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PHARMACOLOGICAL EVALUATION OF ANTI ULCER ACTIVITY OF METHANOLIC LEAF EXTRACT OF *LUPINUS ARBOREUS* ON WISTAR RATS

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ABSTRACT

Peptic ulcers, a prevalent gastrointestinal disorder, necessitate effective treatment options to mitigate symptoms and promote healing. *Lupinus arboreus*, a plant known for its various medicinal properties, was evaluated for its potential to prevent and heal gastric ulcers. This study investigated the pharmacological evaluation of the anti-ulcer activity of methanolic leaf extract of *Lupinus arboreus* on Wistar rats, along with a comprehensive phytochemical analysis of the plant. The study employed a methanol extraction method to obtain the active constituents from the leaves of *Lupinus arboreus*. The phytochemical analysis revealed the presence of flavonoids, tannins, saponins, and alkaloids, which are known to contribute to gastro protective effects. The anti-ulcer potential was assessed using ulcer model induced by ethanol. Various dosages of the methanolic leaf extract were administered to evaluate their efficacy in reducing ulceration, compared to standard anti-ulcer drugs. Parameters such as ulcer index, gastric juice volume, pH, of the stomach lining were analyzed. The methanolic extract exhibited significant anti-ulcer activity, evidenced by reduced ulcer index and enhanced mucus production. These findings suggest that *Lupinus arboreus* leaves possess potent anti-ulcer properties, likely due to its rich phytochemical profile.

Keywords: Anti-ulcer activity, Methanolic leaf extract, *Lupinus arboreus*, phytochemical analysis, Wistar rats.

1. INTRODUCTION

The goal of drug therapy for peptic ulcers is to either stimulate the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide) or counteract aggressive factors (acid, pepsin, activeoxidants, platelet aggravating factor "PAF," leukotrienes, endothelins, bile, or exogenous factors, including NSAIDs) **(Al-Yahya *et al.*, 1990).** One of the most common disorders, gastric ulcers, are thought to result from an imbalance between aggressive and defensive forces. The stomach mucosa is constantly exposed to substances that could be harmful, including medicines, pepsin, acid, bile acids, dietary constituents, and bacterial products like Helicobacter pylori. Enhanced production of stomach acid and pepsin, suppression of prostaglandin synthesis and cell proliferative growth, reduced gastric blood

flow, and decreased gastric motility are among the agents that have been linked to the pathophysiology of gastric ulcer **(Toma *et al.*, 2005).** The global cause of substantial morbidity and a large drain on health care resources is still peptic ulcer disease and its consequences **(Tanih *et al.*, 2010).** Strong anti-ulcer medications are available, but the majority of them have several toxicities, which highlights the need to look for new alternatives. Up to 80% of people on the planet receive primary healthcare from plants, which supports the idea that plant extracts can be a good source of novel medications **(Lavnya *et al.*, 2012; Panda and Sonkamble, 2012)**. Relieving pain, healing the ulcer, and preventing a recurrence of the ulcer are the objectives of treating Peptic Ulcer Disease. As of right now, no affordable medicine achieves each of these objectives. Therefore, research is being done to identify a treatment that works using natural product sources. To get a good result, many spices and herbs have been investigated by different experts for their anti-ulcer properties **(Rafatullah *et al.*, 1990).** Yellow bush is the English name for the common decorative shrub *Lupinus arboreus. L. arboreus* is known as "Chikadoma" in Igbo, Southeast Nigeria **(Ohadoma *et al.*, 2015)**, after the principal investigator Dr. Chika Ohadoma, who spearheaded substantial effort on the groundbreaking study of this plant **(Ohadoma, 2016)**. It can be easily identified as a bushy shrub up to 1.8 meters tall with fragrant, vivid yellow flowers mixed with purple and white hues. Coastal dunes in Northern California are home to *L. arboreus,* an invasive plant **(Pickart *et al.*, 1998).** In traditional medicine, the boiled and macerated leaves of *L. arboreus* are applied to relieve pain and swelling associated with toothaches and chest pain. The ancients used lupines without any scientific evidence to treat scabies, ulcers, skin abnormalities, or other cutaneous diseases **(Lawal *et al.*, 2020).** Flavonoids, terpenoids, glycosides, alkaloids, steroids, and tannins are just a few of the many phytochemicals that are present in *L. arboreus* leaf extract and fractions, which also have antinociceptive, anti-inflammatory, and antibacterial properties **(Ohadoma *et al.*, 2014; SC *et al.*, 2018).**

