AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Gastro Protective and anti oxidant activity of Cow urine betel vine extract in ethanol induced Peptic ulcer in Rats

N.B.Sridharamurthy[1*], B.A. Sowmya[1], Manasa MM[2]
1.Department of Pharmacology, School of Pharmaceutical Sciences, Dayananda Sagar University, Kumarswamy layout, Bangalore-78, Karnataka

ABSTRACT

The present study was designed to investigate the antioxidant and gastro-protective potential of Cow urine betel vine extract. Antioxidant and gastro-protective activity was evaluated by using ethanol induced ulcer model; the animals were fed with Cow urine betel vine extract at the dose of 250 and 500mg/kg b.w orally for a period of 14 days. The pretreated extract reduced the gastric ulcers in a dose dependent manner which was determined by measuring the ulcer index and with marked attenuation in the levels of oxidative stress enzymes like SOD, LPO and CAT. Also Cow urine betel vine increased the gastric mucous content in the selected ulcer model.

Keywords: Antioxidant activity, antiulcer activity and ethanol induced ulcer, Cow urine betel vine extract.

*Corresponding Author Email: murthy.sridhara@yahoo.com
Received 29 April 2016, Accepted 11 May 2016

Please cite this article as: Sridharamurthy NB *et al*., Gastro Protective and anti oxidant activity of Cow urine betel vine extract in ethanol induced Peptic ulcer in Rats. American Journal of PharmTech Research 2016.

INTRODUCTION

Peptic ulcers are a common disorder of the entire gastrointestinal tract which occurs due to the imbalance between aggressive factors such as hydrochloric acid (HCL), refluxed bile, leukotrienes, reactive oxygen species (ROS) and defensive factors which include the function of the mucus bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal, non-enzymatic and enzymatic antioxidants and some growth factors [1,2].

Many synthetic drugs are available in the market for the treatment of ulcers but they are associated with various side effects. Plant kingdom thus emerged as a key target for the development of antiulcer drug with lesser side effects. Piper betel is an important medicinal plant, which finds an extensive use in Ayurveda and Unani system of medicine [3]. *Piper betel* is commonly known as betel vine and belongs to the family *Piperaceae*. The betel vine is reported to have antibacterial, antimicrobial, antidiabetic, anticancer, antioxidant and hepatoprotective and immuno-stimulant activities [4, 5.]

The principle constituents of the extract are tannins, flavonoids, alkaloids, saponins and phenolic compounds [6]. Traditionally in the folk medicine the plant has been used in the treatment of ulcers but no evidence is available that reveal the role of Cow urine piper betel extract in augmenting the peptic ulcers.

Hence, the present investigation was aimed to explore the antiulcer and antioxidant potential of Cow urine betel vine extract in ethanol induced gastric ulcer model.

MATERIAL AND METHOD

The leaves of betel vine was collected from the nearby plantations and authenticated by Dr. Muralidhar, Prof and HOD, Department of biotechnology, Dayananda Sagar Institutions. Later leaves were shade dried and coarsely powdered. Ranitidine (50mg/kg) was used as standard drug.

Preparation of extract:

200gms of powdered leaf was extracted with fresh cow urine by maceration process for 4 days at 40-45^0 C, the filtrate was then and subjected for evaporation to remove moisture content in rotary vacuum evaporator. Later the extract was collected and kept in dessicator for further use.

Qualitative phytochemical investigation

The cow urine betel vine extract was then subjected to phyto-chemical investigation for the presence of various secondary metabolites as tannins, amino acids, alkaloids, flavonoids, saponins and proteins using standard methods [7, 8, 9].

Experimental animals

Wistar rats weighing between 200-250gms were procured from the animal house of Dayananda Sagar College of Pharmacy, Bangalore. They were maintained at standard housing condition at the room temperature of 22^0C ($\pm 3^0$C) and relative humidity of 44-56% with light and dark cycles of 10-14hrs respectively 10,11. The rats were provided with standard rodent pellet diet and the water *ad libitium* 12. The study of the protocol was duly approved by Institutional Animal Ethics Committee (CPCSEA, Reg no: DSCP/M.Pharm/ IAEC/106/Pharmacology).

Acute toxicity studies.

Acute toxicity was carried out as per the CPCSEA and OECD guidelines 425. Group of 6 healthy mice weighing 20-25gms were selected and kept for 3-4hrs fasting with free access to drinking water. Doses were calculated according to the body weight. The test extracts were dissolved in cow urine and administered orally at the starting dose of 2000mg/kg and were kept for observation for any mortality and behavioral changes for 24hrs, further dose was increased up to 5000mg/kg b.w. It was observed that no mortality was observed even at higher dose of the extract and no change in their behavior. As per the OECD guidelines $1/10^{th}$ and $1/5^{th}$ of the maximum tolerated dose was selected. Therefore effective therapeutic doses selected were 250 and 500mg/kg b.w.

Ethanol induced ulcer model [13].

The animals were separated into 5 groups of each containing 6 rats. The groups were as follows:

Group I: Negative control (saline treated).

Group II: Positive control (Cow urine treated).

Group III: standard (Ranitidine 50mg/kg).

Group IV: Test extract 1 (250mg/kg).

Group V: Test extract 2 (500mg/kg).

Negative control received normal saline 1ml/kg b.w orally. Positive control received 1ml/kg b.w cow urine orally; standard group received Ranitidine 50mg/kg b.w orally. The remaining two groups received test extract at the doses of 250 and 500mg/kg b.w orally for 14 days on the 15^{th} day the animals were sacrificed under light ether anesthesia. Alcohol induces the secretion of gastric juice and decrease mucosal resistance due to which protein content of gastric juice is significantly increased. The plasma protein in the gastric juice with weakening of mucosal resistance barrier of gastric mucosa, leads to peptic ulcer.

Procedure:

Wistar rats of either sex weighing between (150-200g) were divided into 5 group. The animals were fasted for 24hrs with free access to drinking water and were fed with test drugs or standard drug. 1 hour later, 1ml/200gm of 99.8% alcohol is administered orally to each animal. 1 hour later

the animals were anesthetized with ether and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the length of each ulcer were measured. Ulcer index was calculated using severity scores and average number of ulcers per animal was done. Severity scores are given as below:

0-Normal stomach

0.5-Red coloration

1-Spot ulcers

1.5-Haemorrhagic streaks

2-Ulcer>3mm but <5mm

3-Ulcers>5mm

Evaluation of ulcer index:

An ulcer index was calculated as:

$UI=UN+US+UP\times10^{-1}$

- UN= average number of ulcers per animal
- UI= ulcer index
- US=average of severity scores
- UP=percentage of animals with ulcers

% Protection= UI (CONTROL) - UI (TREATED) / UI (CONTROL)

Antioxidant parameters:

In vitro antioxidant activity:

Livers of the animals were cleaned with normal saline for the removal blood clots and further homogenized with ice chilled 10% KCl solution and centrifuged at 2000 rpm for 10 minutes. Supernatant was collected for the estimation of antioxidant parameters like Catalase, Superoxide dismutase and Lipid per- oxidation.

