IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, December 2022
Impact Factor: 6.252

Evaluation of Gastro-Protective Activity of Ethanolic Extract of Roots of Vernonia Cinerea Less

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Abstract: ***Background****: Vernonia cinerea Less is a little shrub that belongs to the Asteraceae family and is found all throughout India. It grows to a height of 0.5 to 3 feet and has purple or pink flowers that bloom during the rainy season. Vernonia cinerea root has been reported to be useful against stomach acidity.*
Aim*: To evaluate gastro-protective activity of ethanolic extract of roots of vernonia cinerea less.*
Method*: The gastro-protective activity of ethanolic fraction of root of Vernonia cinerea less plant was tested by using screening technique of anti-ulcer activity.*
Results and Conclusion*: Administration of ethanolic extract of Vernonia cinerea less shows significant Anti-ulcer activity in a dose dependent manner (100mg/kg and 200mg/kg), when compared to control which is evident by decrease inulcer index. When compared to the control, the ethanolic extract of Vernonia cinerea roots exhibits less substantial anti-ulcer action in a dose-dependent manner, as evidenced by a drop in ulcer index. The ulcer index of Vernonia cinerea roots ethanolic extract is 3.16 0.600 at 100 mg/kg and 1.91 0.396 at 200 mg/kg. In comparison, the standard (Lansoprozole) mean ulcer index is 1.83 0.459.This result suggests that ethanolic extract of root of Vernonia cinerea less plant possess gastro protective(anti-ulcer) activity..*

Keywords: Gastro protective activity, Vernonia cinerea Less, Aspirin, lansoprazole, peptic ulcers

I. INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice [1]. A number of factors such as stress, chemical agents, bile salts, hyperosmolar Nacl, NSAIDs, may lead the gastro duodenal ulcer [2]. Ulcers are caused due to imbalance between aggressive and defensive factors of gastric mucosa [3]. The gastric wall mucus is thought to play a role as a defensive factor against gastrointestinal damage [4]. It is known to when the endogenous defense mechanism of the protective mucosal barrier have failed to sufficiently counteract by burning sensation in the abdomen. Duodenal ulcers more frequently (80% of PUDs) than gastric ulcers. The life time prevalence PUDs is about 10% PUDs are recurrent and most clinical studies are shown that approximately 50% of all ulcers [5]. A recent review reported that the anti-ulcerogenic potential of many plant remedies worldwide have been investigated experimentally so far and diverse molecules have been determined as the active ingredients [6]. Ulceration occurs when there is disturbance of the normal equilibrium caused by either enhanced aggression, due to excessive acid secretion or diminished mucosal resistance [7]. At the same time, each of these drugs confers simpler to severe side effects like arrhythmias, gynaecomastia, hematopoietic changes. [8]. A number of drugs are available in the world for the treatment of peptic ulcer. Such as anti-histamines, proton pump inhibitors, anti-cholinergic, prostaglandins analogues, ulcer protective and ulcer healing drugs, but their clinical evaluation has shown incidence of various adverse drug reaction. [9].
Hence the need for the newer and most safer herbal alternate to conventional gastroprotective agents is inevitable.

II. MATERIALS AND METHOD

2.1 Collection and Extraction of Plant Material

The root of Vernonia cinerea less were collected from Western Ghats area Coimbatore district (Tamilnadu). The plant was identified and authenticated by Dr. K. P. Sahu, Department of Botany, Govt. Model science The leaves were shade dried at room temperature and pulverized. college Jabalpur (M.P). A herbarium specimen is deposited in our college museum. Herbarium No. KP/09/1203.

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2.2 Animals

Albino wistar rats of either sex weighing between 150-200 gms and Albino mice of either sex weighing between 20-25gms were produced from registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad). The animals were housed under standard condition of temperature (25 ± 2 ^{0}C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water ad libitum. The experiment were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved at the Institutional Animal Ethics Committee (IAEC) of R.R.K's College of Pharmacy, Bidar was taken for conducting anti-ulcer activities.

2.3 Extraction

The authenticated whole plant of Vernonia cinerea less were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the leaves was Soxhlet extracted with the solvents with increasing order of polarity i.e. petroleum ether (60-80^0 C), chloroform (59.5 – 61.5^0 C), Ethanolic (64.5 – 65.50 C), and distilled water. After defating with petroleum ether, ethanolic extract was also prepared. The extracts so obtained were concentrated under reduced pressure.

