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PHYTOCHEMICAL INVESTIGATIONS OF *ACORUS CALAMUS* PLANT EXTRACT AND ITS STUDY FOR ANTIULCER ACTIVITY

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ABSTRACT

The condition known as ulcer occurs when the gastrointestinal mucosal defense and repair process is disrupted, resulting in erosion of the stomach or duodenal lining. These days, gastric hyperacidity and ulcers are increasingly frequent and cause a great deal of misery for people. There is an imbalance between the gastroduodenal mucosa's defense mechanisms and the lumen's harmful elements. The goal of this study was to determine the pharmacological properties of the Acorus calamus leaf extract. Petroleum ether and ethyl alcohol were used to extract the dry and powdered leaf material. The main goal of this research work was to carry out the preliminary phytochemical studies of ethanolic extract of leaves of Acorus calamus. Lastly, the antiulcer properties of an ethanolic extract of Acorus calamus leaves were further assessed. Ultimately, it may be said that the testing group that received greater dosages of the ethanolic extract of Acorus Calamus leaves may be showing some promise in treating ulcers when compared to the regular group. It may be possible to incorporate additional clinical data and proof from various studies to better validate this statement.

Keywords: Antiulcer Activity, Ethanolic Extract, Photochemical Screening, Acute toxicity studies, Histological Studies, Physicochemical Parameters.

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INTRODUCTION:

Ulcer is a condition, where there is erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair mechanism. Gastric hyperacidity and ulcer are very common, causing tremendous human suffering now a day. It is an imbalance between damaging factors, within the lumen, and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism, however, is still very poorly understood [1,2]. The most common cause of ulcers is infection of the stomach by bacteria called *Helicobacter pylori (H. pylori)*. Most people with peptic ulcers have these bacteria living in their digestive tract. Yet, many people who have these bacteria in their stomach do not develop an ulcer. The main cause of stomach ulcers is believed to be due to a stomach infection by a bacterium called *Helicobacter pyloricus (H. pylori*).

Another major cause of ulcers is the use of chronic anti-inflammatory drugs, often referred to as NSAIDs (antiinflammatory drugs), including aspirin. Smoking is also an important cause of ulcer formation and failure of ulcer treatment. Treatment that can be done to treat stomach ulcers is with antacid drugs that can neutralize stomach acid, histamine H_2 blocker antagonists. Histamine antagonists (H_2 blockers) are drugs designed to block the action of histamine on stomach cells thereby reducing acid output, for example cimetidine, ranitidine and famotidine. It is hoped that we can protect our stomachs from food and drinks that enter the body from being infected by the *Helicobacter pylori* bacteria and also not underestimating stress. Because when stress increases, stomach acid production causes the pH in the stomach to become acidic so that it can damage the stomach lining and it is also advisable to stop smoking because smoking is the cause of failure in the treatment of stomach ulcers [3-11].

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Acorus calamus is a native of Central Asia and Eastern Europe and also it is indigenous to the marshes of the mountains of India. It is cultivated throughout India, ascending to an altitude of about 2200 metres. It is also found in marshy tracts of Kashmir, Shirmaur (Himachal Pradesh), Manipur and in Naga Hills. It is regularly cultivated in the koratagere taluka of Karnataka state in peninsular India [12]. *Acorus calamus* Linn. (Commonly called as 'Sweet flag') of family Araceae, is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, sword shaped leaves and spadix inflorescence. *A. calamus* grows either as wild or cultivated crop throughout India ascending upto 1800 m in the Himalayas [13]. *Acorus calamus* is now also found across Europe, in southern Russia, northern Asia Minor, southern Siberia, China, Indonesia, Japan, Burma, Sri Lanka, Australia, as well as southern Canada and the northern United States where it may be confused with diploid *Acorus americanus*. (The morphological distinction between the *Acorus* species is made by the number of prominent leaf veins. *Acorus calamus* has a single prominent midvein and then on both sides slightly raised secondary veins (with a diameter less than half the midvein) and many fine tertiary veins. This makes it clearly distinct from *Acorus americanus*) [14].

In Asia, Sweet flag has been used for at least the last 2000 years. The ancient peoples of China used it to lessen swelling and for constipation. The parts used are leaves, root (rhizome) and stem. In India, Ayurvedic medicinal practice has used the rhizomes to cure several diseases like fever, asthma and bronchitis, and as a sedative [14]. The Sioux used the whole plant, making aromatic garlands from the leaves and using the root as a tea for bowel pains, or rubbed the chewed root on the skin for a general illness cure [15].

For the Penobscot people this was a very important root. One story goes that a sickness was plaguing the people. A muskrat spirit came to a man in dream, telling him that he (the muskrat) was a root and where to find him. The man awoke, found the root, and made a medicine which cured the people. In Penobscot homes, pieces of the dried root were strung together and hung up for preservation. Steaming it throughout the home was thought to "kill" sickness. While traveling, a piece of root was kept and chewed to ward off illness. The Potawatomi people powdered the dried root and placed this up the nose to cure catarrh [12].

Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions [17-23]. It is widely employed in modern herbal medicine as its sedative, laxative, diuretic, and carminative properties [13]. It is used in Ayurveda to counter the side effects of all hallucinogens [18]. Both roots and leaves of A. calamus have shown antioxidant [20], antimicrobial and insecticidal activities [16]. Acorus Calamus was also known to many early American settlers and used for a number of diseases. Walt Whitman even wrote poetry about his beloved herb in "Leaves of grass"[19].

As mentioned by *Cover TL* et al. (2020) infection usually lasts for years, causing ulcers in 10% to 15% of those infected with *H. pylori* found in more than 80% of patients with gastric and duodenal ulcers. Meanwhile, the mechanism by which *H. pylori* causes ulcers is not well understood. Elimination of these bacteria with antibiotics has clearly been shown to prevent recurrence of ulcers [2]. The development of medicines starts with the identification of active compounds, detailed biological assays and dosage formulations followed by clinical analysis to establish safety and efficacy of the new drug (Ncube et al., 2008) [24]. Plants and fruits are considered as one of the main sources of important phytocompounds. Plants contain large number of secondary metabolites such as flavonoids, phenols, steroids, alkaloids, saponins and glycosides (Shahidi, 2008) [25]. *Acorus calamus* L. (Family: Acoraceae), commonly known as Sweet Flag is a perennial monocot herb and identified as an endangered medicinal plant species. It is an extensively branched, cylindrical rhizome up to 2.5 cm thick, purplish brown to light brown externally and white internally. It is used as a relief to the digestion disorders, appetite, stomach cramps and colic (Balakumbahan et al., 2010) [26]. Plant leaves, rhizomes and its essential oil has various biological actions like antispasmodic, carminative, mental ailments remedy, chronic diarrhea, dysentery, tumors reliever and used for treatment of epilepsy (Devi and Ganjewala 2009) [27]. It has antifungal, antibacterial, antidiarrheal, insecticidal properties (Phongpaichit et al., 2005), tranquilizing, antidyslipidemic, antioxidant, anticholinesterase, spasmolytic, neuroprotective, vascular modulator activities (Shaha and Gilani, 2010) [28]. Various extracts of *A. calamus* are traditionally used for the antidiabetic, immunosuppressive, hypolipidemic, mitogenic, antiproliferative, and anticarcinogenic activity towards human lymphocytes (Palani et al., 2010) [29]. It has been reported that leaves extracts of *A. calamus* show antimicrobial activities (Khatri et al., 2016) [30].

