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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF COLOCASIA ESCULENTA STEMS BARK ON EXPERIMENTAL ANIMALS

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ABSTRACT

This study looked at the anti-inflammatory properties of an aqueous extract of Colocasia esculenta stem bark. An aqueous extract of Colocasia esculenta stem bark stem bark stem bark was tested for anti-inflammatory properties using four in vitro assays: albumin denaturation inhibition, membrane stabilization examination, testing of membrane sustaining activity, radiation heat induced algesia, and carrageenan-induced rat paw edema. The results revealed that orally administering Colocasia esculenta stem bark stem bark at two dosages of 200 mg/kg and 400 mg/kg to the experimental mice considerably [($p<0.05$), ($p<0.01$)] reduced foot edema when compared to the control-group (thirty minutes before the carrageenan administration). The use of Colocasia esculenta stem bark stem bark at a dose of 200 milligrams significantly reduced paw swelling with 66.51% after three hours of treatment and 70.94% after 5 hours, based on our research. Indomethacin caused a decrease of 78.72% and 83.33% after 3h and 5h, respectively, whereas Colocasia esculenta stem bark stem bark at 400mg/kg produced a reduction of 74.40% and 81.98%. Finally, the outcomes indicated that all of the examined leaves have anti-inflammatory properties at varying degrees, which might be attributable to changes in the content and concentration of bioactive chemicals.

KEYWORDS: *Colocasia esculenta stem bark stem bark team bark*, anti-inflammatory activity, aqueous extract, Carrageenan-induced rat paw edema etc.

1. INTRODUCTION

Examining plants utilized by tribal people for narcotic and therapeutic purposes is an exciting area of inquiry that has not proven unrewarding. There is a critical need to record local knowledge before it is fully lost due to these peoples' westernization. The majority of the information on the use of therapeutic floras can be found in magazines, many of which are out of print and not even found in large libraries. Early papers on this topic contained data that had been lifted from the prior literature without any ethical review, and very little experimental work had been done.

1.1 Inflammation

The term "inflammation" originates from the Latin "inflammare," which meant to put happening fire. Swelling was a complex biologically replies of vascular tissues to noxious stimuli like as infections, injured cells, or nuisances, according to (Ferrero et al. 2007).

In the absence of medicines, inflammation has been essential to Homo sapiens' survival as a defense against invasions and damage after injuries. Recent studies have demonstrated that the immune system regulates and restricts inflammation throughout (Serhan et al., 2004). Without inflammation, wounds and infections would not be able to recover. Inflammation can be characterized as acute or chronic.

1.2 MATERIALS AND METHODS

1.2.1 Collection of plant

The Colocasia esculenta stem bark stem bark stem bark were gathered from CCSs in Meerut, Uttar Pradesh, India, in the month of July. Botanical Survey of India, CCS University in Meerut, authenticated and taxonomically identified the plant material (Authentication number CNH/Tech.II/2023).

1.2.3 Preparation of the extract

The harvested components (stem bark) were meticulously dried for 15 days in the shade. The powdered dried components were then exposed to maceration extraction. The marc was thoroughly extracted three times with ethanol and water (70:30) at room temperature for around 15 days while occasionally shaking 3.5 kg of the powdered plant components. Using a rotary evaporator and lowered pressure at 45–50 °C, the individual extract was filtered and concentrated. Colocasia esculenta stem bark stem bark hydro-alcoholic pure extract was made and kept at 4°C for storage.

1.2.4 Drugs and chemicals

All of the chemicals and reagents were purchased in high purity from Bombay, India (India) Ltd. in Mumbai or S.D. fine chemicals Pvt. Ltd. Carrageenan was produced in Mumbai by Hi-Media Research Laboratories Pvt.

1.2.5 Animal Used

For the investigation, Male Swiss-albino rats and adult male Albino Wistar-rats, both weighing 20–25 g and 120–150 g, respectively, were employed. The animals are kept in polypropylene birdcages at a temp of 25° C (with a 12-hour cycle of light and dark). Before the trial, all the animals spent a week becoming used to the lab surroundings. Ordinary food and unlimited admission to water are made available to the animals. The Institutional Ethical Committee, which was established in accordance with the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India, rigorously followed the rules when it came to the care and use of laboratory animals.

1.3 Evaluation of toxicity

1.3.1 In vivo studies

1.3.1.1 Acute toxicity study The median lethal dosage (LD50) of Colocasia esculenta stem bark stem bark in adult male albino wistar rats was determined by an acute toxicity investigation using the up-and-down method recommended by OECD guideline no. 425 (OECD, 2001). Animals were given test material orally and monitored for 4 hours at half-hourly intervals before being checked again for another 24 hours.