2.4 Preliminary Phytochemical Evaluation of the Root of Vernonia Cinerea Less Extract

A. Test of Alkaloids

1. **Mayer's Test:** Take test solution in the test tube adds the Mayer reagent (Potassium mercuric iodide solution). White or yellow precipitate indicates the presence of alkailoids.
2. **Wagner's Test:** Take the test solution in a test tube then add Wagner's reagent (iodine solution). brown or reddish brownprecipitate.

B. Tests of Glycosides

1. **Raymond's Test:** Take the test solution in test tube and add 1 ml of 50% ethanol. Add 0.1% solution of dinitrobenzene in ethanol then added 2-3 drops of 20% sodiumhydroxide solution . Appearance of violetcolor indicated the presence of Glycosides.
2. **Killer Killani Test:** 2 ml of extract in a test tube add glacial acetic acid then add one drop of 5% FeCl3 with conc. H2SO4. Reddish brown color appeared at the junction of the two liquid layers and upper layer appeared bluish green.
3. **Legal Test:** Take the test solution in a test tube add few drops of pyridine and a drop of 2% sodium nitroprus side then add a dropof 20% sodium hydroxide solution. Deepred color appears.

C. Tests for Carbohydrate

1. **Molisch's Test:** 2-3 ml. extract add few drops of α- naphthol solution (20% in ethyl alcohol) then 1 ml. conc. H2SO4 addedalong the side of the test tubes. Violet ring was formed at the junction of two liquids.
2. **Benedict's Test:** To the extract add equal volume of Benedict's reagent. Heat for 5 min. Solution appears green, yellow or red.

D. Tests for Tannins

1. **Vanillin- HCl Test:** To the extract add vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl). Formation of pink or red color
2. **Gelatin Test:** To the extract solution add aqueous solution of gelatin. White buff colorprecipitate are formed
3. Tests for Flavanoids
4. **Lead acetate test:** Filter paper strip was dipped in the alcoholic solution of extract,ammoniated with ammonia solution. Color changed from white to orange.
5. **Shinoda Test:** To the extract add 5 ml. 95% alcohol, few drops of conc. HCl and 0.5 g magnesium turning. Pink color observed.
6. **Alkaline Reagent Test:** Extracts have to be treated with a few drops of sodium hydroxide solution.

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Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of falvonoids.

E. Tests for Resins

1. **Ferric chloride test:** Take the extract in test tube add alcohol with few drops of FeCl3 solution. Green color appears.
2. **Turbidity Test:** Extract solution (2 g of sample in methanol) add 5 ml distilled water, turbidity appears.

F. Test for Steroids

1. **Libermann- Bur chard Test:** To 2 ml. extract add Chloroform, 1- 2ml. acetic acid and 2 drops H2SO4 from the side of the test tube. First red, then blue and finally green color appeared.
2. **Salkowski Reaction:** To 2 ml. of extract add 2 ml. chloroform, 2 ml. conc. H2SO4. Shakewell. Chloroform layer appeared red color and acid layer shows greenish fluorescence.

G. Test for Proteins and Amino-acids

1. **Biuret Test:** Take 3 ml. of extract in a test tube add 4% NaOH and 2-3 drops of 1% copper sulphate solution. Presence ofred/violet coloration.
2. **Precipitation test:** extract then mix with absolute alcohol. White ppt.
3. **Ninhydrin Test:** Extract in a test tube then add ninhydrin reagent in boiling water bath for 10 min. Violet color appeared.
4. **Cysteine Test:** To 1 ml of protein solutionin a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate. − Mix well and put in a boiling water bath for few minutes; a black deposit is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.

