An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

PHYTOCHEMICAL STUDIES AND ANTI-INFLAMMATORY ACTIVITY OF *CAESALPINIA DIGYNA* ETHANOLIC ROOT EXTRACT

Riyazunnisa. D*[1], Md. Sultan Ali[2], K. Suresh[2]

[1]*Department of Pharmacology, Sri Lakshmi Venkateswara Institute of Pharmaceutical sciences Proddatur, Andhra Pradesh, India*

[2]*Safa College of Pharmacy, Kurnool, Andhra Pradesh, India*

Corresponding author E-mail:** ***riyazunnisa.786@gmail.com

ARTICLE INFO

Key Words

Caesalpinina digyna, Carrageenan induced paw edema, Diclofenac

ABSTRACT

The ethanolic extract of *Caesalpinina digyna* Rottler root was studied for anti-inflammatory activity in carrageenan induced paw edema and cotton pellet induced granuloma in rats and by its membrane stabilizing activity and protein stabilizing activity against heat induced lysis of R.B.C cells and protein denaturation in egg albumin. Wistar rats were orally administered RVEE (200 mg/kg and 400 mg/kg) and the standard drug diclofenac sodium (40 mg/kg) 60 min prior to a subcutaneous injection of carrageenan (0.1 ml of 1% w/v) into their right hind paws to produce edema. The paw volumes were measured at various time intervals to assess the effect of drug treatment. In the granuloma model, 1 sterile cotton pellet was implanted in the axilla region of each rat. EECD (200 mg/kg and 400 mg/kg) and the standard drug diclofenac sodium (40 mg/kg) were administered orally for 8 days to the pellet implanted rats. The granuloma tissue formation was calculated from the dissected pellets. A significant reduction in paw edema and cotton pellet granuloma was observed with EECD treatment when compared with the carrageenan treated and cotton pellet implanted animals respectively. There is significance increase in percentage protection of erythrocyte membrane by *Caesalpinia digyna* extracts. It may be concluded that EECD possesses anti-inflammatory activity in a dose dependent manner which may be due to an underlying antioxidant activity and/ or lysosomal membrane stabilization by virtue of its poly phenolic constituents.

INTRODUCTION:

Inflammation is a protective immune vascular response that involves immune cells, blood vessels and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process and tissue repair [1]. Inflammation can be classified as either *acute* or *chronic*.

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic*

inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2]. NSAIDs (non-steroidal anti-inflammatory drugs) are taken to alleviate pain caused by inflammation. They counteract the COX (cyclo-oxygenase) enzyme, which synthesizes prostaglandins which create inflammation. If prostaglandin synthesis can be blocked, pain is either eliminated or reduced. Examples of NSAIDs include naproxen, ibuprofen and aspirin. NSAIDs long-term use causes stomach ulcers, and even severe and life-threatening hemorrhage [3-6].

Some herbs have anti-inflammatory properties: *Harpagophytum procumbens* - also known as devil's claw, wood spider or grapple plant comes from South Africa and is related to sesame plants. European colonists brought devil's claw back home to treat arthritis, fever and pain. According to the British Herbal Pharmacopoeia, Devil's Claw has diuretic, sedative and analgesic properties. Hyssop *Hyssopus* - from the plant family Lamiaceae, is added to eau de Cologne and Chartreuse (liqueur drink). It is also used to color some spirits. Hyssop is mixed with other herbs, such as liqourice for the treatment of some lung conditions, including inflammation. Ginger, also known as ginger root, is the mass of roots (rhizome) of the *Zingiber officinale* plant. It is used as a medicine or a spice. Jamaican ginger was the traditional medical form of this root and has been used as a carminative (to treat gas or wind) and a stimulant. It has been used for hundreds of years to treat dyspepsia, constipation, colic, other gastrointestinal problems, as well as rheumatoid arthritis pain. Researchers from Michigan Medical School reported that ginger supplements were found to reduce the markers of colon inflammation. Chronic colon inflammation is associated with a higher risk of developing colon cancer. They added that ginger supplements may help to prevent colon cancer. Turmeric *(Curcuma longa)* - also a plant of the ginger family. Current research is looking into the possible beneficial effects of turmeric in treating arthritis, Alzheimer's disease, and some other inflammatory conditions. Curcumin, a substance found in turmeric, is under investigation for the treatment of several illnesses and disorders, including inflammation[6-11].