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs. In recent years, there has been growing interest in alternative therapies and

the therapeutic use of natural products, especially those derived from plants.

Acorus calamus leaves, rhizomes and its essential oil has many biological activities like antispasmodic, carminative and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent and tumors. It also has the insecticidal, antifungal, antibacterial [23]. Tranquilizing, anti-diarrhoeal, anti-dyslipidaemia, neuro protective, antioxidant, anticholinesterase, spasmolytic, vascular modulator activities [22]. The various extract of *Acorus calamus* is traditionally used for the antidiabetes, antiproliferative, immunosuppressive, hypolipidemic, mitogenicand anticarinogenic activity towards human lymphocytes. The different extract forms possess the antispasmodic, anthelminthic, antifungal, antibacterial, fish toxin, insecticidal, anti-diabetes, anti-proliferative, immunosuppressant, antidiarrhoeal, and antioxidant and hypo lipidemic activities. The rhizomes and leaf part were found to possess the mitogenic and anticarcinogenic activity towards human lymphocytes [23]. The rhizomes are also used for treatment of epilepsy, mental ailments, chronicdiarrhea, dysentery, intermittent fevers, cough, throat irritations, bronchitis, as expectorant, and tumors [12, 13].

Fig. 1: Plants parts of A*acorus Calamus*

The present study was intended to find out the pharmacological activities of the extract of leaves of *Acorus* calamus. The dry and powder portions of leaves were extracted with petroleum ether and ethyl alcohol. The main goal of this research work was to carry out the preliminary phytochemical studies of ethanolic extract of leaves of *Acorus calamus.* Finally ethanolic extract of leaves of *Acorus calamus* was further evaluated for the antiulcer activity.

MATERIALS AND METHODS:

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Leaves of plant of *Acorus calamus* was collected in the Shirmaur Lake of Nainital District in Uttrakhand, India. The plant material was taxonomically identified and authenticated by Dr. Bapi Ray Sarkar Associate Professor & HOD of Department of Pharmaceutical Technology University of North Bengal, Raja Rammohunpur, District- Darjeeling West Bengal, India Pin 734013. Authentication letter voucher no NBU/PHARM. TECH/003/2025

EVALUATION OF PHARMACOGNOSTIC PARAMETERS

Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials as well documented by some researchers [12-16,21-23].

EXTRACTION OF PLANT MATERIALS

Extraction of *Acorus calamus* leaves

The freshly collected leaves (2 kg) of *Acorus calamus* were washed with potable water and finally rinsed with distilled water and shade-dried. Then collected and cleaned parts of plant were dried in tray drier under controlled conditions at 35^{o}C ±2 ^{o}C and powdered it. The powdered plant materials (500g) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. All chemicals and reagents used including the solvents were of analytical grade [24-30, 31-33].

PRELIMINARY PHYTO-CHEMICAL TESTS

Preliminary qualitative phytochemical screening of 50% ethanolic extract of ***Acorus calamus leaves*** were performed for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resin, saponins, steroids and tannins [27-37]. All the tests were done in triplicate except the foreign matter and moisture content.

PHARMACOLOGICAL SCREENING ON ANIMAL MODELS

ANIMALS AND ENVIRONMENT CONDITION

All the animals used for the study were healthy and active in their cage. Studies were carried out using male Wistar rats weighing 170-200 g. They were obtained from ilisted isuppliers iof ifrom iCPCSEA ilisted iof iShri iRamnath iSingh icollege iof iPharmacy, iGormi, iBhind, iGwalior The rats were group housed in polyacrylic cages (38×23×10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 ^{0}C) and relative humidity 50% (±10%), with a dark and light cycle of 12 ± 1 h. They were allowed free access to standard dry pellet diet (Amrut, India) and water *ad libitum* and kept in quarantine for a week to acclimatize with animal house facility. All procedures described were reviewed and approved by the institutional committee for ethical use of animals [33-39].

ACUTE TOXICITY STUDY

Acute toxicity study was conducted in two animal groups containing 6 male Wistar rats in each group, for two weeks. Animals were fasted overnight and treatments were given in the morning. Group I and II were given single dose of 1000mg/kg b.w. and 2000mg/kg b.w. of ethanolic extract (volume < 2ml) of stem part of each plant through gastric intubation suspended in 5% acacia solution, respectively. Animals were observed for 8 hours on the day of administration and subsequently once daily for the period of 3 days and finally observed up to fourteen days to record possible food-water intake and body weight changes and observed activity [OECD]. No mortality or gross abnormality was observed with the given dose. Hence, based on the acute toxicity study, three oral doses viz. 200 and 400 mg/kg, were selected for anti ulcer activity study and acute toxicity study revealed the nontoxic nature for each selected plant extract [33-39].

ANTI-ULCER ACTIVITY [40-43]

PYLORUS LIGATION INDUCED ULCER MODEL

EEAC (200 and 400 mg/kg) was administered orally to fasted rats, while omeprazole (4 mg/kg) was given p.o to the standard group and control group received distilled water, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia.

Animals were allowed to recover and stabilize in individual case and were deprived of water during post operative surgery.

After 4 h. of surgery, rats were sacrificed and ulcer score was calculated.

The rats were euthanatized after an hour; stomachs were opened along the greater curvature and observed for ulcers in the glandular region. The gastric content was measured.

The stomach and liver samples were collected for biochemical analysis. The surface area of each lesion was measured and scored for ulcer index using the formula

Ulcer index = 10/X

Where, X= total mucosal area/total ulcerated area.

Based on their intensity, ulcer scores were given arbitrarily as,

0: Absence of any detectable lesion,

0.5: Small Haemorrhagic effusion,

1.0: Haemorrhagic effusion,

1.5: Mucosal ulceration of limited diffusion involving more than 1/3 of the whole surface,

2.0: Mucosal ulceration of limited diffusion involving more than 2/3 of the whole surface,

2.5: Mucosal ulceration of generalized diffusion,

3.0: Deep ulcerations of limited diffusion,

3.5: Deep ulcerations of generalized diffusion,

Gastric juice was collected and gastric secretions studied were performed.

EVALUATION OF ANTIULCER ACTIVITY [17,21,25,40-45]

Collection of Gastric Juice

The gastric juice was collected and centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured.

Biochemical Estimation of Antioxidant Enzymes
CAT, SOD, GSH and LPO assays were performed taking both liver and stomach scrapings to study the effect of the extract on anti-oxidant enzymes in the organs. The liver samples were prepared at a concentration of 200 g/L and the mucosal scrapings were prepared at a concentration of 100 g/L in 20 mM Tris buffer (pH 7.4) and centrifuged at 3000 g at 4°C for 30 min. The supernatant was collected to estimate SOD, CAT, GSH and LPO.

SOD (Superoxide Dismutase)
The spectroscopic assay for SOD (ECI, 1.15 was performed by Marklund and Marklund (1974) with slight modification and activity was expressed as units/mg protein. Pyrogallol was used as a substrate and the rate of inhibition of pyrogallol auto oxidation was taken from the increase in the absorbance at 540 nm UV-Vis Spectrophotometer.

Reduced Glutathione (GSH)
Reduced glutathione on reaction with DTNB (5,5' dithiobis nitro benzoic acid) produces a yellow coloured product that absorbs at 412 nm. Estimation was done by using fluorometric method of Cohn and Lyle (1966). The absorbance was read at 412 nm within 5 minutes. Quantity of glutathione in the sample was calculated using standard -glutathione and values represented as microg/mg protein.