Catalase [14]:

Principle:

In the UV range H_2O_2 shows a continual increase in absorption with decreasing wavelength. Catalase catalyses the rapid decomposition of hydrogen peroxide to water. The decomposition of H_2O_2 shows the decrease in absorbance at 240nm. The difference in absorbance per unit is a measure of catalase activity.

Procedure:

1. Preparation of hydrogen peroxide solution (7.5mM):

1.043ml of 30% w/w H_2O_2 was made up to 100ml with sodium chloride and EDTA solution (9g of NaCl and 29.22mg of EDTA was dissolved in 1 liter distilled water).

2. Preparation of potassium phosphate buffer (65mM, pH 7.8):

2.2g of potassium dihydrogen phosphate and 11.32g of dipotassium hydrogen phosphate were dissolved in 250ml and 1 liter of distilled water respectively, and were mixed together. The pH was adjusted to 7.8 with KH_2PO_4.

3. Preparation of sucrose solution:

10.95g of sucrose was dissolved in 100ml of distilled water.

1. To 2.25ml of potassium phosphate buffer 100µl of the tissue homogenate was added and incubated at 25^oC for 30min.
2. For the blank sucrose solution was used instead of tissue homogenate. Then 0.65ml of H_2O_2 was added to initiate the reaction. The change in absorbance of the reaction mixture at 240mm was measured for 2-3min. dy/dx for 1 min for each assay was calculated and the results are expressed at CAT units/mg of tissue (Beer and Seizer, 1952).

$$\textbf{Cat (U) /100 of sample} = (dy/dx) \times 0.003/38.3956 \times 10^{-6}$$

Where,

dy/dx – change in absorbance/ minute

38.3956×10^{-6} – molar extinction co efficient of H_2O_2 at 240nm.

4. Superoxide dismutase[15]:

Principle:

This enzyme is necessary for the survival in all oxygen metabolizing cells. It is found in the cytosol and inter-membrane space of mitochondria of eukaryotic cells. It contains copper and zinc. In normal cells, this radical alone is the precursor of hydrogen peroxide.

Superoxide dismutase scavenges the superoxide and thus provides a free line defense against free radical damage. SOD's are the family of metallo enzyme that catalyzes the disputation of superoxide anion to hydrogen peroxide and molecular oxygen in the following manner.

$$\mathbf{2H_2O_2 + 2O^- \longrightarrow 2H_2O + O_2}$$

In the erythrocytes, the superoxide anion interacts with peroxides to form hydroxyl radicals, which causes haemolysis in the absence of SOD activity. SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome.

Procedure:

1. Preparation of Sodium carbonate buffer solution (0.05M, pH 10.2):

5.3 gm of sodium carbonate and 1.2 g of sodium bicarbonate were dissolved separately in 1 liter of distilled water, which served as a stock solution. Buffer was prepared by mixing 64ml of sodium carbonate and 70ml of sodium bicarbonate solutions. The pH of the buffer was adjusted to 10.2 using the above stock solution accordingly.

a. Adrenaline (9Mm):

0.03g of adrenaline was dissolved in distilled water and the final volume was made up to 10ml with distilled water containing a drop of concentrated HCL (to bring pH down to 2). Adrenaline is light sensitive; therefore, the vial was kept covered with aluminum foil always

b. Sucrose (0.3199M) solution:

10.96 gms of sucrose was dissolved in distilled water and the volume was made up to 100ml.

To 2.8 ml of sodium carbonate buffer, 0.1ml of tissue homogenate was added and was incubated at 30^0 C for 45minutes.

For the blank sucrose solution was used instead of tissue homogenate. Then, the absorbance obtained was adjusted to 0 for the sample. Thereafter, the reaction was initiated by adding 10µl of adrenaline solution. The change in absorbance was recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature was maintained at 30^0C.

Similarly, SOD calibration curve was prepared by taking 10 units/ ml as standard solution, 1 unit of SOD produce approximately 50% of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue.

Calculation:

SOD = C × total volume × 1000/50 × sample volume × mg of protein per ml

Unit: Units/ mg of protein.

4. Lipid peroxidation[16]:

Lipid per-oxidation that occurs in animal tissues has been generally recognized to induce several cardiovascular, pulmonary and hepatic diseases and is a principle cause of aging. The estimation of lipid per-oxidation has been carried out by a number of methods of which TBA–reactive (Thio-barbituric acid) substance is selected because of its high sensitivity and simplicity in operation. The TBA test is often said to measure malondialdehyde (MDA) formed in per oxidizing lipid systems. The results are expressed as micro mate malon-dialdehyde equivalents. The TBA test works well when applied to defined membrane systems. E.g. Microsomes and liposomes.

Procedure:

1. Preparation of thiobarbituric acid solution:

0.8g of thiobarbituric acid was dissolved in distilled water and volume was made up to 100ml. the pH was adjusted to 7.4 with 1N NaOH/ 0.1N HCL solution.

2. Preparation of Acetic acid solution.

20ml of acetic acid was dissolved in distilled water and the volume was made up to 100ml with distilled water. The pH was adjusted to 3.5 with 1 N NaOH/0.1N HCL solution.

3. Preparation of sodium lauryl sulphate solution:

8.1g of sodium lauryl sulphate was dissolved in distilled water and the volume was made up to 100ml with distilled water.

4. Preparation of mixture of n-butanol and pyridine (15:1 v/v)

15ml of n-butanol and 1ml of pyridine were mixed together.

a. To 1ml of tissue homogenate, 0.2ml of sodium lauryl sulphate solution, 1.5ml of 20% acetic acid and 1.5ml of thio-barbituric acid solution were added.

b. The volume was made up to 5 ml with double distilled water and then mixture was heated on a boiling water bath for 30 min.

c. After cooling, the red chromogen was extracted with 5ml of the mixture of n-butanol and pyridine and centrifuged at 4000 rpm for 10 min.

d. The organic layer was taken and its absorbance was measured at 532 nm.

e. The results were expressed as nM of MDA/mg of wet tissue using molar extinction co-efficient of the chromophore 1.56×10^{-5} $mmol^{-1}$ cm^{-1} as 99% of TBARS is MDA.

Calculation:

LPO= Test O.D × total volume × 1/1.56 × 10^{-5} × 10^{-9} × sample volume × mg protein per ml.

Unit: nmol MDA / min × mg protein

Statistical analysis:

The values were expressed as Mean + or – SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnets multiple comparison tests. The p values < 0.05 were considered as significant.

In vitro Antioxidant Activity [17]

1. DPPH Assay (2,2- diphenyl-1-picrylhydrazyl)

Principle:

DPPH radical scavenging assay

Free radical scavenging ability of the extracts was tested by DPPH free radical scavenging assay as described by Shreedhara CS *et al* [17]. The hydrogen atom donating ability of the plant extractives was determined by the discoloration of methanol solution of 2, 2-diphenyl-1-picrylhydrazyl

(DPPH). DPPH produces violet/purple color in methanol solution and fades to yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (2-512µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectro-photometrically at 517 nm. Ascorbic acid was used as reference. Percentage DPPH free

Radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity= {(A0− A1)/A0} ×100

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration.