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Lupinus arboreus* (300 gm) was collected. After cleaning, plant part (leaf) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant part (leaf) was stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Lupinus arboreus* was authenticated by a plant taxonomist (Dr. Jagrati Tripati, Govt. Botanist) in order to confirm its identity and purity, Authentication no.- AC/064/24 .

2.2 Animal

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). The animals used were of either sex weighing 150-250gm. They were kept in six separate cages at a controlled temperature of 22 ± 2°C. All animals were fed normal diet (golden feed, New Delhi) and provided with water on a regular basis.

2.3 Extraction

In the current investigation, plant material was extracted utilizing the continuous hot percolation method with Soxhlet equipment. The powdered substance of *Lupinus arboreus* was placed in a thimble of soxhlet. Soxhlation was carried out at 60°C using petroleum ether as the nonpolar solvent. The exhausted plant material (marc) was dried and then extracted again with methanol solvent. For each solvent, soxhlation was continued until no visible color change was observed in the siphon tube, and extraction was confirmed by the absence of any residual solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and each extract's % yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al.*, 2002).**

2.4 Phytochemical investigation

An experiment was conducted to assess the presence or absence of numerous phytoconstituents utilizing a detailed qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were used **(Kokate *et al.*, 2000).**

2.5 Quantitative Phytochemical Estimation

2.5.1 TPC

The total phenolic content of *Lupinus arboreus* extract was determined using the Folin-Ciocalteu Assay. The *Lupinus arboreus* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture

was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically (**Tangco *et al.*, 2015)**.

2.5.2 TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Lupinus arboreus* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. The calibration curve was used to determine the total flavonoid concentration, which was expressed as mg Rutin equivalent per gram of dry extract weight **(Parthasarathy S *et al.*, 2009).**

2.6 DPPH

The antioxidant activity of *Lupinus arboreus* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared.

To create different concentrations of *Lupinus arboreus* extracts/standard (20-100µg/ml), a 1mg/mL stock solution was mixed with 2mL of 0.1mM DPPH solution. The resultant mixture was vortexed and incubated for 30 minutes at room temperature in a relatively dark atmosphere before being measured at 517 nm with a UV spectrophotometer (Shimadzu 1700). For the control, add 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. Absorbance of the control was taken against methanol (as blank) at 517 nm **(Athavale *et al.*, 2012).**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

2.7 Acute Toxicity Study

The acute toxic class approach specified in the guideline is a step-by-step procedure that involves three animals of the same gender in each phase. Depending on the animals' mortality and/or moribund condition, 2-4 steps may be required to evaluate the test chemical's acute toxicity. The drug is given orally to a group of experimental animals at one of the specified doses. The chemical is tested step by step, with three animals of the same sex used in each phase. The absence or presence of compound-related mortality in the animals dosed in one stage determines the next phase, i.e., no additional testing is required; instead, three additional animals should be given the same dose, followed by three more animals given the next higher or lower dose level. Each step involves three animals. The dose level to be utilized as the initial dose is chosen from one of four predetermined levels: 5, 50, 300, or 2000 mg/kg body weight (**Guideline Document on 1996**).