Catalase
Catalase activity was measured by the method of Aebi (1984). In the UV range, hydrogen peroxide shows a continuous rise in absorption and decreasing wavelength.
The decomposition of hydrogen peroxide can be followed directly by the decrease in extinction at 240 nm. The difference in extinction per unit time is a measure of catalase activity. To 0.1 ml of sample, 2.9 ml of Phosphate buffer-H_2O_2 was added and the absorbance was read for 3 minutes at 240 nm.

Lipid Peroxidation
To 1ml of tissue homogenate, 1ml of normal saline (0.9 per cent w/v) and 2.0 ml of 10 per cent TCA were added and mixed well.
The mixture (3000 g) was then centrifuged at room temperature for 10 min to separate proteins.
Then, 2 ml of supernatant was taken and 0.5ml of 1.0 per cent TBA was added to it followed by heating at 95°C for 60 min. to generate the pink colored MDA. OD of the samples was measured at 532 nm.
The levels of lipid peroxides were expressed as nM of MDA/mg wet tissue using extinction co-efficient of 1.56×105 M-1 cm^{-1}.

Histopathological Evaluation [40-45]
Stomach histopathology:
After the standard processing, the ulcerated gastric tissues were examined under the microscope for histopathological changes such as inflammation, infiltration, and erosion.
For histological studies, tissues were collected and fixed in 10 per cent neutral formalin solution and dehydrated with a series of ethanol-xylene solutions. The materials were processed by conventional paraffin embedding method. Microtome sections were prepared at 6 µm thicknesses, mounted on glass slides, stained with hematoxylin and eosin followed by observation for histopathological changes under light microscope.

Liver histopathology:
Pieces of liver from each liver lobe were fixed in Bouin's fluid for 24 hr and washed in running tap water to remove the color of Bouin's fluid and dehydrated in alcohol in ascending and descending order, embedded in paraffin and cut at 5µm (Automatic Tissue Processor, Lipshaw) in a rotary microtome. These sections were then deparaffinized in xylene, stained with hematoxylin-eosin dye (Merck, India) and mounted with Canada-balasam. The histological slides were examined and photographs were taken.
Statistical Analysis
Results were expressed as ±SEM (n=6). Statistical analysis were performed with one way analysis of variance (ANNOVA) followed by Dunnett's 't' test P value less than < 0.05 was considered to be statistically significant.
*P>0.05, ** P>0.01 and ***P>0.001 when compared with the control group.

RESULTS AND DISCUSSION:
PHYSICOCHEMICAL STUDIES:
Fresh leaves of the selected plant were collected, washed and after drying up properly it was subjected for the analysis of various physicochemical parameters. **% of foreign organic matter** of ***Acorus Calamus*** (AC) was found in the range of 0.22-0.88 and loss **on drying** was found in a range of 5.41-9.33**.** The moisture content plays vital role in storage of the drugs and as it varies a lot the extractive values will vary which will affect the actual dose of the drugs, as well as due to higher level of moisture content that may be affected on the rate detoriation of crude drug. Determination of loss on drying in crude drugs also represents the presence of moisture level in the materials. If the moisture content is very less then that may be preserved the materials up to long duration and also the dose of the crude drugs is requires very lower

level compare to the crude drugs having large amount of moisture.

The **total ash** of the leaves of ***Acorus Calamus*** was found 0.241-0.297 while **acid insoluble ash** and **water-soluble ash values** were in the range of 0.66-6.22and 0.33-0.45. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The **ash value of the leaves of *Acorus Calamus* was** found 3.42. Total ash reflects the care taken in its preparation as all traces of organic matter are removed during ash formation and usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. A higher limit of acid insoluble ash imitates the cases where silica may be present or when the calcium oxalate content of the drug is very high.

Extractive values of crude drugs are supportive for their estimation, particularly when the constituents of a drug cannot be willingly predictable by any other method. Further, these values point out the nature of the constituents present in a crude drug. The percentages of water soluble extractive values and alcohol soluble extractive values (w/w) of leaves of ***Acorus Calamus*** were 3.82-6.77 and 8.64-11.44 respectively.

PERCENTAGE YIELD:

The percentage yield of ethanolic extract of leavess of ***Acorus Calamus*** was found to be 9.2% (w/w). Further the qualitative phytochemical analysis of the ethanolic extract of leavess of the plant were conducted for confirming the presence or absence of alkaloids, tannic acids, glycosides, saponins, phytosterols, flavonoids, terpenoids, triterpenoids, resins, phenolic compounds & tannins, carbohydrates and proteins & amino acids.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

50% ethanolic extract of leaves samples of the selected plant were subjected to various qualitative tests for the identification and the presence of various phytochemical constituents. The present studies were carried out for the ethanolic extract of leaves of ***Acorus Calamus*** and which revealed that the presence of medicinally active metabolites in the samples. The phytochemical character of leaves of the ***Acorus Calamus*** investigated is summarized in table form. It can be clearly stated and concluded from table mentioned for the phytochemical evaluation data of the leaves extract of the plant. It has been sheen that presence of tannic acids, Glycosides, Saponins, Flavonoids, Terpennoids, Carbohydrate, Protein, phenolic compounds and Tannin in ***Acorus Calamus***. Anthraquinone, alkaloids, Cardiac Glycosides, T Sterols, triterpenoids, resins were absent in ***Acorus Calamus***.

STUDIES OF MORPHOLOGY & MICROSCOPIC CHARACTER:

Fresh leaves of the selected plant (***Acorus Calamus***) were collected, washed and after drying up it was subjected for the various morphological and microscopical studies and it was further matched with some earlier reported matters.

Macroscopic & Microscopic character of *Acorus Calamus*

Morphology

Height of the collected leaves of ***Acorus Calamus*** was found smooth, aromatic odor and hight is 22.1-1.2 cm and 1.24 cm wide it was found linear, slender, narrow and long.

Microscopy

Microscopic evaluation

The microscopy of Acorus ***Calamus*** Linn fresh leaves showed that it was a monocot plant. T.S. of leaves shows epidermis with randomly arranged vascular bundles and shows the thick-walled parenchyma and aerenchyma.

PHARMACOLOGICAL SCREENING ON ANIMAL MODELS

ACUTE TOXICITY STUDY

Table 1: Data showing the determination of acute toxicity of 50% ethanolic extract of leaves of *Acorus Calamus* plant

Treatment / Dose	Total mice taken	Mortality (after 72 hr.)
		Inference of ethanolic extract of leaves of *Acorus Calamus*
500mg/kg(25-30gm)	6	0
1000mg/kg(25-30gm)	6	0

ACUTE TOXICITY STUDY:

The 50% ethanolic extract of **leaves of *Acorus Calamus*** has shown 0 % mortality (**Table 1**) at a dose corresponds to 500 & 1000 mg/kg body weight after observing continuously for 8 hours and finally overnight mortality recorded. Behavior of the animals including body weight and any other toxic symptoms also observed for 72 h and the animals were kept under observation up to 14 days.

Acute toxicity study exposed the non-toxic nature of the ethanolic extract of leaves of the plant. There was no mortality or any toxic effects observed at the maximum tested dose level of 1000 mg/kg after 14 days.

From the acute toxicity study, it can be concluded that the ethanolic extract of leaves of the selected plant (***Acorus Calamus***) has no fatal effect up to 1000mg/kg body weight after oral administration in rats. Hence, as per the literature guidelines 1/10th of the dose was set to assess the anti ulcer activity of the selected plant (***Acorus Calamus***).