1.3.1.2 Cytotoxicity assay (MTT); A colorimetric test employing MTT was used to investigate the cytotoxic effects of Colocasia esculenta stem bark stem bark on human PBMC (Peripheral blood mononuclear cells). Using the BDV acutainer CPTTM (Cell preparation tube, BD BioScience, USA), PBMC were extracted from blood (from healthy participants) and suspended in RPMI medium (Jansky et al. 2003). PBMC (5 X 105 cells/well of 96-well tissue culture plate) were pre-incubated with varying concentrations of Colocasia esculenta stem bark stem bark (100 to 12.5 g/ml) or doxorubicin for 24 hours at 37°C (in 5% CO2) in a final volume of 180 l. 20 l of MTT (5 mg/ml) were put to each well of the plate after 24 hours. The plates underwent a gentle shake and a four-hour incubation period at 37°C in a 5% CO2 environment. After the incubation period was through, the supernatants were delicately taken out (without jarring the formazan crystals) and 150 l of DMSO were added to each well. Until the crystals were dissolved and the absorbance was read at 544nm, the plate was shaken continuously.

1.4 Selection of dose

In an acute toxicity investigation, Colocasia esculenta stem bark stem bark didn't some harmful results up to 2000 mg /kg body mass, orally. Therefore, the two doses of Colocasia esculenta stem bark stem bark were chosen for biological screening from the LD50 (2000 mg/kg) in a way that the initial dose (low dose) was roughly one tenth of the LD50, i.e., 200mg/kg, and a high dose was twice that of the starting dose, i.e., 400mg/kg.

1.5 Evaluation of anti-inflammatory activity

1 In vivo studies

Carrageenan-induced rat paw edema

Carrageenan-induced paw edema in rats was used to test Colocasia esculenta stem bark stem bark's anti-inflammatory effects (Winter et al., 1962). Four groups of five experimental rats, weighing between 100 and 140gm, were created. Different groups received oral doses of the control substance (normal saline), Colocasia esculenta stem bark stem bark (200 mg/kg and 400 mg/kg), and the reference medication (indomethacin 100 mg/kg) 30 minutes before receiving a carrageenan injection. The plantar side of the right hand paw of each rats received an intradermal injection of carrageenan (Type I; 1% w/v in normal saline).

After injecting carrageenan, the paw volumes were measured using a plethysmometer (Bhattat et al., 1977) at 0, 1, 3, and 5 hours. Comparing the paw volumes to the control group allowed researchers to assess the percentage of paw edema inhibition.

Analysis of PGE2 in carrageenan induced paw edema by LC-MS/MS

Paw tissue processing

Using the technique described by Peiying et al. in 2006, mediators of inflammation were isolated from paw edema. Rat hind paws were isolated and pulverized into a fine powder using a mortar that had been cooled with liquid nitrogen after 5 hours of edema formation. A sealed micro centrifuge tube containing ice-cold phosphate buffer saline (PBS), 0.1% butylated hydroxytoluene (BHT), and 1mM ethylenediamine tetraacetic acid (EDTA) was then used to transfer the samples. The volume of the samples was three times larger than that of the ice-cold PBS. The sample was then homogenized for 3 minutes at 0°C by an ultrasonic processor from Misonix, Farmingdale, New Jersey. A modified version of the procedure was used to extract the eicosanoids from a 100-ml aliquot of the homogenate that was transferred to a glass tube of Kempen et al. (Kempen et al., 20015ml of hexane: ethyl acetate (1:1, v/v) was used for extraction, and the mixture was vortexed for 2 minutes. The samples underwent a 10-minute, 1800-g centrifugation at 4 °C. the top biological coating is reduced, the biological stages from three extraction tubes were combined and dried at room temperature using a nitrogen stream. All extraction processes were carried out in frigid (4°C) lighting conditions. Before loading into LCMS/MS, samples were reconstituted in 100 l of methanol: 10mM ammonium acetate buffer at pH 8.5 (70:30, v/v).

Mass spectrometry

The triple quadrapole mass spec API2000 (AB-SCIEX) hyphenated system from Shimadzu HPLC, Shimadzu

Controller, and Shimadzu was used to develop the tandem LC-MS/MS method for PGE2 measurement. The target analytic was ionized in negative mode using an ESI ionization source. Source temperature was 400 °C, Ion spray voltage was -4500 volts, Decluster potential was -30 volts, and Collision Energy was -20 volts. Multiple reactions monitoring (MRM) mode was used to monitor the sample, and the parent ion 351.2 and product ion 271.2 were discovered. An internal standard (IS) was employed, which was tolbutamide.

Acetic acid-induced peritoneal inflammation

Male mice weighing 18 to 22gram are applied for the experimentation. Four groups of the experimental animals were created (n=5).Different groups received oral doses of the control substance (normal saline), Colocasia esculenta stem bark stem bark (200 mg/kg and 400 mg/kg), and the reference medication (indomethacin 100 mg/kg). Each animal received 0.25ml of 1.2% acetic acid (in normal saline) intraperitoneally 30 minutes later. The animals were killed three hours later, their abdominal viscera were exposed, and the peritoneal exudates were collected. The total white blood cells (WBC) and protein content of the peritoneal exudates were then measured using the Bradford reagent. (Whittle et al., 1964).