Review of literature: Namita *et al* in 2012, studied evaluation of anti-inflammatory activity of leaf extract of Kigelia pinnata on wistar rats. Anti-inflammatory activity of the leaf extract of Kigelia pinnata at a dose of 200mg/kg & 400mg/kg was evaluated against the standard drug indomethacin at a dose of 10 mg/kg i.p. Wistar rats of either sex of five numbers in each group was undertaken for study and evaluated by carrageenan-induced paw edema and cotton pellet-induced granuloma methods. Deepthi *et al* in 2011, Gardenia taitensis is a medium sized evergreen tree belongs to Rubiaceae family. The petroleum ether extract of whole plant of Gardenia taitensis (PGT) was investigated for the evaluation of anti- inflammatory and analgesic activity. Toxicity signs and symptoms were not observed. Anti-inflammatory activity was established by carrageenan induced paw edema and cotton pellet induced granuloma at the dose of 200 and 400 mg/kg. Analgesic activity was carried out by tail immersion method in mice. Shekar *et al* in 2009, The ethanolic and acetone extracts of Bauhnia variegate bark was examined for anti-inflammatory in experimental animals. In this study both acute and sub-acute inflammation models were used to evaluate the anti-inflammatory activity of the Bauhnia variegate. In acute model carrageenan was used to induce inflammation in rat hind paw and in sub-acute inflammation, cotton pellet induced granuloma was performed [12-19].

The purpose of the present study was to navigate and collate the state-of-the-art knowledge of a herbal plant Caesalpinia digyna Rottler, To observe and document the phytochemical findings of ethanolic extract of root of Caesalpinia digyna[EECD], To evaluate *in-vitro* anti-inflammatory activity of ethanolic extract of root of Caesalpinia digyna against heat induced lysis of R.B.C cells and protein denaturation in egg albumin.

Materials and Methods: The pure drug Diclofenac sodium was received as a gift pack from yarrow chemicals. Drabkin's reagent: It is a solution used in the cyan methemoglobin method of measuring hemoglobin. It consists of sodium bicarbonate, potassium cyanide, and potassium ferricyanide. Normal saline: 0.9%

of sodium chloride solution. Instruments: Tabletop centrifuge, UV spectrophotometer, Hot air oven, Hot plate.

Plant extraction: The roots of *Caesalpinia digyna* are shade dried and pulverized and stored in an air tight container for future use. Ethanolic extraction of phytoconstituents were done by using soxhlation method [20]. The authenticated roots of *Caesalpinia digyna* are cut in to bits and dried under the shade for 10 days to ensure complete dryness at room temperature. The dried roots were then subjected to milling in order to make fine powder. A weighed quantity (80 gm) of the powder was extracted with ethanol using soxhlation until it become colorless. The extract subsequently concentrated by evaporation until all the solvent has been removed to give an extract sample known as semisolid mass, kept in a petridish and stored in refrigerator until use. The ethanolic extract was then used for anti-inflammatory study.

Thin-layer chromatography: (TLC) is a chromatography technique used to separate non-volatile mixtures [21]. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase.

Phytochemical study: Qualitative screening of ethanol extracts of root of *Caesalpinia digyna* [EECD] is performed by using different standard methods for the detection of various classes of active phytochemical components [22].

Test for Carbohydrates: The extract solution was made by suspending small amount of the extract in 10 ml of distilled water and filtered. The test solution was subjected to test for carbohydrates. Molisch's test, Fehling's test, Caramelisation, Bromine water test, Borntrager's test, Benedict's test and Selwinoff's test.

Test for Alkaloids: To the extract solution add few drops of dilute hydrochloric acid, mix and filtered. The filtrate was subjected to different alkaloidal reagent tests. Dragendorff's test, Wagner's test, Mayer's test, Hager's test, Tannic acid test.

Test for Flavonoids: To the extract solution add concentrated sulphuric acid yellowish orange colour indicates anthocyanins, orange to crimson indicates flavonones, yellow to orange colour indicates flavones. To the extract solution add sodium hydroxide solution blue to violet colour indicates the presence of anthocyanins, yellow to orange colour indicates flavonones. Millon's reagent test, Ferric chloride test, Alkaline reagent test, Lead acetate test, Ammonia test, Shinoda test, Zinc hydrochloride test.