2.8 Experimental work

The rats were obtained from animal house of Pinnacle Biomedical Research Institute, Bhopal (M.P). They were kept in appropriate environment and on a 12- hour light-dark cycle with free access to pellet food and water up to the time of experimentation. The animals were acclimatized to laboratory condition for 5 days prior to the actual experiments. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals and Organization for Economic Cooperation and Development (OECD) guidelines. Protocol approval CPCSEA number is **PBRI/IAEC/11-03-24/030.**

2.8.1 Induction of ulcer in rats:

Male Wistar rats weighing 200±50 were fasted for 24 hr with free access to water and rats randomly divided into five groups. The control group received a vehicle (distilled water, 5 ml/kg, through oral route) and Second group is inducer group which was treated with Ethanol 20 mg/kg through oral route. And treatment groups III and IV were given Ethanol 8 ml/kg and test sample (*Lupinus arboreus* extract- 100, 200 mg/kg body weight) and Standard group (V) was treated with the standard antiulcer drug (Ranitidine 20 mg/kg through oral route). After one hour of ethanol delivery, the rats were slaughtered, and their stomachs were taken and opened along the larger curvature**.** Rats (n=30) were randomized into following groups:

Group 1- Normal control

Group 2- Inducer group Ethanol 8 ml/kg bw

Group 3- Treated with *Lupinus arboreus* extract 100 mg/kg bw

Group 4- Treated with *Lupinus arboreus* extract 200 mg/kg bw

Group 5- Treated with standard drug (Ranitidine) 20 mg/kg bw

Parameters assessed for anti-ulcer activity

- Determination of Ulcer Index
- Determination of pH and Volume of gastric juice
- Free acidity determination

2.8.2 Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. The stomachs were then dissected along the bigger curvature, cleansed with normal saline to remove gastric contents, and examined under a 10x magnifying lens for the existence of ulcers. Ulcers were examined using the Kulkarni approach (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = hemorrhagic streaks, 3 = ulcers > 3 but < 5, and 5 = ulcers > 5). The ulcer index and the percentage of ulcer inhibition were calculated as follows:

$$\textbf{Ulcer index (UI) = UN + US + UP} \times \textbf{10}^{-1}$$

Where,

UN = Average number of ulcers per animal,
US = Average of severity score,
UP = Percentage of animals with ulcers

2.8.3 Volume, pH of gastric juice

Each animal's stomach juice volume was measured and examined following centrifugation at 1000 rpm for 10 minutes. The volume of the centrifuged sample was calculated as mL per 100g body weight.

A pH meter is used for determining pH after diluting 1 ml of gastric juice aliquot with 1 ml of distilled water.

2.8.4 Determination of free acidity

1 ml of distilled water was used to dilute 1 ml of gastric juice aliquot and then transferred to a conical flask (50 ml) with the addition of 2 drops of phenolphthalein indicator. 0.01 N NaOH was used for titration until a permanent pink color was resulted; its consumed volume was determined**.** The free acidity was calculated by the formula:

$$Acidity = \frac{Volume of NaOH \times Normality of NaOH \times 100}{0.1}$$

3. RESULTS

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used.

Table 1: Percentage Yield of crude extracts of *Lupinus arboreus* extract

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Lupinus arboreus*	Pet ether	289	1.65	0.57%
2		Methanol	297	6.34	2.13%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Absent	Present
Glycoside		
Borntrager test	Present	Present
Legal's test	Present	Present
Killer-Killiani test	Present	Present
Carbohydrates		
Molish's test	Absent	Present
Fehling's test	Absent	Present
Benedict's test	Absent	Present
Barfoed's test	Absent	Present
Proteins and Amino Acids		
Biuret test	Absent	Absent
Flavonoids		
Alkaline reagent test	Absent	Present
Lead Acetate test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Saponin		
Foam test	Present	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Absent	Absent
Libbermann-Burchard's test	Absent	Absent

3.3 Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

3.3.1 Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

Table 3 Standard table for Gallic acid and Rutin

S. No.	Concentration (µg/ml)	Absorbance (Gallic acid)	Absorbance (Rutin)
1.	20	0.146	0.173
2.	40	0.178	0.201
3.	60	0.190	0.277
4.	80	0.234	0.316
5.	100	0.273	0.331