Inhibition of the albumin denaturation

For this specific experiment, the methodology from Sakat et al., 2010, and Mizushima et al., 1968, was used with a few minor adjustments. The response combination was composed of examination extracts at various concentrations and 1% bovine albumin portion in aqueous solution. A little amount of 1N HCl was used to modify the pH of the reaction mixture. The samples were heated to 57°C for 20 minutes after 20 minutes of incubation at37°C. The samples are cool before the turbidity at 660nm is determined spectrophotometrically. The experiment was carried out three times. The following equation was used to determine the % inhibition of protein denaturation: Inhibition percentage is equal to 100 /Abs control.

Membrane stabilization test

Preparation of bovinered blood cell

A solution containing dextrose (2%), sodium citrate (0.8%), citric acid (0.05%), and sodium chloride (0.42%) was used to collect fresh cow blood samples. On a Bench centrifuge Model 800D, blood samples were centrifuged for 10 min. at room temperature at 3000 rpm. The packed red blood cell was carefully separated from the supernatants (plasma and leucocytes) and washed in new normal saline (0.85% w/v NaCl). Up till the supernatants were clear, washing and centrifugation were done five times. Then, as previously reported, bovine erythrocytes (2% v/v) were produced. (Oyedapo et al., 2004).

Assay of membrane stabilizing activity

With 2% v/v bovine erythrocyte suspension, indomethacin as the ordinary medication, the sheath stabilizing examine was conducted as explained through Oyedapo et al. (2004) and Sadique et al. (1989). The final reaction mixtures were created up to 4.5 ml with isohaline, and the assay mixtures were made up of 2ml of hypo saline(0.25 %w/v) sodium chloride, 1.0 ml of0.15M sodium-phosphate buffer, pH 7.4, 0.5ml of 2%(v/v) bovine erythrocyte solution, and 0.0 - 1.0ml of medicines (standard, extracts/fractions). The reaction mixtures were heated to 56 degrees Celsius for 30 minutes in a water bath, then centrifuged for 10 minutes at room temperature at 5000 rpm on a Gallenkamp Bench Centrifuge. At a wavelength of 560 nm, the released hemoglobin's absorbance was measured. The following formula was used to compute the hemolysis's % inhibition:.

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) \times 100/\text{Abs Control}$$

1.6 Evaluation of analgesic activity

In vivo studies

Radiant heat induced algesia (Tail flick method)

Colocasia esculenta stem bark stem bark (200mg/kg and 400mg/kg,p.o.) and the common opioid morphine (5 mg/kg, i.p.) were given to experimental mice, which were split into four groups of five (n=5). Using an analgesiometer (Techno Electronics, India), it was determined how long it took the mice to retract their tails at intervals of 30, 60, 120, and 180 minutes. Significantly longer reaction times in rats given Colocasia esculenta stem bark stem bark or morphine compared to the control group suggested the existence of analgesia. (Connor et al., 2000; Matheus et al, 2005).

Acetic acid-induced writhing

Different groups of mice (n=5) received either the usual medication Indomethacin (10 mg/kg) or the control substance Colocasia esculenta stem bark stem bark (200mg/kg and 400mg/kg) orally. Thirty minutes later, each mouse received an intraperitoneal injection of 3% (v/v) acetic acid (10ml/kg). For a 20 minute period, the number of writhing responses from each animal was counted. (Koster et al., 1959).

1.7 Statistical analysis

For in vivo and in vitro experiments, the outcomes are presented as mean S.E.M. (n=5) and mean S.D. (n=3), respectively. For the in vivo investigations, one-way analysis of variance (ANOVA) was employed, followed by the post hoc Dunnett's test, whereas for the in vitro experiments, the Student "t" test was utilized. Unless otherwise stated, statistical significance was defined as "p" values 0.05.

1.8 RESULTS AND DISCUSSION

1.8.1 Evaluation of toxicity of *Colocasia esculenta stem bark stem bark* stems bark

In-vitro Evaluation

Acute toxicity Studies

Evaluation of toxicity of hydro alcoholic Extract of *Colocasia esculenta stem bark stem bark* stems bark:

The Colocasia esculenta stem bark stem bark toxicity study's test animals handled it well, and there were no fatalities up to the highest experimental dose (2000 mg/kg) within 24 hours. During the observation period, abnormalities such skin changes, morbidity, hostility, increased oral secretion, sensitivity to sound and pain, as well as breathing motions and death, were also missed.

In-vitro Studies

Cytotoxicity studies of *Colocasia esculenta stem bark stem bark*

A 100 g/ml concentration of *Colocasia esculenta stem bark stem bark* is not cytotoxic, according to the experiment's findings. It should be underlined that rather than the extract's toxicity, *Colocasia esculenta stem bark stem bark* inhibitory effect on cytokine production (in LPS-induced PBMC) may be caused by a potential reduction of the inflammation signalling cascade. Figure 2 and Figure 1).