DPPH + H-A ⟶ DPPH-H + A

(Purple) (Yellow)

DPPH shows a strong absorption band at 517nm. Antioxidant react with DPPH, which is a stable free radical and reduces DPPH to DPPH-H (2, 2-diphenyl-1-picrylhydrazine) and as consequence the absorbance decreases from the DPPH radical (purple) to DPPH-H (yellow) form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Preparation of ascorbic acid stock solution (standard drug)

Ascorbic acid was used as standard. Ascorbic acid stock solution was prepared in distilled water in the concentration of 1000µg/ ml, from the stock solution various dilutions *viz*. 2, 4, 8, 16, 32, 64, 128, 256, 512 µg /ml were prepared using distilled water and used for antioxidant studies.

Preparation of Cow urine Betel vine extracts stock solution

Extract stock solution was prepared in distilled water in the concentration of 1000µg/ml, from the stock solution various dilutions *viz.* 2, 4, 8, 16, 32, 64, 128, 256, 512µg/ml were prepared using distilled water and were used for antioxidant studies.

Procedure:

1) To 1ml of various concentration of extract, 1ml of solution of DPPH 0.1 Mm (0.39mg in 10ml methanol) was added.
2) An equal amount of water and DPPH was added and was used for control.
3) Ascorbic acid was used as standard for comparison.
4) After incubation for 20min in dark, absorbance was recorded at 517 nm.
5) % scavenging was calculated using the formula

Scavenging = OD (Control) – OD (Test)/ OD (Control)

6) Graph was plotted with concentration (µg/ml) on X axis and % scavenging on Y axis and IC_{50} values was calculated.

Nitric oxide Scavenging Assay 18

Principle:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be measured using a modified Griess-Illosvoy method. Nitric oxide generated in this manner was converted into nitric acid and nitrous acid in contact with dissolved oxygen and water. The liberated nitrous acid is estimated using Griess reagent, which forms a purple Azo dye in presence of a test compound likely to be the scavenger and the amount of nitrous acid will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging.

Aqueous solution

SODIUM NITROPRUSSIDE ⟶ NITRIC OXIDE

Dissolved O_2 / water

NITRIC OXIDE ⟶ NITRIC ACID + NITROUS ACID

NITROUS ACID + SULPHANILIC ACID ⟶ DIAZONIUM SALT

NAPTHYL ETHYLENE DIAMINE ⟶ AZODYE

DIHYDROCHLORIDE DIAZONIUM SALT (NEDD) (purple colored dye)

Procedure:

1, Preparation of ascorbic acid stock solution (standard drug):

Ascorbic acid was used as standard. Ascorbic acid stock solution was prepared in distilled water in the concentration of 1000µg/ ml, from the stock solution various dilutions viz. 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/ml were prepared using distilled water and used for antioxidant studies.

2. Preparation of Cow urine Betel vine extracts stock solution

Extract stock solution was prepared in distilled water in the concentration of 1000µg/ml, from the stock solution various dilutions viz. 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/ml were prepared using distilled water and used for antioxidant studies.

3. Preparation of Sodium nitroprusside solution:

0.2998g of sodium nitroprusside is dissolved in distilled water, and volume was made up to 100ml.

4. Preparation of sulphanilic acid:

0.33g of sulphanilic acid was heated with 20% glacial acetic acid, and volume was made up to 100ml using 20% glacial acetic acid.

1) Preparation of NEDD (N-1-Naphthylethylene diamine dihydrochloride) solution:

0.1g of NEDD was heated with 50% glacial acetic acid, and volume was made up to 100ml using 50% glacial acetic acid.

2) To 0.5ml of various concentrations of Cow urine extract, 2ml of sodium nitroprusside solution and 0.5ml of phosphate buffer was added.
3) To 2ml of sodium nitroprusside solution 0.5ml of phosphate buffer was added and used as control.
4) The reaction mixture was incubated at 25^0C for 180minutes.
5) To 0.5ml of incubated reaction mixture 1ml of sulphanilic acid was added and allowed to stand for 5 minutes, then 1ml of NEDD was added and incubated for 30 minutes.
6) Ascorbic acid was used as standard for comparison.
7) After incubation absorbance was measured at 540nm.
8) % scavenging was calculated using the formula.

% SCAVENGING = OD (Control) – OD (Test)/ OD (Control)

9) Graph was plotted with concentration (µg/ml) on X axis and % scavenging on y axis and IC_{50} values was calculated.

RESULTS AND DISCUSSION

Ethanol induced gastric ulcers.

The ulcer index of the negative control (saline treated) is 30±5.52, Positive control (Cow urine treated) is 19.6±2.9 and that of extract treated 250mg/kg and 500mg/kg is 13.4±2.44* ($p<0.01$) and 12.2±2.57** ($p<0.001$) respectively. The % protection is 34.6%, 55.3% and 59.3% respectively. For the reference standard ranitidine the ulcer index is 11.6±2.48** ($p<0.001$) and the % protection is 61.3%.The results are tabulated in Table: 1 and Figure 1.

Table 1: The effect of Cow urine Betel vine extract on ulcer index in Ethanol induced Peptic ulcer model

Sl.no	Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
I	Negative control(saline treated)	-	30±5.52	-
II	Positive Control (Cow urine treated)	-	$19.6±2.9^{ns}$	34.6
III	Standard (Ranitidine)	50	11.6±2.48**	61.3
IV	Test extract 1	250	13.4±2.44*	55.3
V	Test extract 2	500	12.2±2.57**	59.3

All the values are expressed in mean ± SEM. Six animals in a group (n=6),*: value significantly different at $p<0.01$ v/s. Ethanol induced negative control,**: value significantly different at $p<0.001$ v/s. Ethanol induced negative control.

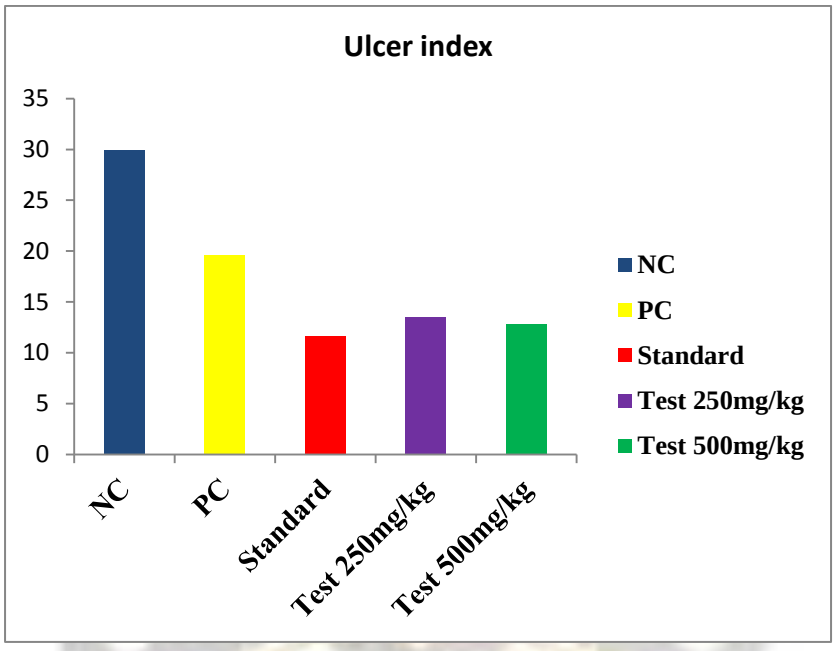

Figure 1: The effect of Cow urine Betel vine extract on ulcer index in Ethanol induced Peptic ulcer model

Effect of Cow urine Betel vine extract on Lipid peroxidation (LPO), Superoxide dismutase (SOD) and Catalase (CAT) in Ulcer models.