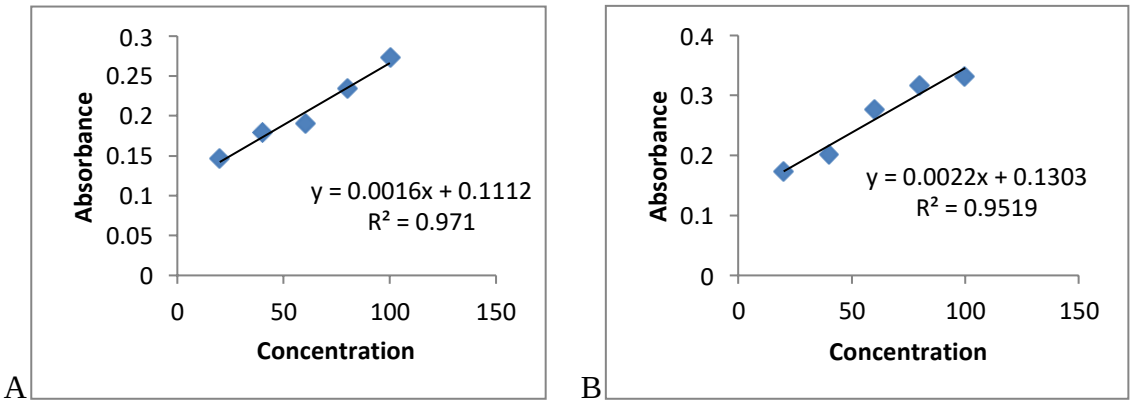


Figure 1: Represent standard curve of Gallic acid (A) and Rutin (B)

3.3.1.1 Total Phenolic Content in extract

Table 4: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.136	56 mg/gm
2	0.179	
3	0.186	

3.3.1.2 Total Flavonoid Content in extract

Table 5: Total Flavonoid Content

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.147	17.66 mg/gm
2	0.158	
3	0.191	

3.4 *In vitro* Antioxidant Assays

In the present investigation, the *in vitro* anti-oxidant activity of extracts of *Lupinus arboreus was* evaluated by DPPH radical scavenging activity. The results are summarized in Tables

3.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

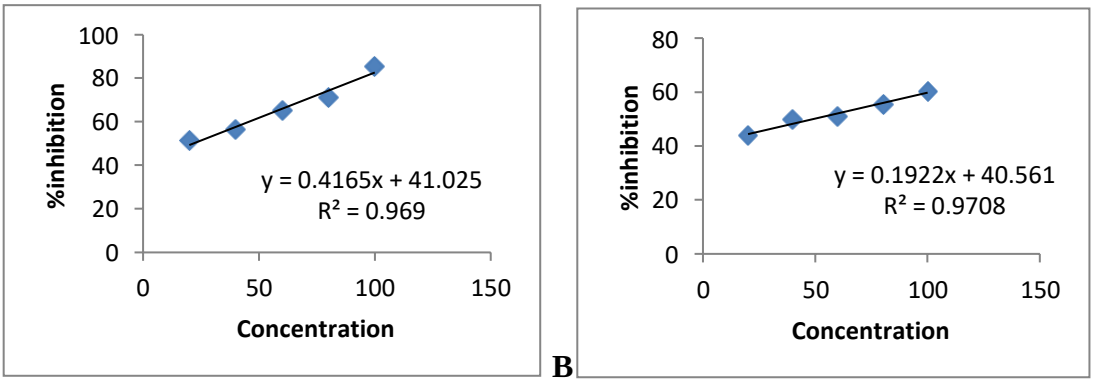
Table 6: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	51.307
40	0.432	56.539
60	0.345	65.291
80	0.284	71.428

100	0.144	85.513
Control	0.994	
IC50	21.58	

Table 7: DPPH radical scavenging activity of methanol extract of *Lupinus arboreus*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.519	43.952
40	0.465	49.784
60	0.454	50.971
80	0.413	55.399
100	0.367	60.367
Control	0.926	
IC50	49.16	