Assessment of anti-inflammatory activity of *Colocasia esculenta stem bark stem bark*

Numerous in vivo models are applied to examine of ***Colocasia esculenta stem bark stem bark*** anti-inflammatory properties. The outcomes are shown below.

In-vivo studies

Carrageenan-induced rat paw edema

In this investigation, it was discovered that pre-treating the experimental animals with *Colocasia esculenta stem bark stem bark* at its two doses of 200 mg/kg and 400 mg/kg orally significantly [(p<0.05), (p0.01)] reduced the paw edema as associated to the control-group (30 minutes before to carrageenan injection). Our results showed that *Colocasia esculenta stem bark stem bark* at a dosage of 200mg/kg significantly reduced paw edema through 66.51% after 3 hours and by 70.94% after 5 hours. The usual medication indomethacin generated an inhibition of 78.72% and 83.33% after the periods of 3h and 5h, whereas *Colocasia esculenta stem bark stem bark* at 400mg/kg dose produced an inhibition of 74.40% and 81.98%.

Table 1.1: Effect of Colocasia esculenta stem bark stem bark on carrageenan-induced rat paw edema.

	Dose	1 hrs		2 hrs		3 Hrs	
		Mean paw volume (ml)	% Inhibition	Mean paw volume (ml)	% Inhibition	Mean paw volume (ml)	% Inhibition
Normal	-	2.07±0.07	-	2.07±0.09*	-	1.77±0.67	-
Control	0.1ml	2.09±0.09		2.55±0.07*	-	1.55±0.07*	-
Indomethacin	10 mg/kg	1.34±0.08*	36.45	0.48±0.49*	77.88	0.243±0.04**	84.44
Colocasia esculenta stem bark stem bark stem bark extract	200 mg/kg	1.55±0.07*	27.77	0.76±0.04***	77.06	0.44±0.03**	71.99
Colocasia esculenta stem bark stem bark stem bark extract	400 mg/kg	1.43±0.09	29.09	0.58±0.08**	78.09	0.268±0.08**	82.88

Standards was showed as mean±S.D; (n =5), *p< 0.05,**p < 0.01(vs.-control)

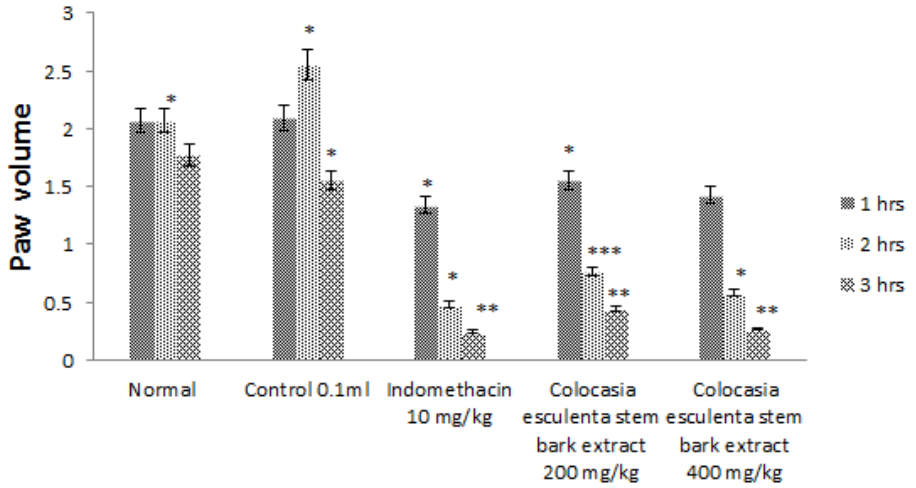

Figure1.1: Effect of *Colocasia esculenta stem bark stem bark* on carrageenan-induced rats paw edema Standards was expressed as mean± S.D,(n = 5),*p < 0.05,p < 0.01 (vs.-control)**