H. Test for Fats

1. **Sudan Red test:** To a test tube, add equal parts of test sample and water to fill about half full. Add 3 drops of Sudan III stain to each test tube. Shake gently to mix. A red- stained oil layer will separate out and float on the water surface if fat is present.
2. **Spot test:** Take a small strip of filter paper. Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.
3. **Saponification test:** To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

I. Phenol Test

1. **Ferric chloride Test:** To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

J. Diterpenes Test

1. **Copper acetate test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

K. Test for Saponins

1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
2. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

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III. EXPERIMENTAL SCHEDULE

In-vitro anti-oxidant Activity

A. Reducing Power

Different doses of 99% ethanolic extracts of Vernonia cinerea less root were mixed in 1 ml of distilled water so as to get 20µg, 40µg, 60µg, 80µg, and 100µg concentrations. This was mixed with phosphate buffer 2.5 ml, 0.2 M, pH 6.6 and potassium ferric cyanide 2.5 ml, 1. The mixture was incubated at 50^0 C for 20 min. A portion 2.5 ml of trichloroacetic acid 10% was added to the mixture, which was then centrifused at 3000 rpm for 10 min. The upper layer of the solution 2.5 ml was mixed distilled water 2.5 ml and FeCl3 0.5 ml, 0.1%, and the absorbance OD was measured at 700 nm. Increase in absorbance of the reaction mixture indicate increase in reducing power. The % reducing power was calculated by using the formula. [10]

% increase in absorbance = Test OD - Control OD / Control OD × 100

B. Superoxide Anion Scavenging Activity

Various concentration of 99% ethanolic extract of Vernonia cinerea less root solutions were prepared such that each 0.1 ml contains 20, 40, 60, 80, and 100µg. About 1 ml of Nitroblue tetrazolium (NBT) solution (156µM In 100mM phosphate buffer , pH 7.4) and 1 ml of Nicotinamide adenine dinucleotide (NADH) solution (468µM PMS in 100 mM phosphate buffer, pH 7.4) were mixed 0.1 ml of various concentrations of sample of ethanolic extract of Vernonia cinerea less root and standard in water was mixed. The reaction was initiated by adding 10µl of Phenazine Methosulphate (PMS) solution (60µM PMS in 100 mM phosphate buffer, pH 7.4) to the above mixture and was incubated at 25^o C for 5 min. The absorbance was at measured 560nm against blank. Decrease absorbance of the reaction mixture indicate superoxide anion scavenging activity. [11]

% increase in absorbance = Test OD - Control OD / Control OD × 100

C. Hydroxyl Radical Scavenging

In biochemical systems, superoxide radical and H2O2 react together to form the hydroxyl radical, OH, this can attack and destroy almost all known biochemical hydrogen peroxide occurs naturally at low concentration level in environment and air. In the body, H2O2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radical (OH) that can initiate lipid per oxidation and cause damage to DNA and erythrocytes. Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation assay of Hathwell and Gutteride. In 50 mM phosphate buffer (pH 7.4) 1mM deoxyribose, 0.2 mm phenyl hydrazine hydrochloride were prepared 0.6 ml of 1 mM deoxyribose and 0.4 ml of ethanolic extract of Vernonia cinerea less (varing doses 20, 40, 60, 80, and 100µg) or sodium metabisulphate (25µg Std) were mixed and phosphate buffer was added to make the volume to 1.6 ml. The reaction mixture was incubated for 10 min and 0.4 ml of 0.2 mM phenyl hydrazine HCl was added and incubated for 1 hr and 1 ml each of 2.8% TCA and 1 % (w/v) of thiobarbituric acid were added. The reaction mixture was heated for 10 min on a boiling water bath. The tubes were cooled and absorbance was taken at 532 nm. The absorbance of the reaction mixture was inversely proportional to the hydroxyl radical scavenging activity. [12]

D. Toxicity Studies

Acute toxicity studies for Ethanolic extracts of root plant of Vernonia cinerea less belonging to the family 'Asteraceae' were conducted as per OECD using Albino Swiss mice. Each animal was administered Ethanolic, aqueous Ethanolic extracts solution of the extract by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD50, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

IV. INVESTIGATION OF GASTROPROTECTIVE MECHANISM

4.1 Pylorus Ligation Ulcer Model

Albino wistar rats of either sex weighing between 150-200 gm were divided into four groups of 6 animals each.