Finally it can be concluded from the acute toxicity study that the ethanolic extract of leaves of the selected plant (***Acorus Calamus***) were safe and sound still at doses as high as 1 g/kg b.w. of rats.

PHRMACOLOGICAL EVALUATION

Acute toxicity study following OECD guidelines 423(2001)

Acute toxicity studies carried out prior to pharmacological evaluation studies of 50% **ethanolic extract of leaves of *Acorus Calamus*** using OECD [39] Test Guideline 423(2001). 50% **ethanolic extract of leaves of *Acorus Calamus*** was found to be safe up to 1000 mg/kg body weight.

Anti-Ulcer Activity

PYLORUS LIGATION INDUCED ULCER MODEL

Table 2: Ulcer score, ulcer index, gastric content, pH of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant

Sl. No.	Ulcer score	Ulcer Index	Gastric content (ml)	pH
Control Group	3.95±0.64	3.41±0.48	6.61±0.89	2.42±0.30
Standard 4 mg/Kg b.w.	0.31±0.012	0.16±0.02	1.57±0.23	4.64±0.40
100 mg/Kg b.w.	0.49±0.22*	0.29±0.08*	3.55±0.45*	4.04±0.53*
200mg/Kg b.w.	0.41±0.22**	0.21±0.06***	2.55±0.42*	4.5±0.55**

Results were expressed as ±SEM (n=6). Statistical analysis were performed with one way analysis of variance (ANNOVA) followed by Dunnett's 't' test P value less than < 0.05 was considered to be statistically significant.*P>0.05, ** P>0.01 and ***P>0.001 when we compared with the control group

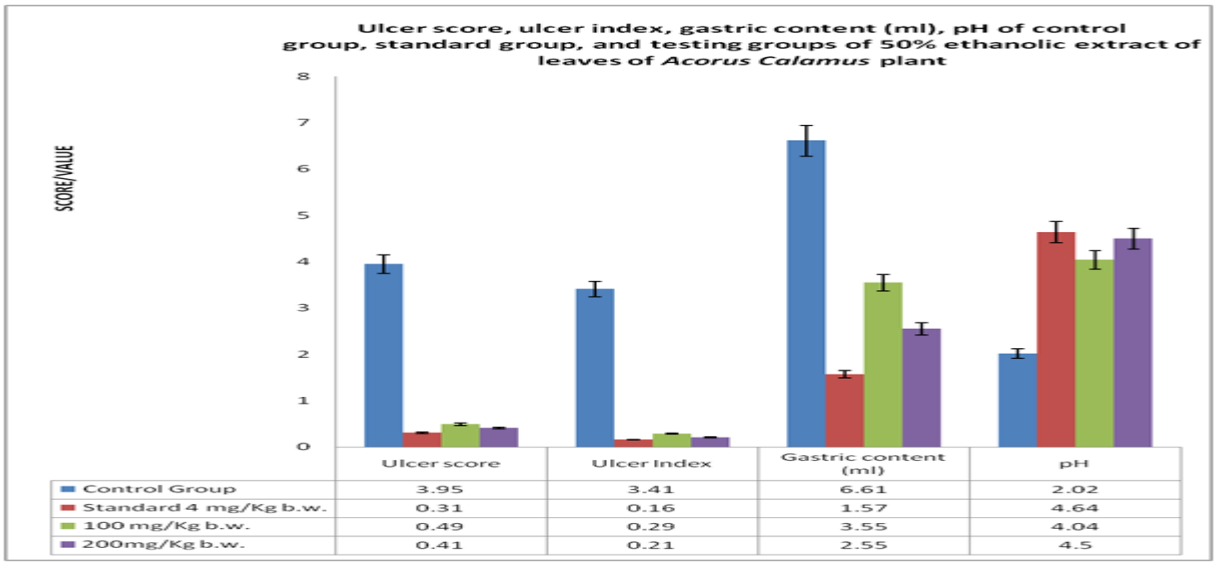

Figure 2: Ulcer score, ulcer index, gastric content, pH of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant.

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Table 3: LPO and SOD of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant. SOD (Superoxide Dismutase) in the sample was calculated using SOD and values represented as microg/mg protein and the levels of lipid peroxides were expressed as nM of MDA/mg wet tissue using extinction co-efficient of 1.56 × 105 M-1 cm^{-1}.

Sl. No.	LPO (MDA/gm weight of tissue)		SOD (units/mg of protein)	
	stomach	liver	stomach	liver
Control Group	7	15	0.002	0.005
Standard Group (4mg/Kg b.w.)	1	7	0.005	0.018
EELAC-100mg/Kg b.w.	4*	11*	0.004***	0.012**
EELAC-200mg/Kg b.w.	3**	9***	0.004***	0.014***

Results were expressed as ±SEM (n=6). Statistical analysis were performed with one way analysis of variance (ANNOVA) followed by Dunnett's 't' test P value less than < 0.05 was considered to be statistically significant.*P>0.05, ** P>0.01 and ***P>0.001 when we compared with the control group