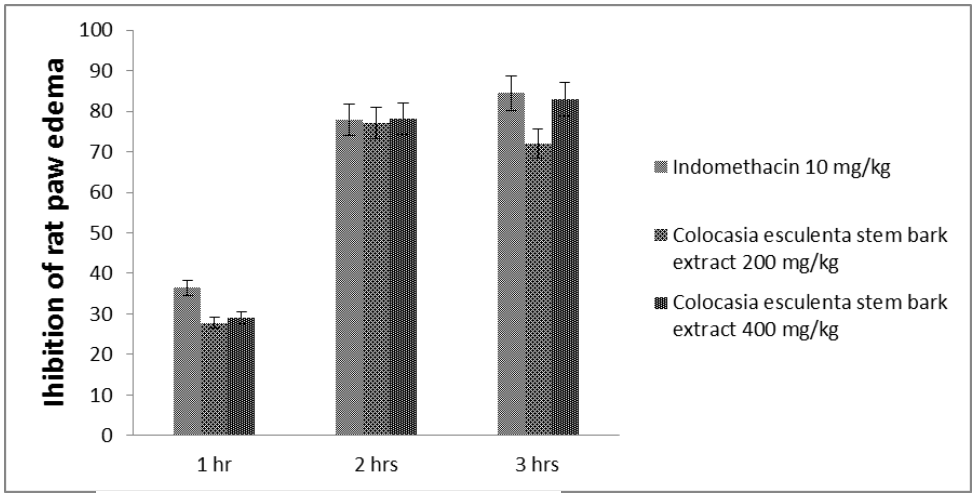

Figure 1.2: Effect of *Colocasia esculenta stem bark stem bark* on time course of carrageenan-induced rat paw edema. Values are expressed as mean ± S.D; (n = 5), *p < 0.05, **p < 0.01 (vs. control)

Table 1.2: Peak area of PGE2 determined by LCMS in different samples.

Sample	Peak Area
NS	1549
Carrageenan 0.01 ml	4750
Colocasia esculenta stem bark stem bark stem bark extract 200 mg/kg	2555
Colocasia esculenta stem bark stem bark stem bark extract 400mg/kg	2116
Indomethacin 10 mg/kg	322

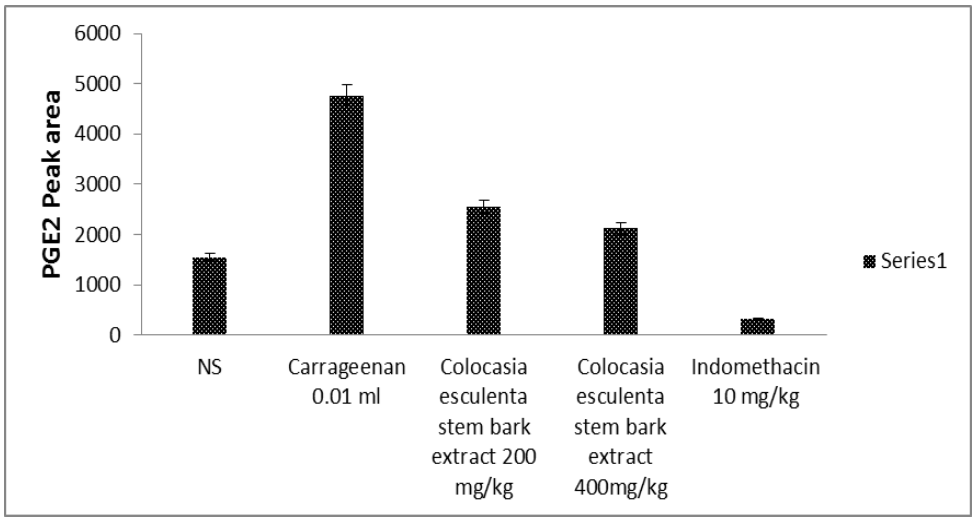

Figure 1.3: Peak area of PGE2vs samples.

Acetic acid-induced peritoneal inflammation

Table 1.3: Effect of *Colocasia esculenta stem bark stem bark* on Acetic acid-induced peritoneal inflammation in mice.

Treatment groups	Dose	Conc. Of protein in peritoneal exudate (µg/ml)	%Inhibition	Leukocyte countml of exudates
Normal	Normal slain	738.2±11.04	-	845.00±12.09
Control	0.1 ml	839.3±20.5	-	11915±136
Indomethacin	10 mg/kg	330.67±19.3*	61.09	8365±104**
Colocasia esculenta stem bark stem bark stem bark extract	200 mg/kg	556±50.40	34.99	9410±116**
Colocasia esculenta stem bark stem bark stem bark extract	400 mg /kg	359.09±19.20	58.40	8340±93**

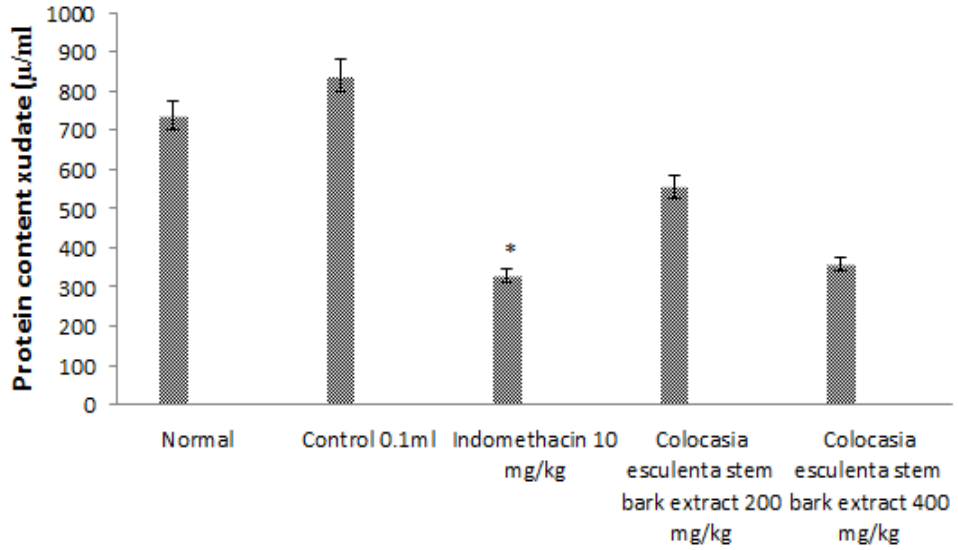

Figure 1.4: Effect of *Colocasia esculenta stem bark stem bark* on Acetic acid-induced peritoneal inflammation in mice. Standards were stated in terms of the mean ±S.E.M;(n = 5),p < 0.01 (vs.-control).**