Group 1: Control

Group 2: Standard (Lansoprozole 8 mg /kg)

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Group 3: Test drug EEVCL 100 mg/kg
Group 4: Test drug EEVCL 200 mg/kg
In this method albino wistar rats were fasted in individual cages for 24hrs. Care was being taken to avoid caprophagy. Ethanolic extract and standard drugs were administered 30 min prior to pylorus ligation as mention above. Rats were sacrificed by an over dose of anesthetic ether after 4 hrs of pyloric ligation, the abdomen was opened, cardiac end of stomach was dissected out and the content were drained in centrifuge tube. The volume of gastric was measure and centrifuged at 2000rpm for 10 min. From this supernatant aliquots were taken for determination of pH, total and free acidity. [13]

4.2. Aspirin induced ulcer model

Albino wistar rats of either sex weighing between 150-200 gm were divided into four groups of 6 animals each.
Group 1: Control
Group 2: Standard (Lansoprozole 8mg/kg)
Group 3: Root ethanolic extract (100mg/kg)
Group 4: Root ethanolic extract (200mg/kg)
The animals are fasted for 24 hrs. The test drugs are administered orally 30 min prior to aspirin at the dose of 200mg/kg. After four hours the rats are sacrificed by using anesthetic ether and examined the gastric ulcer. [14]

4.3 Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test by using Graph pad Instant software. P value less than 0.05 was considered to be statically significant.*P<0.05, **<0.01, and ***<0.001, when compared with control.

V. RESULTS AND DISCUSSION

5.1 Preliminary Phytochemical Investigation

Table 1: The phytochemical study that Alkaloids, Glycosides, Flavanoids, Tannins and Saponins are present in ethanolic extract. Glycosides and steroids are present in chloroform and petroleum ether extract is given below.

Constituents	Petroleum ether extract	Chloroform extract	Ethanolic extract
Alkaloids	Absent	Absent	Present
Carbohydrates	Absent	Absent	Absent
Glycosides	Absent	Present	Present
Steroids	Present	Absent	Absent
Flavanoids	Absent	Absent	Present
Saponins	Absent	Absent	Present
Fixed oil and Fats	Absent	Absent	Absent
Tannins	Absent	Absent	Present
Protein & Amino acids	Absent	Absent	Absent
Mucilage	Absent	Absent	Absent

5.2 In-vitro anti-oxidant activity

A. Reducing power activity of Vernonia cinerea less whole plant

It is observed that extracts of Vernonia cinerea less root have demonstrated dose dependent increase in the reducing property. Whereas 25µg sodium metabisulphate (std) has 69.54% reducing property. However, the Vernonia cinerea less extract at dose 80µg and 100µg shows better activity. Maximum activity was seen at dose 100µg in roots of ethanolic extract in percentage of inhibition is 38.50.

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Table 2: Reducing power activity of ethanolic extract of Vernonia cinerea less.

Groups	Absorbance mean ± SEM	% Inhibition
Control	0.174 ± 0.002	-----
Control + Standard	0.295 ± 0.001***	69.54
Control + Ethanolic extract 20µg	0.178 ± 0.003**	2.29
Control + Ethanolic extract 40µg	0.187 ± 0.002***	7.47
Control + Ethanolic extract 60µg	0.198 ± 0.003***	13.79
Control + Ethanolic extract 80µg	0.227 ± 0.001***	30.45
Control + Ethanolic extract 100µg	0.241 ± 0.001***	38.50

Values are mean ± SEM ., n = 3, *P<0.05, **P<0.01, ***P<0.001, compared with control group.