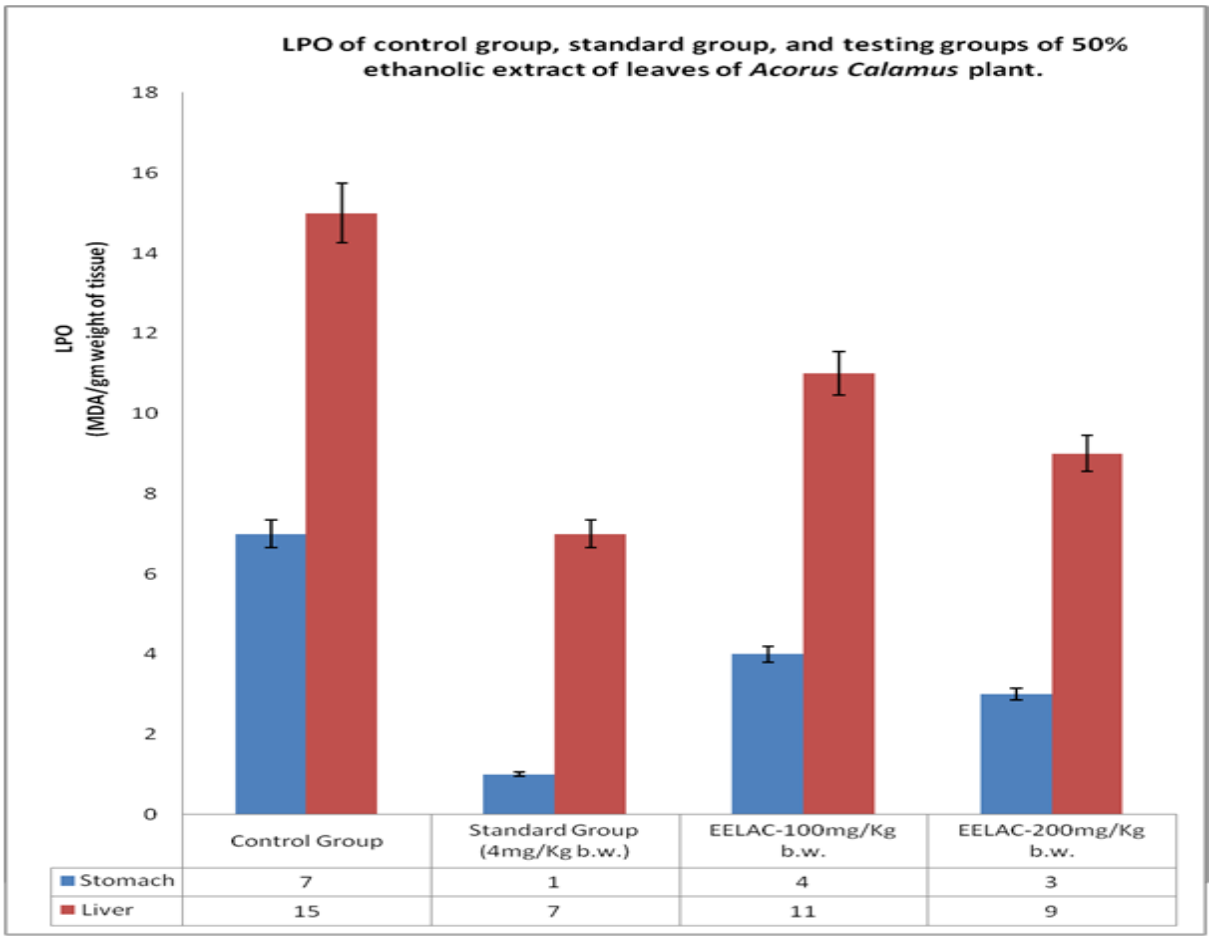


Figure 3: LPO of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant.

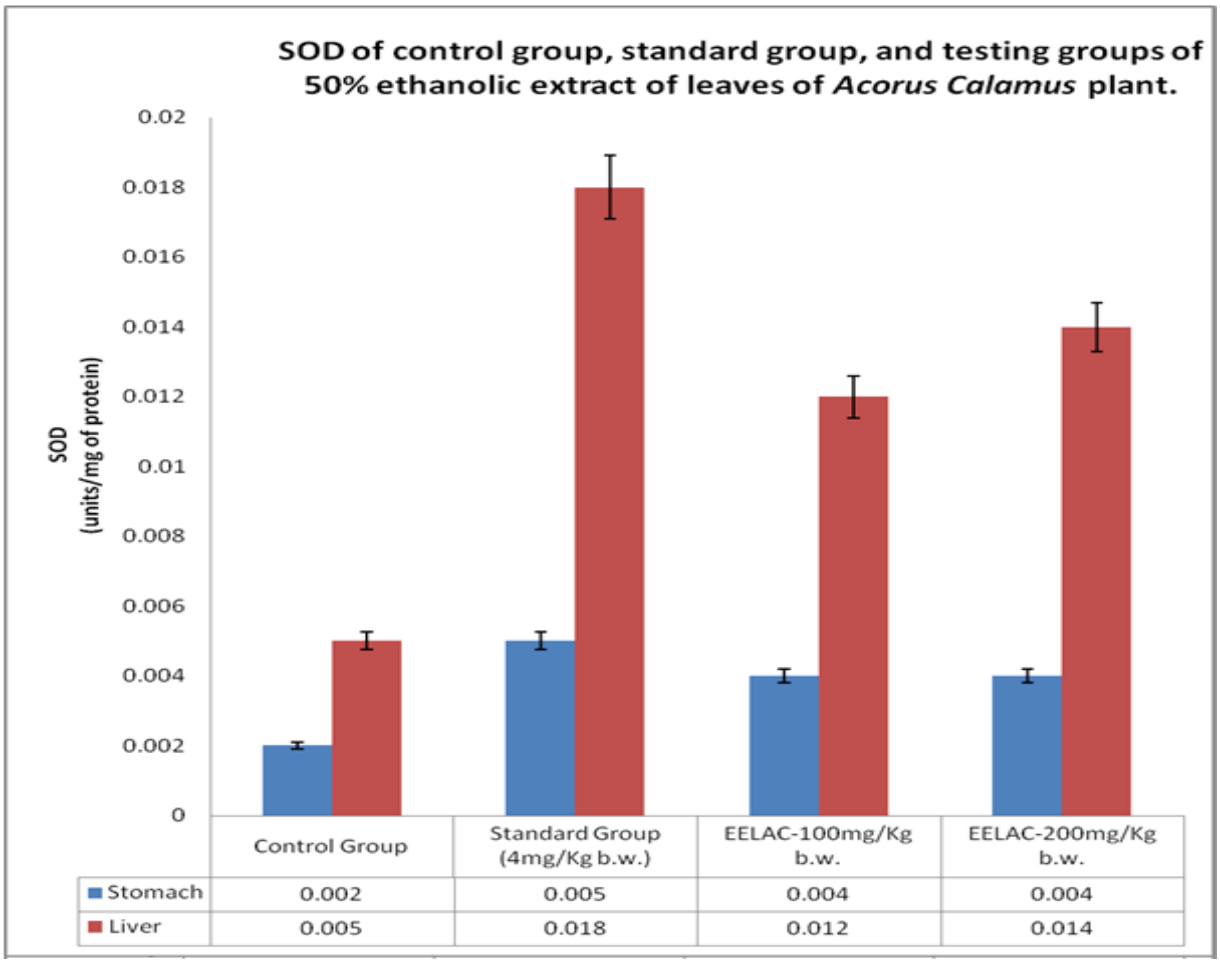


Figure 4: SOD of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant.

Table 4: GSH and CAT of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant. Quantity of glutathione (GSH) in the sample was calculated using standard-glutathione and values represented as microg/mg protein. The difference in extinction per unit time is a measure of catalase activity.

Sl. No.	GSH (mcg/ mg of protein)		CAT (units/mg of protein)	
	stomach	liver	stomach	liver
Control Group	5	5	0.1	0.2
Standard Group (4mg/Kg b.w.)	80	90	0.3	0.7
100mg/Kg b.w.	38**	45**	0.2***	0.4**
200mg/Kg b.w.	45**	56**	0.2***	0.5***

Results were expressed as ±SEM (n=6). Statistical analysis were performed with one way analysis of variance (ANNOVA) followed by Dunnett's 't' test P value less than < 0.05 was considered to be statistically significant.*P>0.05, ** P>0.01 and ***P>0.001 when we compared with the control group