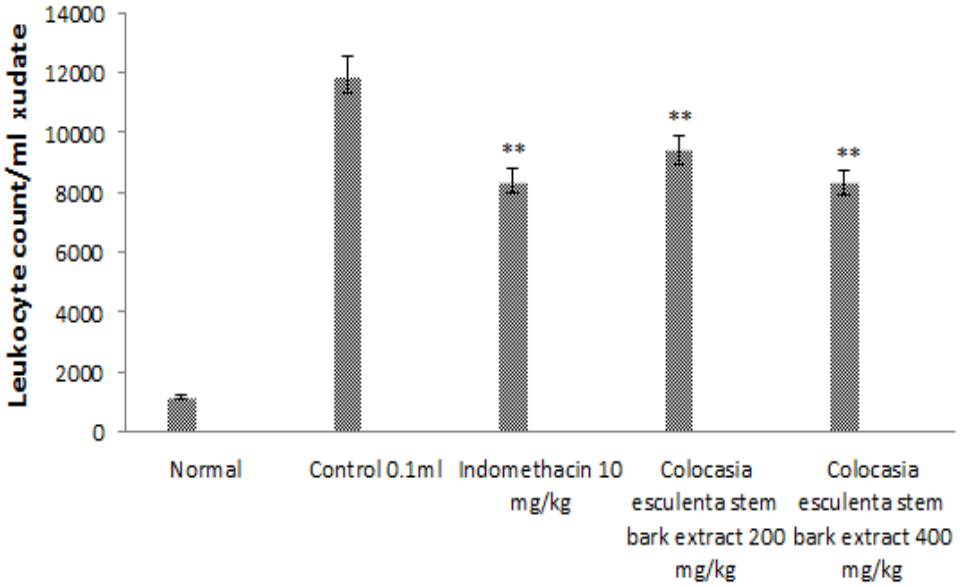

Figure 1.5: Effect of *Colocasia esculenta stem bark stem bark* on Acetic acid-induced leukocyte count of peritoneal exudates in mice. Standards were showed as mean± S.E.M; (n =5),p < 0.01 (vs. control)**

In vitro studies

Table 1.4: Result of *Colocasia esculenta stem bark stem bark*. on heat-induced albumin denaturation.

Treatment	Concentration (µg/ml)	Absorbance at 660 nm	% Inhibition of albumin denaturation
Control	-	0.37±0.30	-
Colocasia esculenta stem bark stem bark stem bark extract	100	0.38±0.053	26%
Colocasia esculenta stem bark stem bark stem bark extract	200	0.29±0.031*	40%
Colocasia esculenta stem bark stem bark stem bark extract	300	0.22±0.031*	54%
Colocasia esculenta stem bark stem bark stem bark extract	400	0.16±0.023***	62%
Indomethacin	10	0.12±0.23***	73%

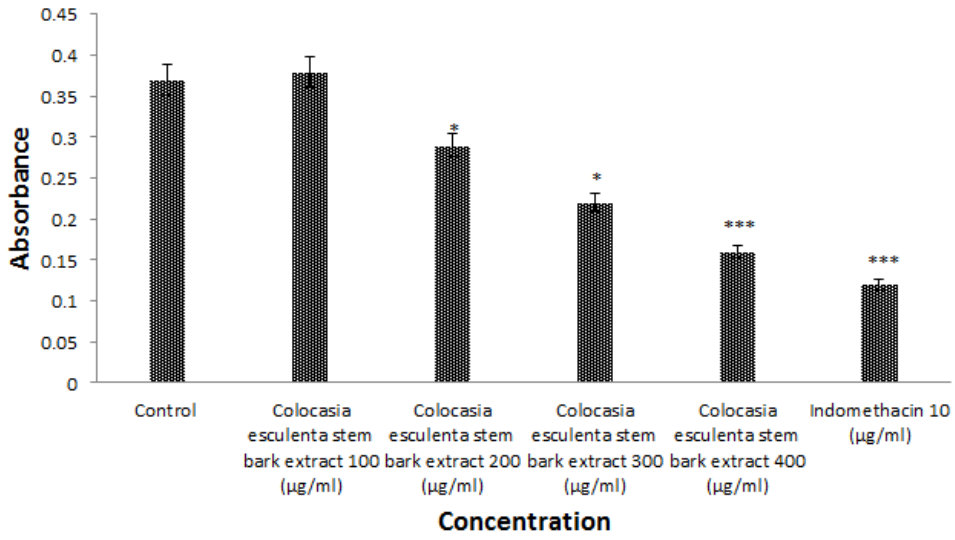

Figure 1.6: Effect of the Hydro-alcoholic extract of Colocasia esculenta stem bark stem bark on heat induced albumin denaturation. Each value represents the Mean ± SD. N=3, Experimental groups were compared to control *p < 0.05, **p < 0.01.