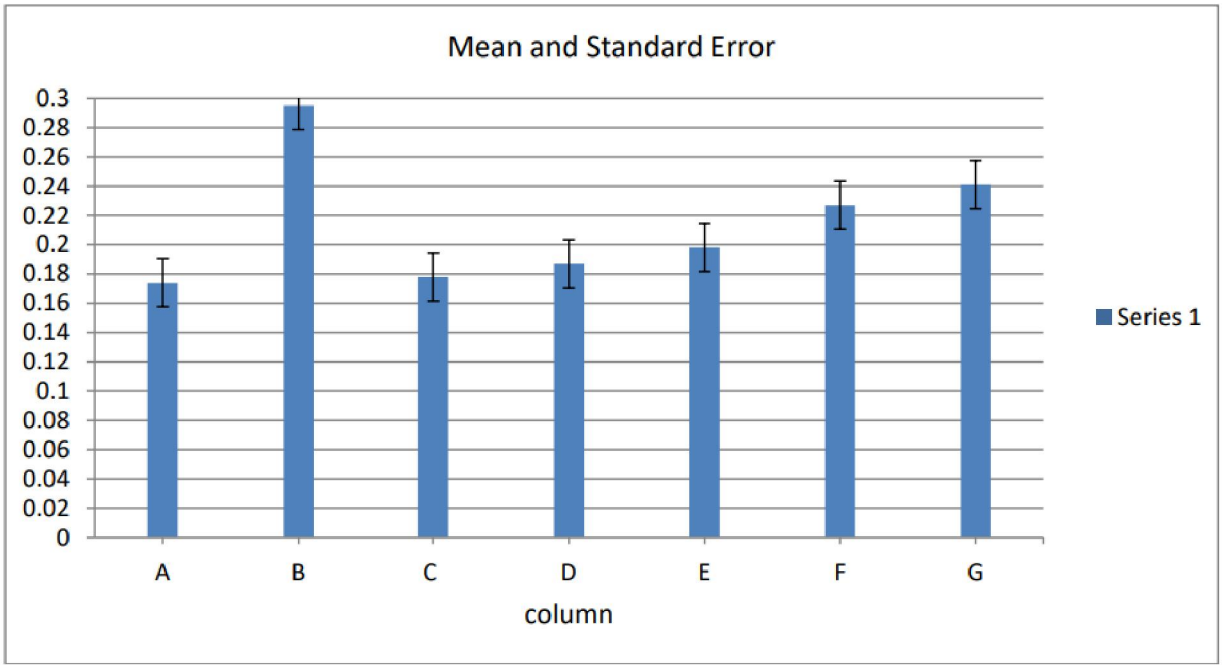

Figure 1: Reducing power activity of ethanolic extract of Vernonia cinerea less.

B. Superoxide anion scavenging activity of roots Vernonia cinerea less.

It is observed that the ethanolic extracts have demonstrated dose dependent increase in the superoxide anion scavenging activity. Where as 25µg sodium metabisulphate (std) has 71.31% activity. Where 100µg of ethanolic extract showed 51.10% activity. The maximum activity was seen at dose 100µg in root of ethanolic extract in which percentage inhibition is 51.10 Where as in the standard the percentage inhibition is 71.31.

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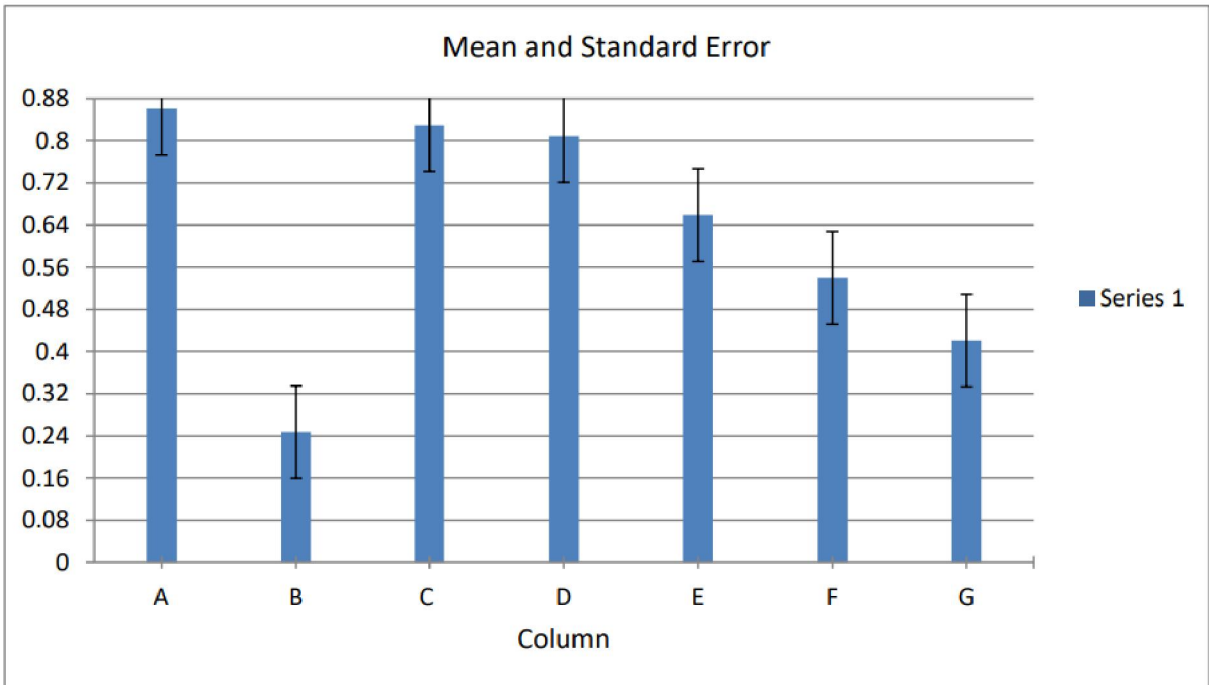