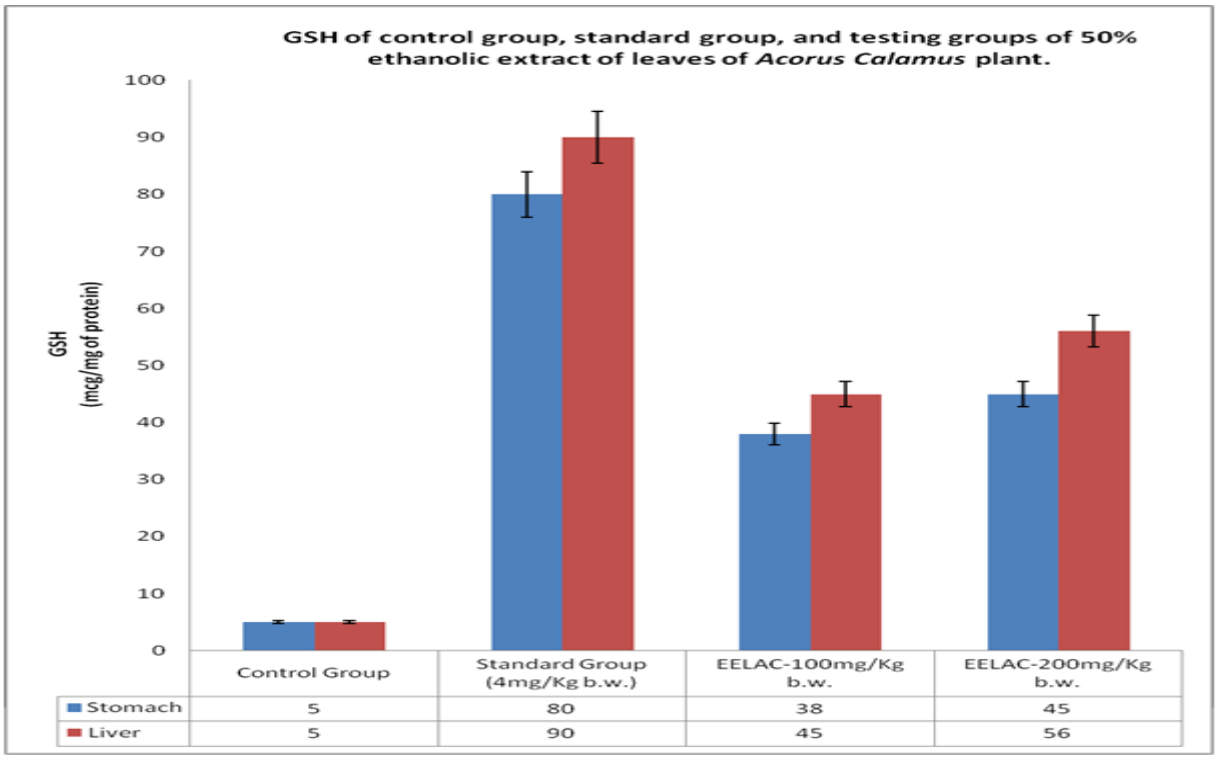


Figure 5: GSH of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant.

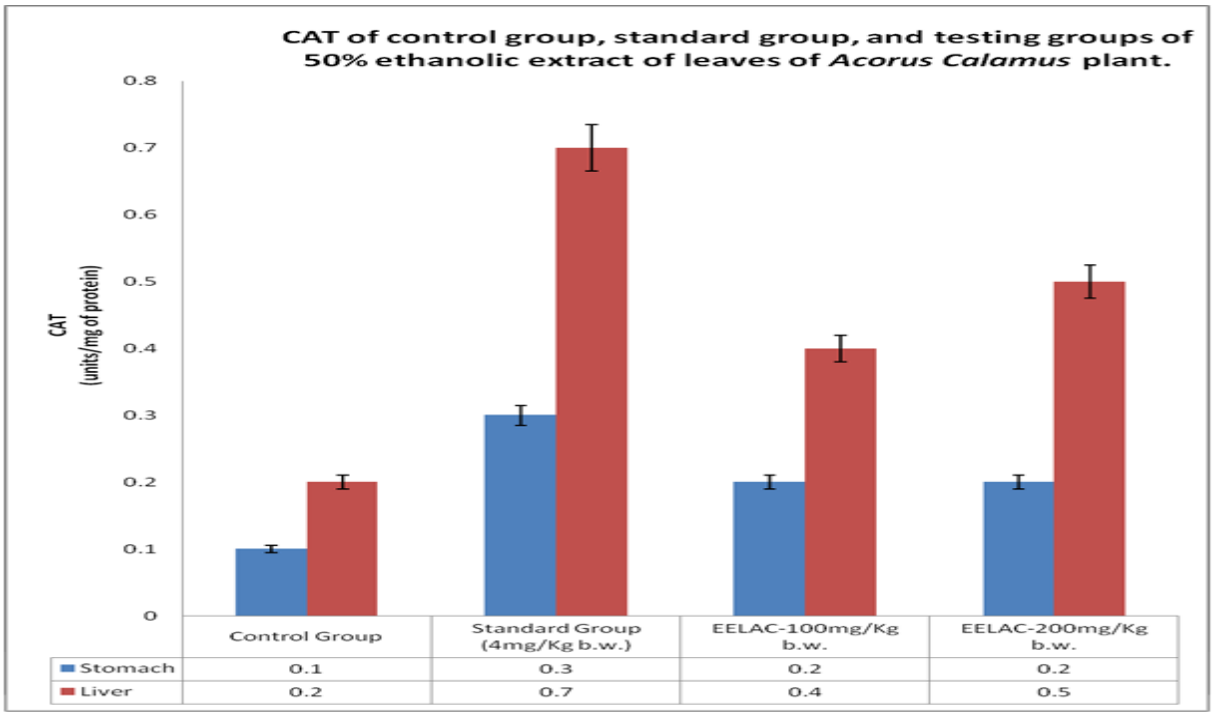


Figure 6: CAT of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant.

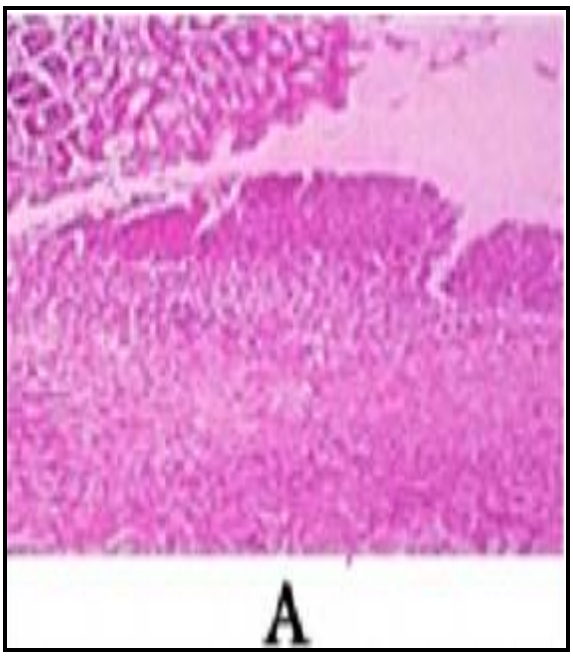
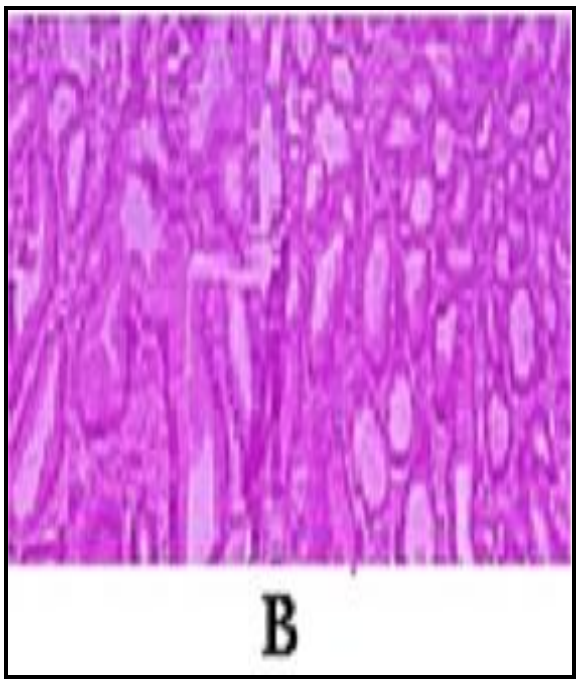
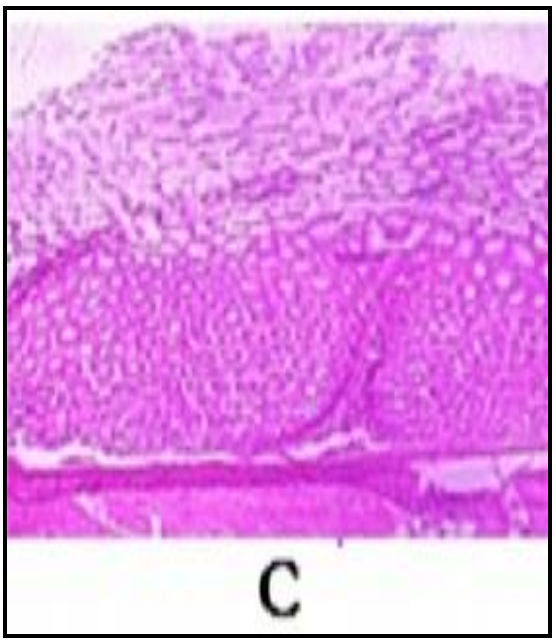


Figure 7A: Pylorus ligation induced ulcer: Necrosis of the mucosal epithelial cells of gastric mucosa/villi of the control group.