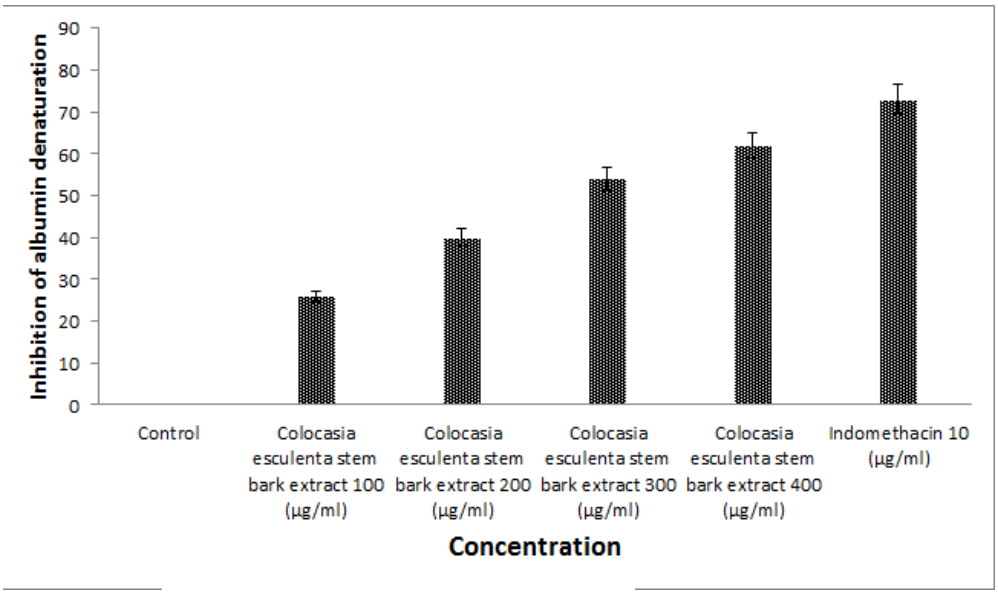

Figure 1.6: Effect of the *Colocasia esculenta stem bark stem bark* on % inhibition of heat induced albumin denaturation. Each value represents the Mean ± SD. N=3, Investigational groups are related to control *p< 0.05,p < 0.01, (***p<0.001 are considered as extremely significant).**

Table 1.5: Effect of the *Colocasia esculenta stem bark stem bark* on heat induced haemolysis of erythrocyte.

Treatment	Concentration (µg/ml)	Absorbance at 660 nm	% Inhibition of albumin denaturation
Control	-	0.29±0.30	-
Colocasia esculenta stem bark stem bark stem bark extract	100	0.25±0.053	19%
Colocasia esculenta stem bark stem bark stem bark extract	200	0.23±0.031*	26%
Colocasia esculenta stem bark stem bark stem bark extract	300	0.21±0.31**	33%
Colocasia esculenta stem bark stem bark stem bark extract	400	0.17±0.023***	44%
Indomethacin	10	0.14±0.23***	74%

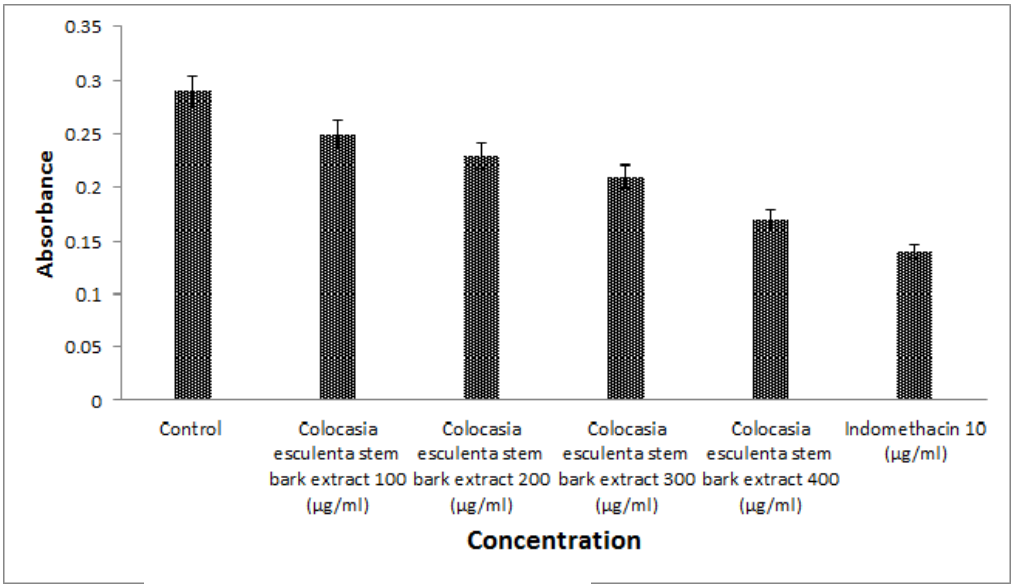

Figure 1.7: Effect of the *Colocasia esculenta stem bark stem bark* on heat induced haemolysis of erythrocyte.