Figure 2: Superoxide anion scavenging activity of roots Vernonia cinerea less

C. Hydroxyl radical scavenging activity of Ethanolic extract of Vernonia cinerea less.

It is observed that both the ethanolic extract have demonstrated dose dependent increase in the Hydroxyl radical scavenging activity. Where as 25µg of sodium metabisulphate (std.) showed 69.11% (for 1 hr incubation period) and has 54.96% activity (for 4 hr incubation period). Where as 100µg Ethanolic extract showed lesser activity in case of 1 hr incubation period i.e. 67.94% but showed higher in case of 4 hr incubation period i.e. 61.57%

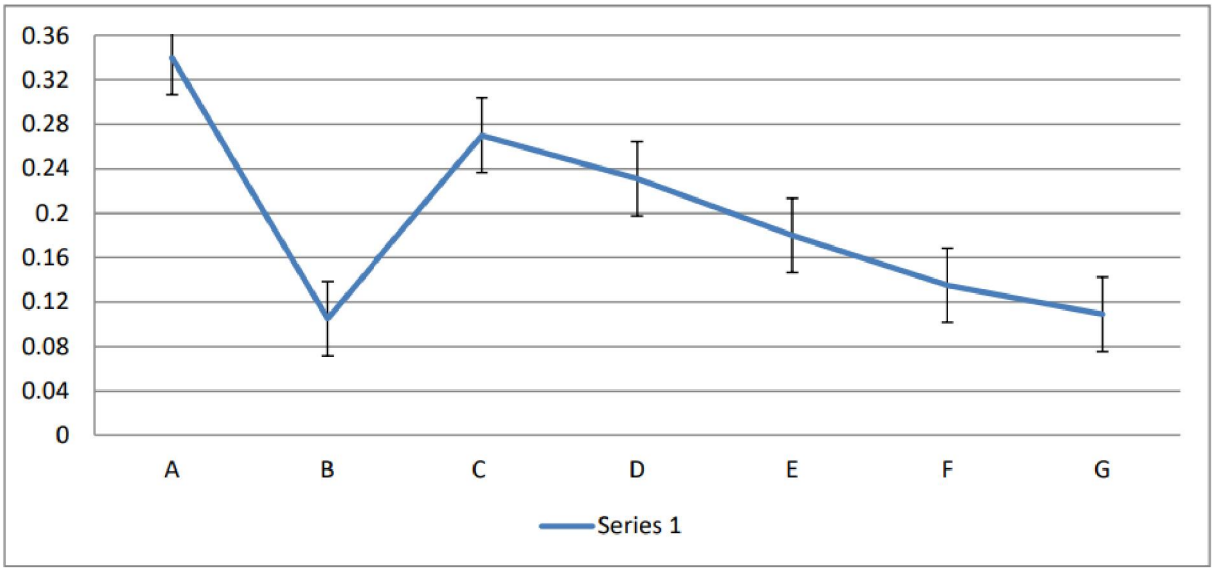

Figure 3: Hydroxyl radical scavenging activity of Ethanolic extract of Vernonia cinerea less

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Gastroprotective Activities of Ethanolic extract of Vernonia cinerea less

Effect of EEVCL on Pylorus ligation induced ulcer in rats

The ethanolic extract shows significant anti-ulcer activity in a dose dependent manner, when compared to control which is evident by decrease in ulcer index. The control group with an ulcer index of 5.66±0.210. The received treatment with standard is 1.66±0.401 (70.67%). The ulcer index of ethanolic extract of Vernonia cinerea less at a dose of 100 mg/kg is 3.83 ± 0.542 (32.33%) and 200 mg/kg is 2.66 ± 0.210 (53.00%).