There is decrease in the lipid peroxidation activity in the Cow urine treated group and is (15.3±0.0001*). The Lipid per oxidation for the extracts 250mg/kg, 500mg/kg and the standard treated groups are13.5±0.00008**, (12.8±0.0001**) and (11.5±0.00008**) respectively which are tabulated in Table 2 and Figure 2.

The super oxide dismutase activity for the Cow urine, extracts 250mg/kg, 500mg/kg and standard treated animals are 62.85±0.014**, 58.2±0.028*, 65.18±0.031**, 51.21±0.022*** respectively. The results are tabulated in Table 2 and Figure 3.

The catalase activity for the control, extract I and II and standard treated animals are 13.98±0.017***, 20.93±0.024**, 26.72±0.013*, 24.22±0.016*** respectively. The results are tabulated in Table 2 and Figure 4.

Table 2: Effect of Cow urine Betel vine extract on Lipid per-oxidation (LPO), Superoxide dismutase (SOD) and Catalase (CAT) in Ulcer induced rats.

Treatment	Dose (mg/kg)	LPO (nmol of MDA/100mg protein)	SOD (% inhibition)	CAT (units/mg protein)
Negative control (saline treated)	-	9.7±0.0001	88.46±0.010	32.5±0.009
Positive control (Cow urine treated)	-	15.3±0.0001*	62.85±0.014**	13.98±0.017***
Standard (Ranitidine)	**50**	11.5±0.00008**	51.21±0.022***	24.22±0.016***
Test extract 1	**250**	13.5±0.00008**	58.2±0.028*	20.93±0.024**
Test extract 2	**500**	12.8±0.0001**	65.18±0.031**	26.72±0.013*

N=6 animal in a group;*: Value significantly different at p<0.05 vs. Negative control, **: Value significantly different at p<0.01 vs. Negative control, ***: Value significantly different at p<0.001 vs. Negative control. Data were analyzed by using one way ANOVA followed by Dunnet multiple test.

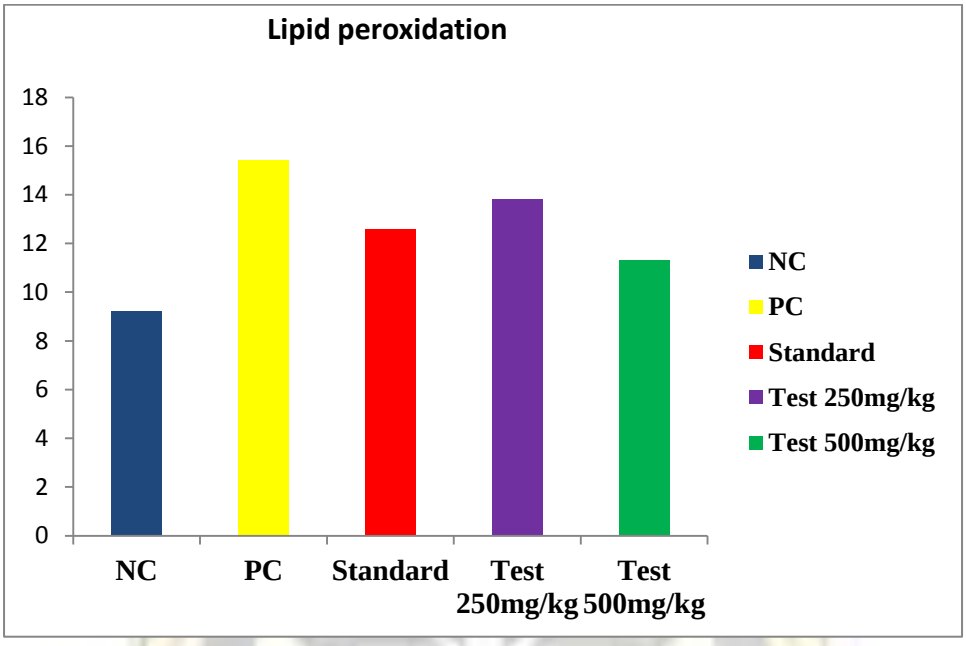

Figure 2: Effect of Cow urine Betel vine extract on Lipid Peroxidation (LPO) in ulcer induced rats.

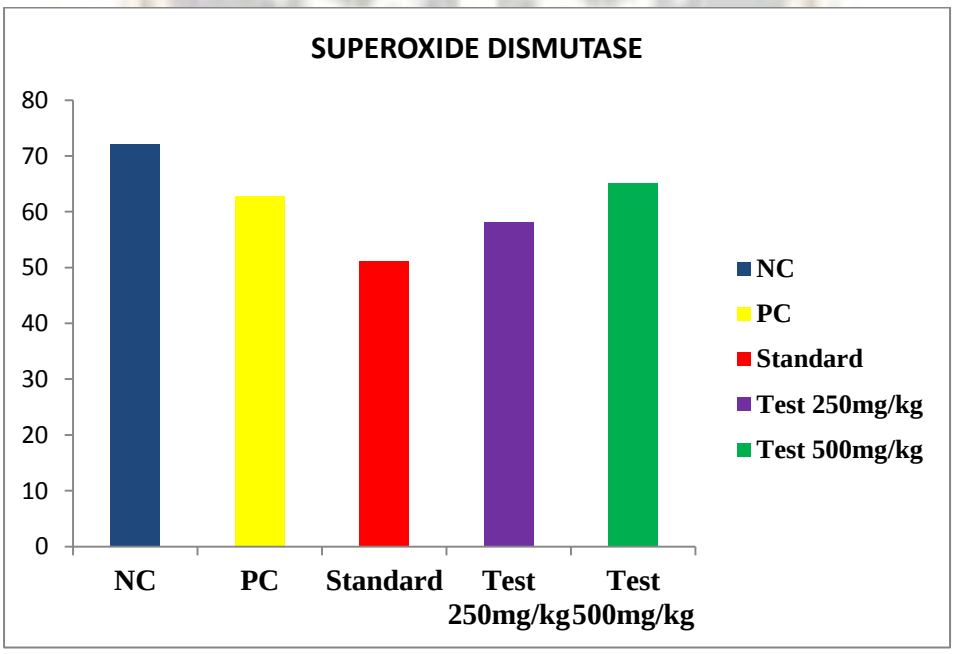

Figure 3: Effect of Cow urine with Betel vine extract on Superoxide Dismutase (SOD) in ulcer induced rats.