Figures 7 B: Pylorus ligation induced ulcer: The standard group showed lesser necrosis and sloughing of mucosal epithelial cell of the gastric mucosa and it was found almost normal.



Figures 7C: Pylorus ligation induced ulcer: Necrosis of the mucosal epithelial cells of gastric villi in 100 mg/kg b.w. dose of EEAC, the gastric mucosa show much alteration (In 100 mg/kg b.w. dose of EEAC, the gastric mucosa showed necrosis and sloughing of mucosal epithelial cells).

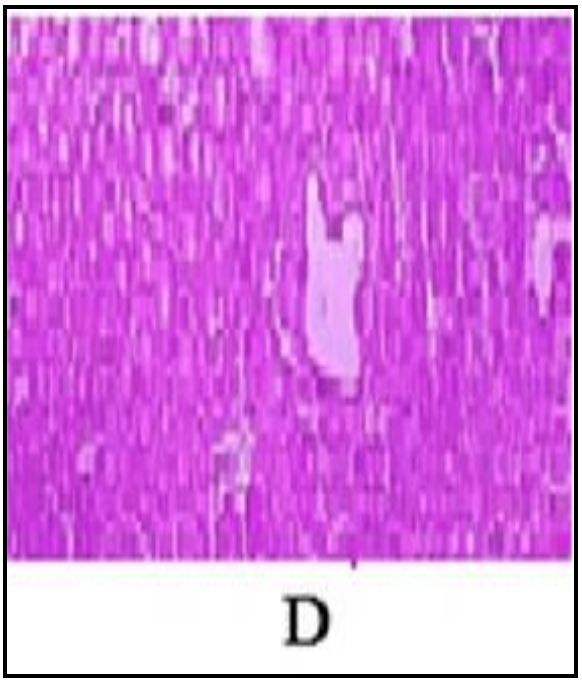


Figure 7D: Pylorus ligation induced ulcer: No visible histological alternation (200 mg/kg b.w. dose of EEAC) and was found almost normal and very less necrosis.

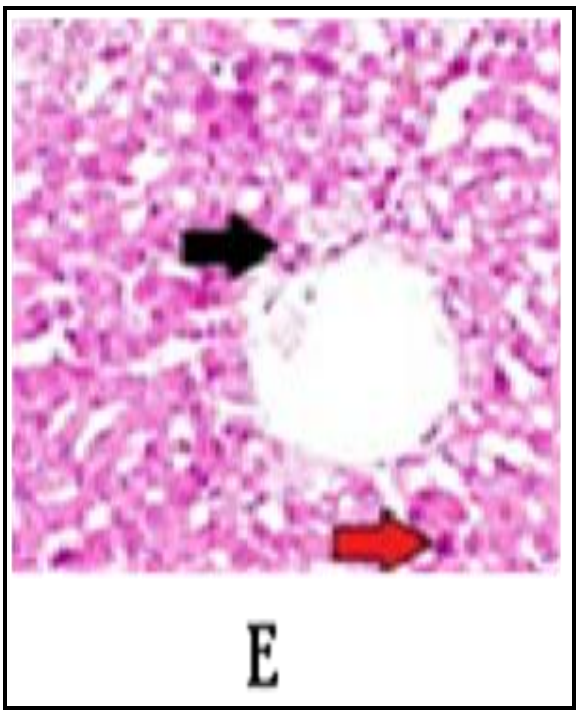
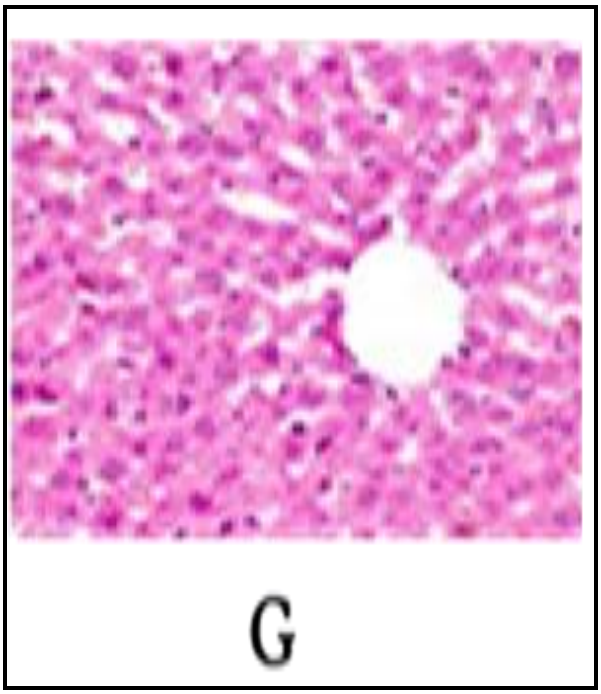
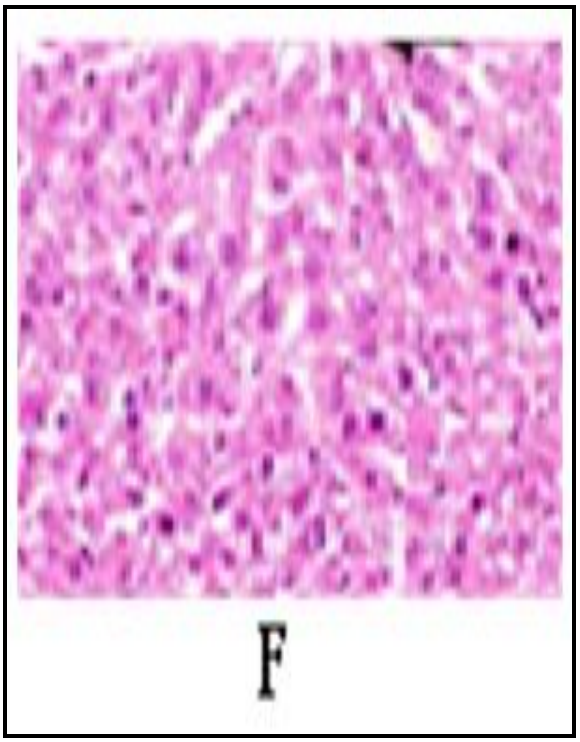


Figure 7E: Pylorus ligation induced ulcer: Necrosis of the mucosal epithelial cells of liver of the control group.



Figures 7G: Necrosis of the mucosal epithelial cells of liver cell in 100 mg/kg dose of EEAC, the gastric mucosa shows much alteration.



Figures 7F: Pylorus ligation induced ulcer: The standard group showed lesser necrosis of liver cell and it was found almost normal.