Table 3: The ulcer index of pylorus ligation induced gastric ulcer of Ethanolic extract of root of Vernonia cinerea less.

Group	Treatment	Dose mg/kg	Mean ulcer index ± SEM	% Protection
1	Control	----	5.66±0.210	----
2	Standard	8	1.66±0.401***	70.67
3	EEVCL	100	3.83±0.542***	32.33
4	EEVCL	200	2.66±0.210***	53.00

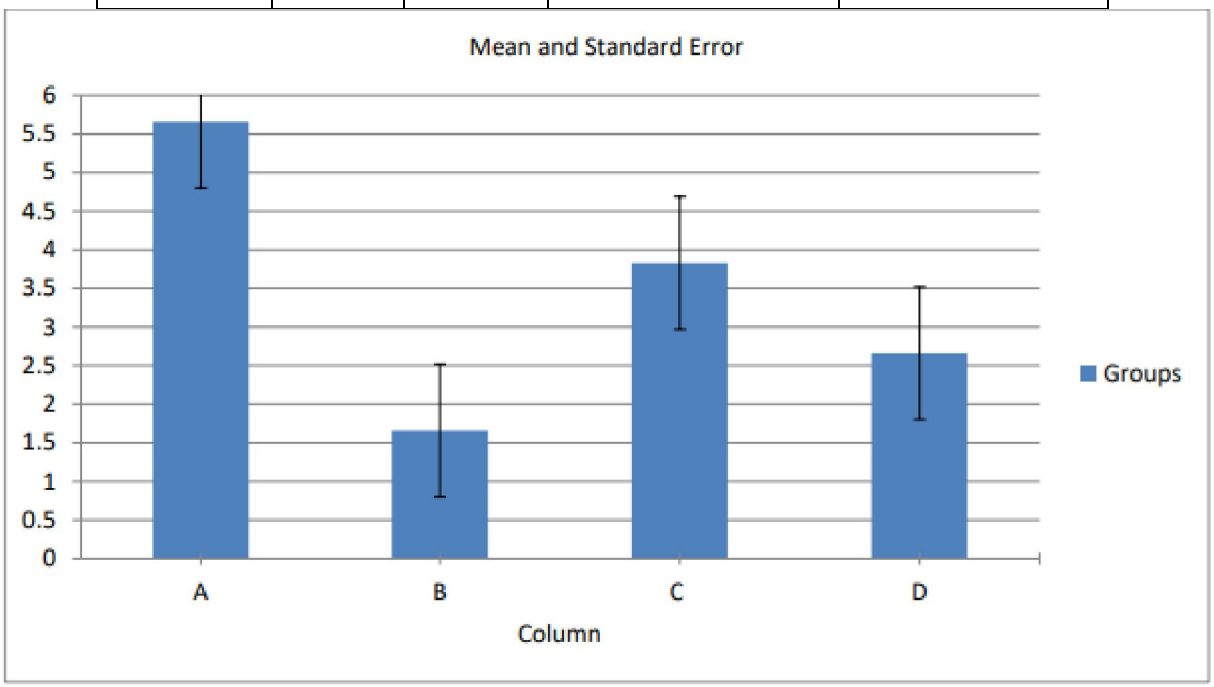

Figure 4: Effect of Ethanolic extract of root of Vernonia cinerea less. on ulcer index followed by pylorus ligation method

Effect of EEVCL on Aspirin induced ulcer in rats

The ethanolic extract of roots of Vernonia cinerea less shows significant anti-ulcer activity in a dose dependent manner, when compared to control which is evident by decrease in ulcer index. The ulcer index of ethanolic extract of roots of Vernonia cinerea less at dose of 100 mg/kg is 3.16 ± 0.600 and 200 mg/kg is 1.91 ± 0.396. Whereas standard (Lansoprozole) mean ulcer index is 1.83 ± 0.459

Table 4: The ulcer index of aspirin induced gastric ulcer of Ethanolic extract of root of Vernonia cinerea less.