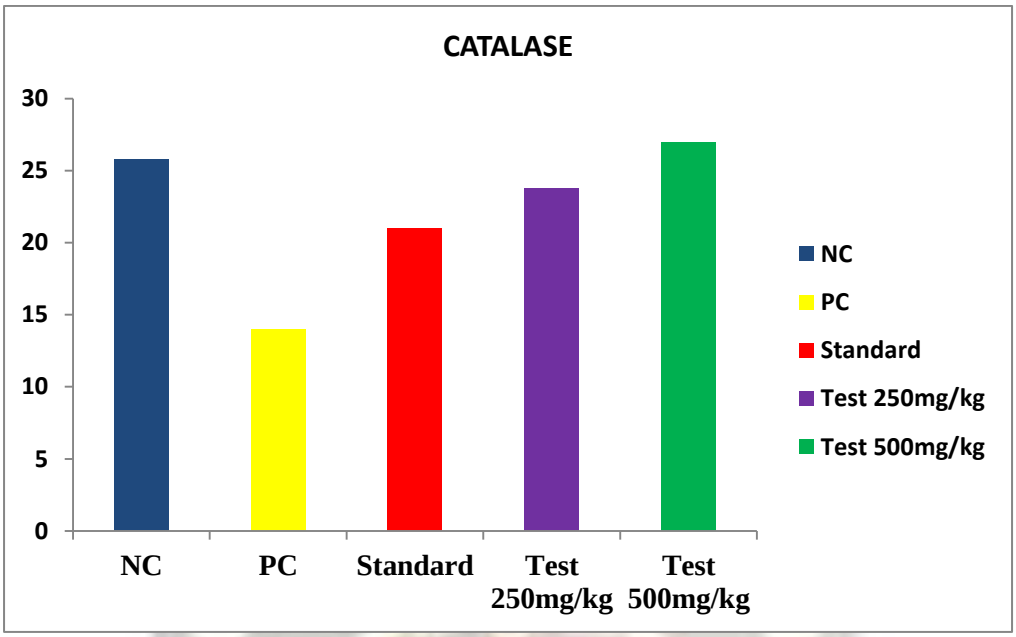

Figure 4: Effect of Cow urine Betel vine extract on Catalase (CAT) in ulcer induced rats.

Effect of Cow urine Betel vine extract on 1,1-diphenyl 2 picrylHydrazyl (DPPH) free radical scavenging activity and Nitric oxide scavenging activity in ulcer induced rats.

1, 1-Diphenyl 2 picrylhydrazyl (DPPH) radical scavenging activity.

The free radical scavenging activity of Cow urine extract is expressed in terms of percentage inhibition. The decrease in percentage of inhibition shows increased absorbance. The decrease in optical absorbance was measured at 512nm after addition of test compound. The percentage inhibition for the test extracts from 18.91% to the maximum of 44.5%. IC_{50} value was found at 3.2mcg/ml.

Table 3: Effect of Cow urine Betel vine extract on DPPH free radical scavenging activity

Conc of extract/std mcg/ml	% Inhibition of Ascorbic acid	% Inhibition of Test extract
2	19.4±0.12	18.91±0.2
4	21.6±0.15	20.94±0.2
8	51.49±0.13	22.97±0.34
16	54.47±0.71	27.07±0.14
32	55.97±0.22	31.08±0.33
34	58.95±0.25	35.81±0.59
128	60.44±0.27	37.83±0.28
256	62.88±0.19	39.86±0.27
512	55.22±0.23	44.5±0.25

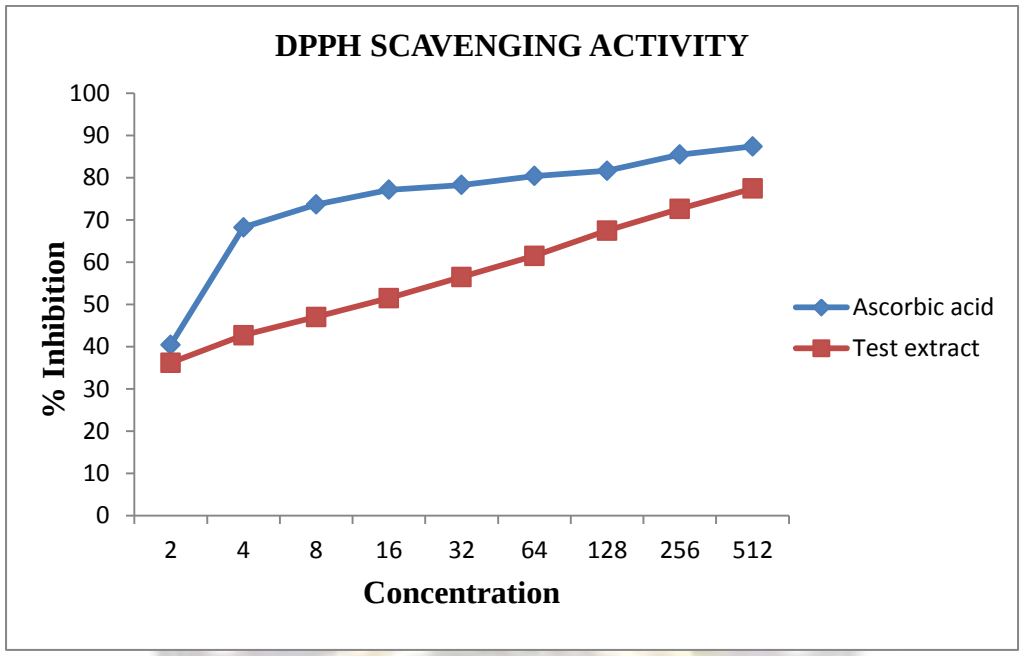

Figure 5: Effect of Cow urine Betel vine extract on DPPH free radical scavenging activity.

Nitric oxide scavenging method

The nitric oxide anion scavenging activity of the crude extract of the leaves is expressed in terms of percentage inhibition. Cow urine betel vine extract is found to possess good scavenging activity on nitric oxide anion at all concentrations under test. The Cow urine extract at concentration range from 2-512mcg/ml inhibited the production of nitric oxide anion radical by 36.17% to 77.41%. On the other hand the standard ascorbic acid showed significant scavenging activity in a dose dependent manner. The maximum inhibition of the superoxide anion was observed at 512mcg/ml concentration and is 77.41%. The Cow urine extract exhibited a moderate inhibition scavenging activity, with 50% inhibition concentration at 7.8mcg/ml. The results are tabulated in Table: 4 and Figure 6.