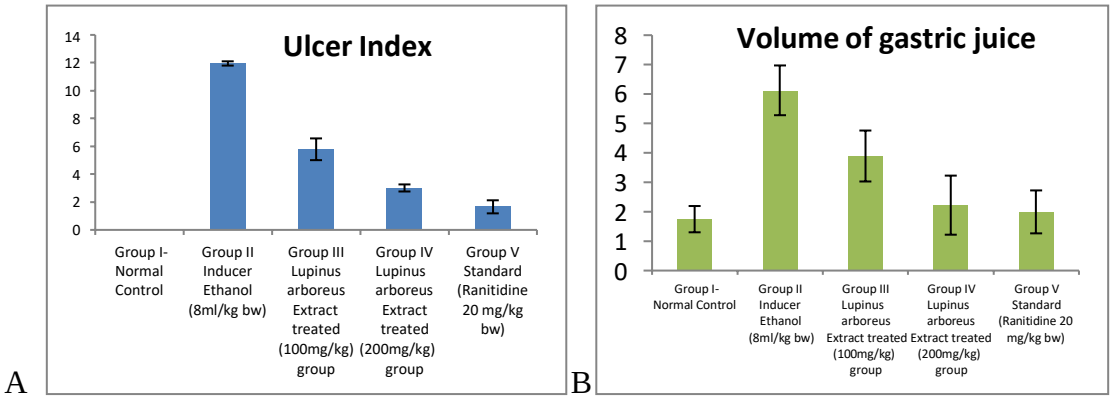
A B

Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid (A) and extract of *Lupinus arboreus* (B)

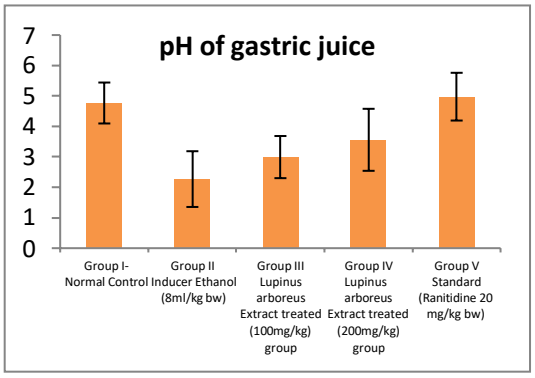
3.5 Determination of Ulcer Index, Volume and pH of gastric juice

Table 8: Observation of Ulcer Index, Volume and pH of gastric juice

Treatment Group	Ulcer Index (Mean)	Volume of gastric juice	pH of gastric juice
Group I- Normal Control	0	1.745± 0.448	4.766±0.673
Group II Inducer Ethanol (8ml/kg bw)	11.945±0.155	5.123± 0.846	2.267±0.915
Group III *Lupinus arboreus* Extract treated (100mg/kg) group	5.786±0.782	3.892± 0.865	2.988±0.693
Group IV *Lupinus arboreus* Extract treated (200mg/kg) group	3.004±0.253	2.224± 1.004	3.559±1.018
Group V Standard (Ranitidine 20 mg/kg bw)	1.656±0.468	1.993± 0.73	4.976±0.784



A B

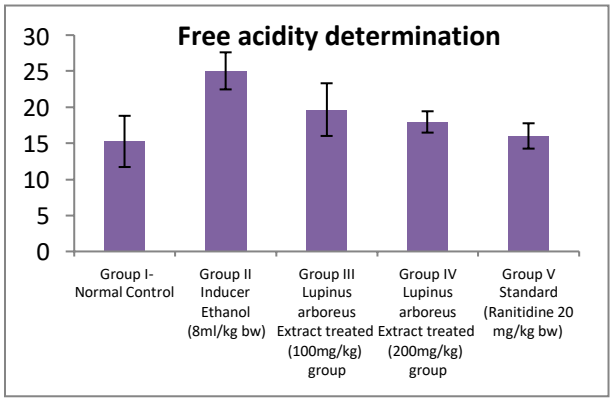
C 

Graph 1: Bar chart represents ulcer index (A), gastric volume (B), pH of gastric juice (C) in Ethanol induced ulcer in rats

3.6 Free acidity determination:

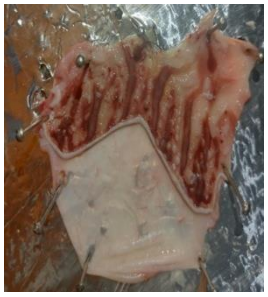
Table 9: Observation of free acidity in Ethanol induced peptic ulcer in rats:

Treatment Group	Free acidity determination (mE/L)
Group I- Normal Control	15.278±3.544
Group II Inducer Ethanol (8ml/kg bw)	25.054±2.563
Group III *Lupinus arboreus* Extract treated (100mg/kg) group	19.667±3.656
Group IV *Lupinus arboreus* Extract treated (200mg/kg) group	17.976±1.473
Group V Standard (Ranitidine 20 mg/kg bw)	16.034±1.754



Graph 2: Bar chart represents free acidity determination in Ethanol induced ulcer in rat

3.7 Images

1. 
2. 
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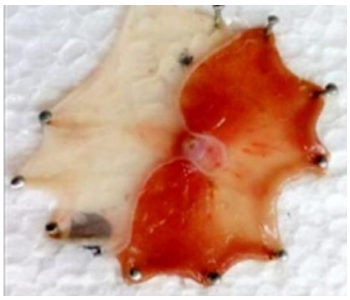
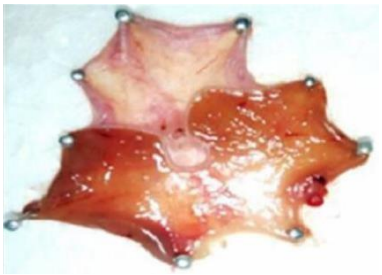
4.  5. 

Fig 3: 1. Normal Stomach, 2. Induced, 3. Standard, 4. 100mg/Kg extract and 5. 200mg/Kg extract

4. DISCUSSION

The phytochemical examination of a methanolic extract of *Lupinus arboreus* revealed the presence of alkaloids, phenolic, saponins, glycoside, saponin, carbohydrates, tannin, and phenolic. TPC and TFC were calculated as part of a quantitative phytochemical experiment. The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. Results shown in Table 4 & table 5. DPPH radical scavenging activity of *Lupinus arboreus* extract exhibited percent inhibition 60.36% and its IC 50value was found to be 49.16µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 85.51% and showed IC 50 value of 21.58µg/ml. In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study. Five groups of adult albino wistar rats were taken for the study. Rats were divided into five groups each containing 6 animals. Group first is normal group received the saline for 7 days. Group second is Ethanol inducer group (8 ml/kg bw). Group third is *Lupinus arboreus extract* (100 mg/kg bw). Fourth group is *Lupinus arboreus* extract (200mg/kg bw) and Group fifth is standard (Ranitidine 20 mg/kg bw). The extract of *Lupinus arboreus* was evaluated by using ethanol induced peptic ulcer model. Ulcer produced in this model was seen as red sores. The stomachs of rats in the ethanol induced peptic ulcer showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs. There was a significant decrease in the measured gastric ulcer index in the stomach of *Lupinus arboreus* (200 mg/kg bw) treated animals when compared with the *Lupinus arboreus* (100 mg/kg bw) treated. The volume of gastric juice was observed as 2.224 ml of *Lupinus arboreus* (200 mg/kg bw) in decreased level as compared to *Lupinus arboreus* (100 mg/kg bw) treated group showed gastric volume of 3.892. The pH of gastric juice was observed as 3.559 of *Lupinus arboreus* (200 mg/kg bw) treated group and it showed the reduction in acidic pH as compared to *Lupinus arboreus* (100 mg/kg bw) showed 2.988 pH. The free acidity was observed as mE/L

of *Lupinus arboreus* (200 mg/kg bw) treated group 17.976 and it showed the reduction in acidity as compared to *Lupinus arboreus* (100 mg/kg bw) showed 19.667 mE/L.

5. CONCLUSION

Thus, from the present study it was concluded that the treatment of *Lupinus arboreus* leaf extract maintains the normal range of acidity and also maintain the pH level of stomach. Present study supports the use of *Lupinus arboreus* extract by local healers as traditional medicine in treatment of ulcer. This effect can be attributed to presence of various bioactive components present on extract and also be due to protective potential of extract confirms the mechanism of anti-ulcer activity against ethanol induced peptic ulcer.

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