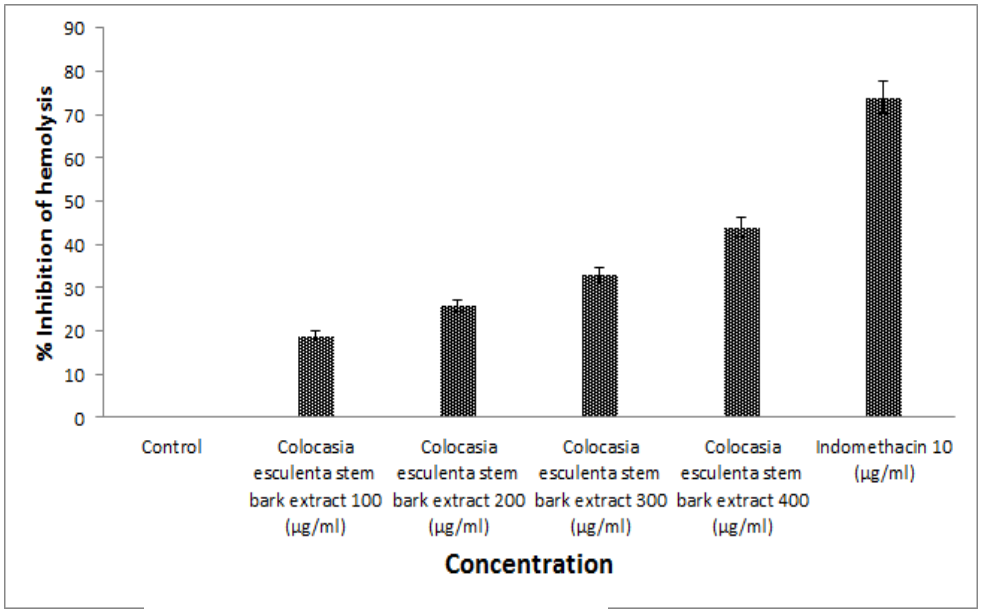

Figure 1.8: Effect of the *Colocasia esculenta stem bark stem bark* on % inhibition of hemolysis of erythrocyte Each value represents the Mean ± SD, N=3.Experimental groups were compared to control **p<0.01, considered extremely significant. *p<0.05, considered significant.

CONCLUSION

The late phase of the carrageenan-induced inflammation model was established in the rat paw tissue, and as a result, the mechanism of action of *Colocasia esculenta stem bark* was sufficiently established. The acetic acid-induced peritoneal inflammation model was used to quantify the degree of inflammation, and the results of this experiment again pointed to the need for NSAID-like PGE2 suppression of eicosanoid production. As a result, *Colocasia esculenta stem bark* mode of action was reinforced. The writhing reaction was significantly inhibited by *Colocasia esculenta stem bark*.

It again suggests a potential mechanism for the antinociceptive action by preventing the production of prostaglandins and the release of inflammatory mediators, both of which are known to stimulate pain fibers similarly to NSAIDs. According to the study, *Colocasia esculenta stem bark* exhibited considerable analgesic and anti-inflammatory effects, as well as significant free radical scavenging activities. It was also discovered to have anti-ulcer effects. Based on these results, efforts were made to use the bioactivity-guided purification method to separate the active component(s) from *Colocasia esculenta stem bark* both solvent extraction and chromatographic methods were used in the bio-activity-guided isolation and purification of the *Colocasia esculenta stem bark*. The majority of current approaches for isolating compounds from medicinal plants are biological activity-guided, which has, for instance, resulted in the isolation, identification, and discovery of significant medications. The highest active

molecule among the 4 bands recovered using preparative TLC was found in band-3, which was acquired through the 9:1 ethyl acetate-methanol sub-fraction of the ethyl acetate fraction of *Colocasia esculenta stem bark*. When the substance in band 3 was chemically characterized, it was discovered to be a Terpenoid. A chemical reported to be present in Styrax japonica S. et Z. was discovered to match the molecular weight of band 3 (466 Da), which suggests that the band 3 of *Colocasia esculenta stem bark* may contain the same substance. Thus, in stark contrast to the widely used NSAIDs, the current study was able to demonstrate Bauhinia acuminate's combined anti-inflammatory and antiulcer action.

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