Table 4: Effect of Cow urine Betel vine extract on Nitric oxide scavenging method

Conc of extract/std mcg/ml	% Inhibition of Ascorbic acid	% Inhibition of Test extract
2	40.43±0.03	36.17±0.05
4	68.23±0.02	42.68±0.03
8	73.64±0.03	46.99±0.08
16	77.13±0.02	51.46±0.06
32	78.25±0.01	56.49±0.03
64	80.39±0.03	61.47±0.01
128	81.64±0.04	67.48±0.01
256	85.45±0.02	72.63±0.02
512	87.4±0.03	77.41±0.03

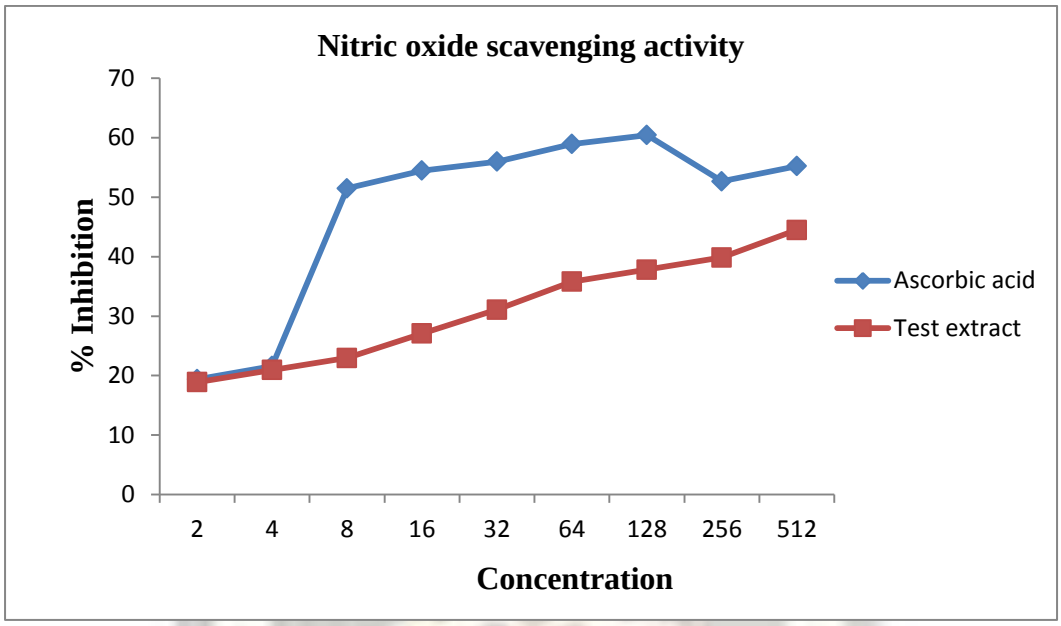

Figure 6: Effect of Cow urine Betel vine extract on Nitric oxide scavenging method.

Histopathological studies:

Group-I: Negative control (saline treated)

The histopathological studies of negative control group in ethanol induced ulcer model shows intact gastric mucosa with ulcerated mucosa. The epithelial cells and vascular spaces show necrotic changes with mixed inflammatory infiltration. The sub-mucosal layer shows severe edema with inflammatory cells infiltration. The muscularispropria appears intact and it is shown in the Figure 7.

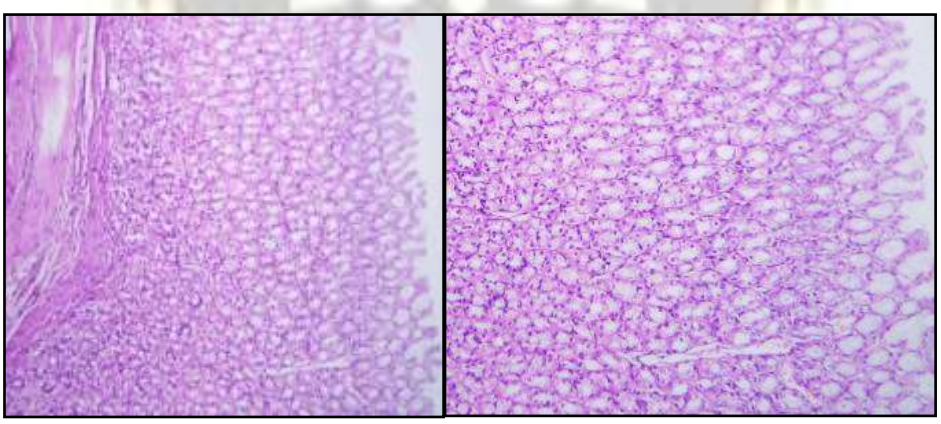

Figure 7: Histo-pathological study of Negative control (saline treated) group in Ethanol induced ulcer model.

Group-II: Positive control (Cow urine treated).

The histopathological studies of Positive control group in ethanol induced ulcer model shows intact gastric mucosa comprising of epithelium, lamina propria and muscularis mucosa. The

epithelium consists of columnar cells with underlying parietal cells, moderate edema, and mild inflammatory infiltration. The sub-mucosal and muscular layers appear intact and it is shown in the Figure 8.

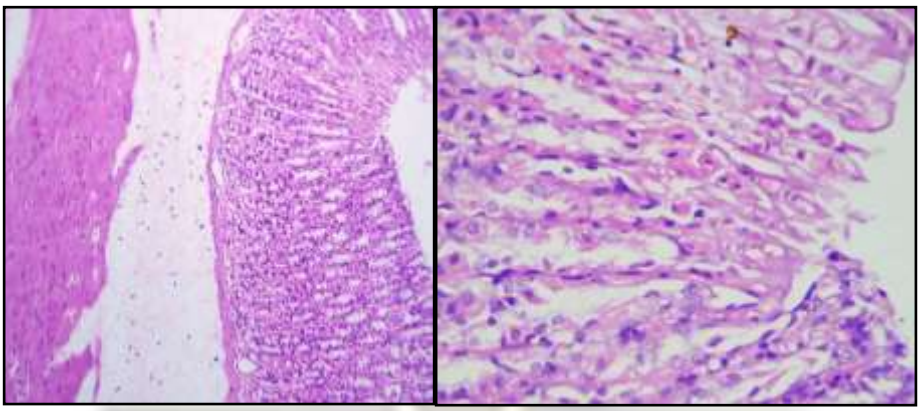

Figure: 8: **Histopathological study of Positive control (Cow urine treated) group in Ethanol induced ulcer model.**

Group-III: Standard drug (Ranitidine).

The histopathological studies of Standard group in ethanol induced ulcer model shows intact gastric mucosa with focal ulcerated epithelium, lamina propria and muscularis mucosa. The epithelium shows increased vascularity. The sub-mucosal shows moderate edema with scattered mononuclear inflammatory infiltration. The muscularispropria appears intact and is shown in the Figure 9.