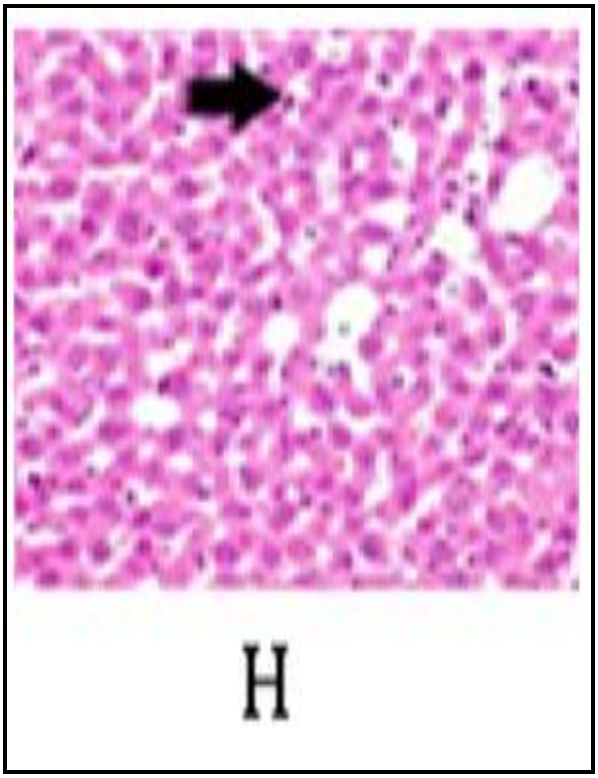


Figure 7H: No visible histological alternation (200 mg/kg dose of EEAC) and was found almost normal and very less necrosis.

PYLORUS LIGATION INDUCED ULCER MODEL

Ulcer score, ulcer index, gastric content, pH of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant is tabulated in **table 2 and Fig. 2**. It is clearly observed that ulcer score of control group were much more greater as compared to standard groups and testing group with hisgh dose also having significant effect as compared to standard group (P>0.01). Ulcer Index also showing similar resulta as in ulcer score, but gastric content volume is 4 times greater as compared to standard group but for tisting groups with very high dose showing 2 times more in case of control group. pH of the gastric fluid was very less for control groups but for both the test and standard significant effected value was found.

LPO and SOD of control group, standard group, and testing groups of 50% ethanolic extract of leaves of *Acorus Calamus* plant. SOD (Superoxide Dismutase) in the sample was calculated using SOD and values represented as microg/mg protein and the levels of lipid peroxides were expressed as nM of MDA/mg wet tissue using extinction co-efficient of 1.56 × 105 M-1 cm^{-1} and which was mentioned in table 3, Fig. 3 and 4.

GSH and CAT of control group, standard group, and testing groups of 50% ethanolic extract of leaves of Acorus Calamus plant. Quantity of glutathione (GSH) in the sample was calculated using standard-glutathione and values represented as microg/mg protein. The difference in extinction per unit time is a measure of catalase activity and which was mentioned in table 4, Fig. 5 and 6. Levels of significant of each results of testing groups were mentioned on the tabulated data as compared to the standard groups and some similar results were found for the testing groups.

Stomach histology:

Figures 7 shown necrosis of the mucosal epithelial cells of gastric villi: in control group (A), standard group (B), 100 mg/kg dose of EEAC(C), 200 mg/kg dose of EEAC(D) and Necrosis of the cells of liver: in control group (E), standard group (F), 100 mg/kg dose of EEAC(G), 200 mg/kg dose of EEAC(H).

In pylorus ligation induced ulcer model, necrosis of the mucosal epithelial cells of gastric villi was observed in the control group (**Figure 7A**). In the treated group with 100 mg/kg b.w. dose of EEAC, (**Figure 7C**) the gastric mucosa showed necrosis and sloughing of mucosal epithelial cells whereas, the standard group and 200 mg/kg b.w. dose of EEAC of treated showed lesser necrosis and sloughing of mucosal epithelial cell of the gastric mucosa (**Figures 7B and 7D**).

The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucous content as one of the involved factors. It is also associated with significant production of free radicals, leading to an increased oxidative stress and damage to the cell and cell membrane. Aspirin causes a dose-dependent reduction in mucosal prostaglandin E_2 and prostaglandin I_2 biosynthesis accompanied by an increase in the mean of gastric ulceration. It is therefore reasonable to assume that the observed gastric mucosal lesion induced by aspirin is due to deficiency of mucosal prostaglandin [45]. The present study reveals that both the doses of EEPF significantly ($P < 0.01$) reduced the ulcer index and increased the gastric pH of ethanol and aspirin-induced ulcer models.

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of free radicals, the uptake of oxygen and rearrangement of double bond in unsaturated lipids, which eventually result in destruction of membrane lipids. The release of alkaline phosphatase enzyme has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration such as absolute alcohol and aspirin-induced ulcer. Increased activity of this enzyme may be found in damaged tissues [40-45].

Liver histology:

Figures 7 Ato D shown necrosis of the mucosal epithelial cells of gastric villi: in control group(Gr.I), standard group (Gr. II), 100 mg/kg dose of EEAC(Gr. III), 200 mg/kg dose of EEAC(Gr. IV) and **Figures 7 Eto H** shown Necrosis of the cells of liver: in control group (E), standard group (F), 100 mg/kg dose of EEAC(G), 200 mg/kg dose of EEAC(H).

Histological study of liver tissue of rats of different groups at the end of study were carried out and figures of the all groups were represented in **Figure 7 E-H,** in which section **'E'** represents for control group (Group I) having almost normal hepatic structure (hepatocytes ordered around the central vein and presence of von Kupffer cells lining the sinusoidal spaces; section **'F'** represents for standard group (Group II) having nearly normal radially arranged hepatocytes around the central vein (it also shows that blood sinusoidal spaces and their von Kupffer cells are like those of the NC group).

Section **'G'** represents with ethanolic extract of AC treated group at a dose of 100mg/kg b.w.; section

'H' represents ethanolic extract of AC treated group at a dose of 200mg/kg b.w. shows almost normal hepatic structure with radially arranged recovered hepatocytes around the central vein with the absence of any degenerated apoptotic cells (it also shows that hepatic sinusoidal spaces and von Kupffer cells are like those of the NC group);

Extract treated liver showed (**Figure 7 G & H)** improved normal structural design of hepatocytes with very less number of recovered degenerated hepatocytes and central vein without any kind of prominent focal degeneration.

Histological study of liver tissue of rats of standard drug-metformin treated groups shows (**Figure 7F)** the evidence of almost normal hepatic construction with radially arranged hepatocytes around the central vein with the recovered apoptotic cells or the absence of degenerated apoptotic cells. The hepatic sinusoidal spaces and von Kupffer cells are similar to those of the NC group (**Figure 7 F& H)**. Figures 6.7 shown the necrosis of the mucosal epithelial cells of gastric villi: in control group (A), standard group (B), 100 mg/kg dose of EEAC(C), 200 mg/kg dose of EEAC (D) and Necrosis of the cells of liver: in control group (E), standard group (F), 100 mg/kg dose of EEAC (G), 200 mg/kg dose of EEAC (H).

CONCLUSION

Finally it can be concluded that as compared to standard group the testing group with higher doses of ethanolic extract of leaves of *Acorus Calamus* may be somewhat showing effective against to treat the ulcers. Further clinical data and by different studies for proofing the same may be incorporated to confirm this statement further accurately.

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