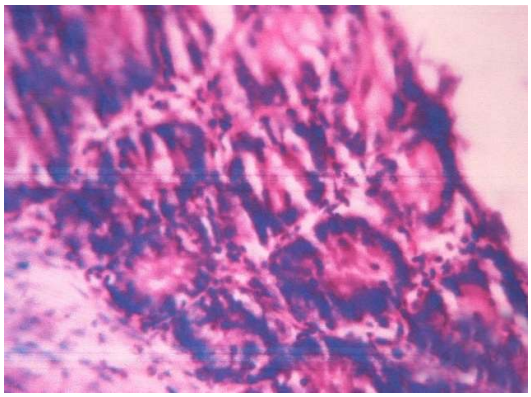
S. NO.	COMPOUND	PETROLEUM ETHER	ETHANOL
1	**Alkaloids**	**-ve**	**-ve**
2	**Glycosides**	**-ve**	**+ve**
3	**Carbohydrates**	**-ve**	**+ve**
4	**Tannins / Phenols**	**-ve**	**+ve**
5	**Flavonoids**	**-ve**	**+ve**
6	**Sterols**	**+ve**	**+ve**
7	**Tri-terpenoids**	**-ve**	**-ve**
8	**Proteins**	**-ve**	**-ve**
9	**Amino acids**	**-ve**	**-ve**
10	**Fixed oils**	**-ve**	**-ve**
11	**Fats**	**+ve**	**-ve**
12	**Mucilage**	**-ve**	**-ve**
13	**Saponins**	**+ve**	**+ve**

Table 2. Effect of *W.trilobata* (twice daily for five days) on pylorus ligation induced gastric ulcers.

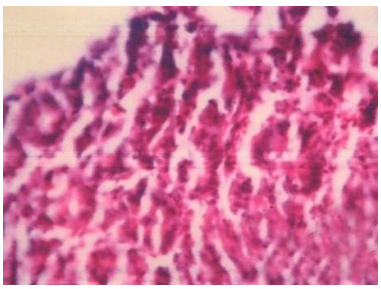
Groups	Treatment	Dose(Mg/Kg)	Ulcer index(mm^2/rat)	Percent Protection(%)
I	Normal	10 ml/kg	19.4±2.4	-
II	Negative control	-	13.8±1.91	35.74%
III	*W.trilobata*	100 mg	8.4±2.09	59.49%
IV	*W.trilobata*	200 mg	4.6±0.96*	76.43%
V	Ranitidine	50 mg	3.6±0.84	84.19%

HISTOPATHOLOGICAL STUDIES

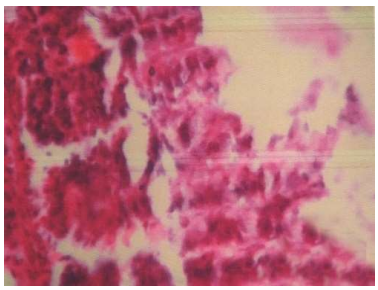
HISTOPATHOLOGY OF STOMACH



Normal (10ml/kg) fig.no:1.



NegativeControl(fig.no:1.2)



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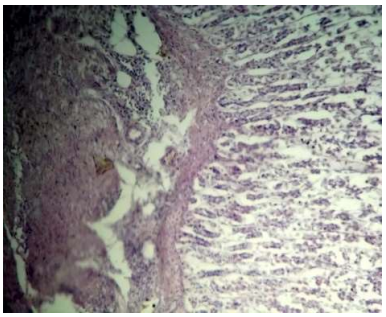
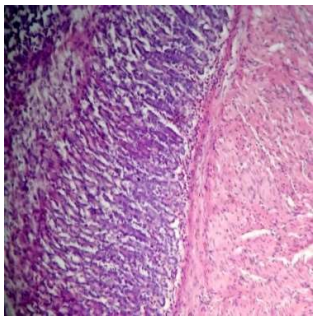


Ethanolic extract of *Wedelia trilobata* (200mg/kg) fig no:1.4



Ethanolic extract of *Wedelia trilobata* (100mg/kg) fig no:1.5

Conclusion

Based on the traditional uses, the plant was selected. The phytochemical and pharmacological studies were done on leaves of ***"Wedelia trilobata (L)A.Hitchc."*** The leaves of the plant were separated, dried under shade and subjected for Extraction process. The phytochemical constituents were extracted by successive solvent extraction. The phytochemical constituents were identified by chemical tests and these tests showed the presence of numerous active compounds like phenolic compounds and flavonoids. It may showed the good pharmacological activities.

Ethanolic extracts of leaves of ***"Wedelia trilobata (L)A.Hitchc.***"showed the presence of many phytoconstituents such as carbohydrate, glycosides, phytosterols, fixed oils and fats, saponins, flavonoids, gums and mucilage and also of sufficient quantity necessary for the studies.

In the pharmacological studies ethanolic extracts of leaves of ***"Wedelia trilobata (L)A.Hitchc***." showed significant antiulcer activity. The antiulcer activity was evaluated by using Pylorus ligated induced ulcer models in rat. It shows that flavonoids present in these extracts may be responsible for Antiulcer activity.

The histopathological results are shown in fig no 1.1 to 1.5. In case of histopathological analysis the recovery of the ulcer shown by the Ethanolic Extract 200 mg was much better than 100 mg ethanolic extract .It was nearly equal to the standard .

Future Aspects:

As per the overall study conducted we can conclude that the use of the plant ***"Wedelia trilobata (L)A.Hitchc."*** is much more beneficial for ulcer curing. In future it may be a good

anti ulcer curing. In future it may be a good Antiulcer treatment which can cure various types of ulcer completely, so it is necessary to have a detailed study on the same.

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