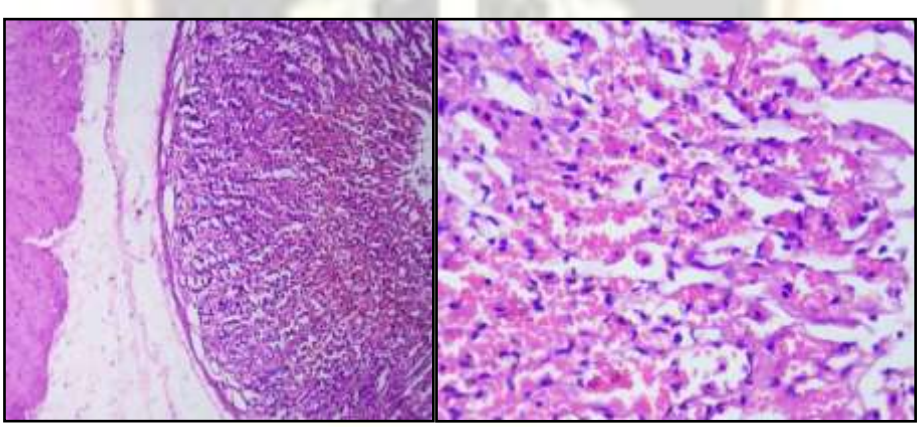

Figure 9: Histopathological study of Standard (Ranitidine treated) group in Ethanol induced ulcer model.

Group-IV: Test Extract I (250mg/kg b.w).

The histopathological studies of Test Extract I treated group in ethanol induced ulcer model shows intact mucosa consisting of some regenerated epithelial cells with intervening scattered

inflammatory infiltration. The submucosa shows mild edema with little inflammatory infiltration. The muscularis propria appears intact and it is shown in the Figure 10.

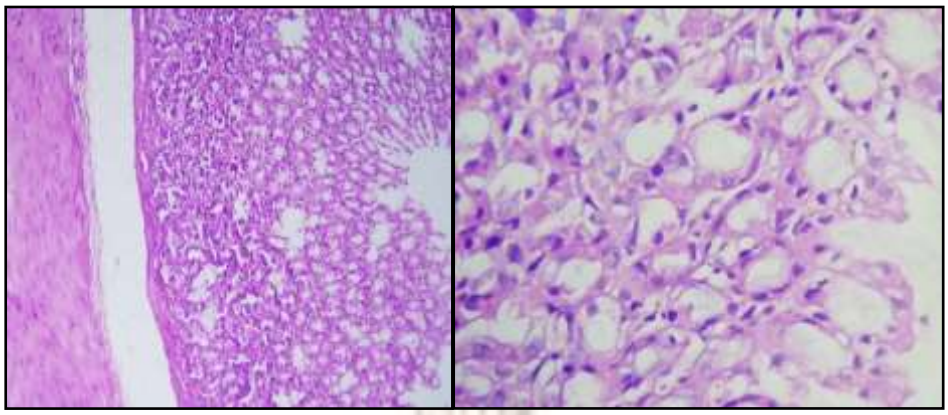

Figure 10: Histo-pathological study of Cow urine Betel vine extract (250mg/kgb.w.) in Ethanol induced ulcer model.

Group-V: Test Extract II (500mg/kg b.w)

The histopathological studies of Test Extract II treated group in ethanol induced ulcer model shows intact mucosa consisting of some regenerated epithelial cells with few congested blood vessels with mild inflammatory infiltration. The sub-mucosal appears intact. The muscularispropria appears intact and it is revealed in the Figure 11.

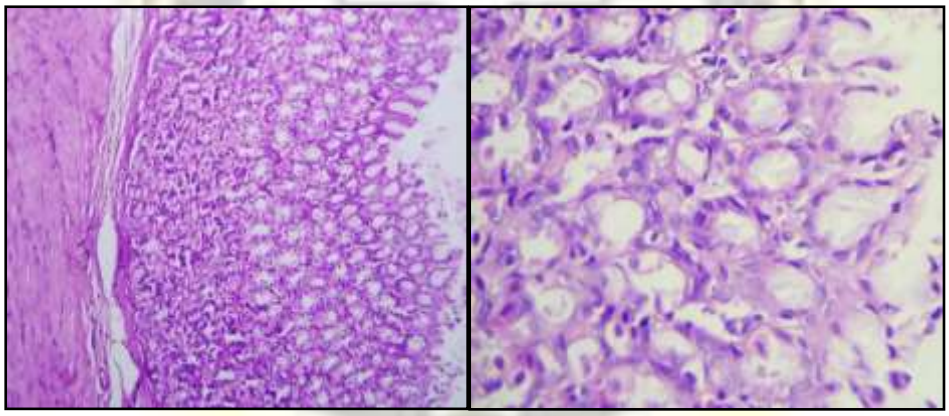

Figure 11: Histo-pathological study of Cow urine Betel vine extract (500mg/kg b.w) in Ethanol induced ulcer model.

DISCUSSION:

Peptic ulcer is leading cause of mortality and morbidity in developing countries, characterized by imbalance in between aggressive gastric luminal factor and defensive mucosal barrier. This disease is mainly associated with increase in gastric acid secretion. Numerous factors like diet, smoking, drugs like aspirin and infection are responsible for augmentation of ulcers. Still, no therapeutic intervention has been found successful. So, in the present study efforts has been made to review

and to explore various animal models to find out a suitable medication for the treatment of peptic ulcer.

Various synthetic and herbal drugs like tulsi, *Areca catechu* are employed in the treatment and management of the ulcers but still no complete curative treatment is available. So this review has been designed to explore the effects of Cow urine betel vine extract for the treatment of peptic ulcer. The extract at the dose of 500mg/kg b.w shows significant reduction ulcer count when compared to control and negative control and is almost equal to the selected standard ranitidine in ethanol induced ulcer models

Piper betel (family Piperaceae) has been traditionally used to treat pathological ailments like bacterial infections, liver disorders, ulcers etc 19. Cow urine is a divine medicine and is used for treatment of diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, AIDS, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, gynecological problems 20. Based on the review of literature of the two substances the current study was taken by combining the above two substances in the treatment of gastric ulcers induced by ethanol. Phyto-chemical screening of the extract showed the presence of alkaloids, tannins, flavonoids and triterpinoids.

Literature review indicates that tannins are involved in the antiulcer activities 21. Further, research reveals that betel vine increases secretion of several immune associated substances that protects the mucus membrane of normal intestine indicating a possibility to abate the injury of intestine [22].

Ethanol is involved in the progression of ulcer 23. Generation of free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa by ethanol [24]. Ethanol has been reported to stimulate the formation of leukotriene C4 (LTC4), histamine and reactive oxygen species that has been responsible to smash up the gastric mucosa [25, 26].

Administration of ethanol resulted in severe damage to goblet cells resulting in decrease in mucus. Moreover, ethanol resulted in increased ulcer index to 4 and 5 score with perforations. In addition, ethanol administration increased generation of ROS estimated by LPO, SOD and CAT.

Treatment with Cow urine betel vine extract significantly ($p<0.01$) attenuated the ulcer index with ulcer protection. Further extract significantly reduces the generation of ROS indicating antioxidant potential of the extract.