Group	Treatment	Dose mg/kg	Mean ulcer index ±SEM	% Protection
1	Control	----	5.41±0.271	----
2	Standard	8	1.83±0.459***	66.17
3	EEVCL	100	3.16±0.600***	41.58
4	EEVCL	200	1.91±0.396***	64.69

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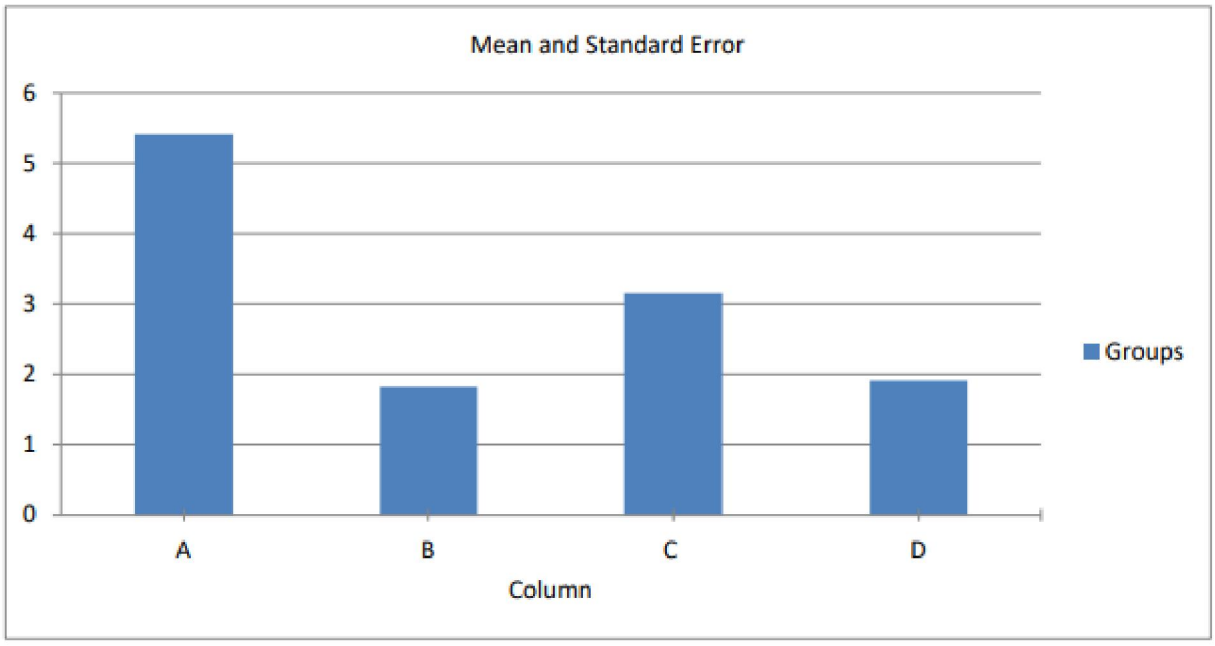

Figure 5: Effect of Ethanolic extract of root of Vernonia cinerea less. on ulcer index followed by aspirin method.

VI. CONCLUSION

When compared to the control, administration of an ethanolic extract of Vernonia cinerea exhibits considerable anti-ulcer action in a dose dependent manner (100mg/kg and 200mg/kg), as evidenced by a drop in ulcer index. The ethanolic extract of Vernonia cinerea roots demonstrates less significant anti-ulcer effect in a dose-dependent manner when compared to the control, as demonstrated by a decrease in ulcer index. Vernonia cinerea roots ethanolic extract had an ulcer index of 3.16 0.600 at 100 mg/kg and 1.91 0.396 at 200 mg/kg. In comparison, the conventional ulcer index (Lansoprozole) is 1.83 0.459. According to the findings, an ethanolic extract of the root of the Vernonia cinerea less plant has strong gastroprotective (anti-ulcer) effect. These findings corroborate the long-held belief that the effects are positive.These results support the traditional belief about the beneficial effects. Thus, the study validated a native herbal practitioner's claim that the roots have anti-ulcer and anti-diarrheal activity.

VII. ACKNOWLEDGEMENTS

The authors are thankful to Dr. K. S. Rao Principal RRK's College of Pharmacy Bidar, Karnataka for providing the facilities to carry out the work and Prof. Rajshekar Bhande Head, dept. of Pharmacology for his valuable suggestion and guidance and also sincere thanks to Dr. Md Faooq Ahmed for his moral support.

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