Table 1 shows the results of peptic ulcer induced by ethanol. The ulcer index in Test I and Test II treated animal are 13.4 and 12.2 respectively and when compared to control treated animals the ulcer index is reduced to about 21% and 25% respectively. The ulcer index of the standard treated

animals is 11.6. This indicates that the ulcer index of Test II animals is almost equal to the standard.

Table 2 indicates the effects of Test extract I and Test extract II on various anti-oxidant enzymes. Test I and Test extracts has reduced the lipid per-oxidation to about 2% and 3% respectively. The Test extracts did not altered the SOD values to the higher extant and hence the effect of Test extracts in reducing the SOD is not much. The Test I and Test II extracts has reduced the Catalase activity to about 7% and 14% respectively. Hence the result clearly indicates that the Test extract played a significant role in reducing the formation of free radicals and prevents the gastric ulcer formation.

Table 3 Shows the DDPH free radical scavenging activity of Test extract. The Maximum free radical scavenging activity was observed at the concentration of 512mcg/ml which is 44.5% and for the standard ascorbic at this concentration is 55.22%.

Table 4 shows the free radical scavenging activity of nitric oxide. The maximum nitric oxide free radical scavenging activity was seen at 512mcg/ml which is 77.41% and that of the standard ascorbic acid is 87.4%. From the result it is clear that the free scavenging activity of the Test extract is almost equal to that of the pure ascorbic acid. The free radical scavenging of the Test extract is because due to the presence flavonoids and phenolic compounds in the extract thus confirm the phytochemical chemical investigation of the extract.

CONCLUSION

The above finding thus suggest the antiulcer and the antioxidant potential of Cow urine betel vine extract, thus support the traditional use of the extract for controlling the gastric ulcers.

REFERENCES

1. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Gastric toxicity and mucosal ulceration induced by oxygen derived reactive species, protection by melatonin. Current Molecular Medicine 2001: 501-513.
2. Konturek P.C, Konturek S. Role of Helicobacter pylori infection in gastric - duodenal secretion and in pathgenesis of peptic ulcer and gastritis. Journal of Physiology and Pharmacology 1994; 45: 333-350.
3. Babu K S, Srinivas P V, Praveen B, Kishore K H, Murthy S, Rao J M. Antimicrobial constituents from the rhizomes of Rheum emodi. Phytochemistry 2003; 62: 203-207.

4. Radhika R, Krishna K, Sudarsanam D. Antidiabetic activity of Rheum emodi in Alloxan induced diabetic rats. International Journal of Pharmaceutical Sciences and Research 2012; 8: 296-300.
5. Ibrahim M, Khaja MN, Aara A, Khan AA, Habeeb MA, Devi YP. Narasu ML. Hepatoprotective activity of Sapindusmukorossi and Rheum emodi extracts: In nitro and in vivo studies. World Journal of Gastroenterology 2008; 14: 2566-2571.
6. Stevens P.F. Angiosperm Phylogeny Website Version 9, June 2008. http://www.mobot.org/mobot/research/apweb/welcome.html.
7. Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: Cassia tora, Momordicacharantia and Calendula officinalis. International Journal of Applied Research in Natural Products 2008; 1(3):20-28.
8. Obsai NL, Egbunonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samaneasaman* pods. African Journal of Pure and applied Chemistry 2010; 4(9):206-212.
9. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolate* (Ochanaceae). Life Science Journal 2007; 4(4):75-79.
10. Gujrati V, Patel N, Rao VN, Nandakumar K, Gouda TS, Shantakumar SM. Hepatoprotective activity of alcoholic and aqueous extract of leaves of Tylophoraindica (Linn) in rats. Indian J Pharmacol 2007; 39: 43-47.
11. Ray DK and Thokchom IS. Antiyretic, antidiarrhoeal, hypoglycemic and hepatoprotective activities of eethyl acetate extract of Acacia Catechu wild, in albino rats. Indian J Pahrmacol 2006; 38: 408-413.
12. Shivkumar SI, Suresh HM, Vrushabendraswamy BM, Kumar GS, Dhanpal R, Ashokbabu VL, Venkat Rao N. Hepatoprotective activity of fruits of Coccinia Grandis Linn against carbon tetrachloride induced hepatotoxicity. AdvPharmacolToxicol 2006; 7(1): 7-9.
13. , Rimpi Arora, Shaba Parveen. Study of different experimental models used in peptic ulcer. International Journal of Recent Advances in Pharmaceutical Research 2015; 5(2):31-37.
14. Gornall AG, Baradawill CJ, David MM. Determination of serum proteins by means of Biuret reaction Biol. Chem 1949; 177:151.
15. Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for determination of superoxide dismutase activity in tissue extracts. Anal Biochem 1986; 154: 536-541.

16. Esterbauer H, Cheeseman KH. Detremination of aldehydic lipid peroxidation products: Malondialdehyde and 4-hydroxynonenal. Meth. Enzymol 1990: 186: 407-42.
17. Shreedhara CS, Aswatha Ram HN, Sachin B, Zanwar. Free radical scavenging activity of aqueous root extract of*Argyreia nervosa*. J Nat Remedies 2009; 9: 216-223.
18. Balakrishnan N, Panda AB, Raj NR, Shrivastava A, Prathani R. The evaluation of Nitric oxide scavenging activity of *Acalyphaindica Linn* root. Asian Journal of Research and Chemicals 2009; 2: 148-150.
19. Kounsar F, Afzal ZM. Rheum emodi includes nitric oxide synthase activity in Murine macrophages. American Journal of Biomedical Sciences 2010; 2(2): 155-163.
20. Rajkumar V, Gunjan G, Rangasamy AK. Antioxidant and Anticancer potential of Rheum emodi rhizomes extract. e CAM Advance 2010; 3: 1-9.
21. De Jesus NZ, de Souza Falcao H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, Barbosa- Filho JM, Tavares JF, da Silva MS, de AthaydeFilho PF, Batista LM. Tannins, peptic ulcers and related mechanisms. Int J MolSci 2012; 13(3): 3203-3228.
22. Xu N, Chen XL, Lu LJ, Ciren SZ. Effects of rhubarb on the immune substance secreted from intestine in mice. ZhongguoZhong Yao ZaZhi 2005; 30: 1441-1443.
23. Kaur A, Singh R, Sharma R, Kumar S. Peptic ulcer: A review on etiology and pathogenesis. IRJP 2012; 3: 34-36.
24. Tendon R, Khana HD, Dorababu M, Goel RK. Oxidative stress and antioxidants status in Peptic ulcer and Gastric Carcioma. Indian Journal of Physiology and Pharmacology 2004; 48:115-118.
25. Kumar A, Sharma A, Vijayakumar M, Rao Ch V. Antiulcerogenic effect of ethanolic extract of Portulacoleracea Experimental study. Pharmacology online 2010; 1: 417-432.
26. Hussain Md T, Verma AR, Vijayakumar M, Sharma A, Mathela CS, Rao Ch V. Rutin, a natural flavonoid, protects against gastric mucosal damage in experimental animals. Asian Journal of Traditional Medicines 2009; 4: 1-10.