Test for Glycosides: The extract solution was hydrolyzed by dilute hydrochloric acid solution and neutralized by dilute sodium hydroxide solution. To this add 0.5 ml of Fehling's A and B solution, a red precipitate indicates the presence of glycosides. **Test 1:** To 20 mg of dried extract add 5 ml of 10% dilute sulphuric acid boil for two minutes and filter. The filtrate was neutralized with equal volume of 5% sodium hydroxide solution. Add 0.1 ml of Fehling's A and B solution until becomes alkaline and boil for two minutes. The quantity of red precipitate appeared was noted and compared with that formed in Test 2. **Test 2:**To 20 mg of dried extract add 5 ml of distilled water boil for two minutes and filter. Add equal volume of distilled water and 0.1 ml of Fehling's A and B solution to the filtrate until becomes alkaline and boil for two minutes. The quantity of red precipitate appeared was noted and compared with that formed in Test 2. The quantity of red precipitate appeared was noted. Compare the Test 2 precipitate with Test 1. If the Test 2 precipitate is more than that of Test 1, indicates the presence of glycosides. Because Test 1 signifies the amount of free reducing sugar previously present in the crude drug, whereas Test 2 signifies the glycoside after acid hydrolysis.

Test for different glycosides: Keller Killani test, Legal's test, Baljet's test, Bromine water test, Raymond's test, Borntrager's test, Modified Borntrager's test,

Test for Saponins: Foam test, Haemolysis test.

Test for Sterols and Triterpenoids: About 0.5 g of extract is treated with chloroform, filtered and the filtrate was subjected to tests for sterols and triterpenoids. Libermann Buchard test, Salkowski test.

Test for Tannins: Ferric chloride test, Gelatin test, Lead acetate test, Match stick test, Alkaline reagent test, Vanillin hydrochloride test.

Test for Phenols: Ferric chloride test, Zinc hydrochloride test, Shinoda test.
Test For proteins and Amino Acids: Millon's test, Ninhydrin test and Biuret test
Test for Quinones: About 0.2 g of the extract was treated with concentrated hydrochloric acid formation of yellow precipitate indicates the presence of quinones.

***In-Vitro* Anti Inflammatory Activity**

Membrane stabilizing activity [23]: Fresh venous blood (5 ml) from a healthy volunteer was collected in a tube containing 5ml of sterile Alsevier solution (2% Dextrose, 0.8% Sodium Citrate, 0.05% Citric acid, and 0.42% Sodium chloride). Separation of red-blood cells (RBC) was performed by centrifuging the blood samples at 3500 rpm for 10 min with three washes in freshly prepared sterile Alsevier solution. Finally, a 10% RBC working solution was prepared by adding 45 ml of sterile normal saline solution to the pellet remaining in the tube. A reaction mixture (4.5 ml) consisting of 2ml hypotonic saline (0.25% w: v NaCl), 1ml 0.15M sodium phosphate buffer (pH 7.4), and varying concentration of the test drugs (50, 100, 150, 200, 250 and 300 µg/ml) in 1ml of normal saline was added with 0.5ml of 10% HRBC in normal saline. Two controls were performed: one with 1.0ml of isotonic saline without drug (control1) and the other one with 1ml of drug solution without red-blood cells (control2). The mixture was incubated at 56◦C for 30 min. The tubes were cooled under running water for 20 min, the mixture was centrifuged, and then 100µl supernatant with 4ml drabkin's solution was mixed in a tube, & was allowed for 10 mins to complete the reaction. Then absorbance was taken at 560nm. In blank cuvette 4ml drabkin's solution was kept.

Protein Inhibitory Activity [24] Aliquots (1 ml) extract (50, 100, 150, 200, 250 and 300 µg/ml) was mixed with equal volume of egg albumin solution (5%) and incubated at 27±1°C for 15 min. The mixture was then placed at 70°C in a water bath for 10 min for denaturation of egg albumin, and the turbidity of the mixture was measured spectrophotometrically at 660 nm after cooling. Each experiment was carried out in triplicate to get the average; and the percentage inhibition of albumin denaturation.

Carragenan induced paw edema method: [25]. Animals were weighed and numbered; Hind paws were marked on both the (right & left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume. The initial paw volume (both right & left) of each rat was measured by mercury displacement method. Animals were divided into four groups each comprising of at least six rats. To one group saline was injected and to the second group diclofenac sodium subcutaneously. The third group was injected with low dose of test drug [EECD 200mg] and the fourth group was injected high dose of test drug[EECD 400mg]. After 30 minutes injected 0.1ml of 1% (w/v) carrageenan in the plantar region of the left paw of controlled as well as diclofenac-treated group. The right paw serves as reference non-inflammed paw for comparison. Paw volume of control and diclofenac treated rats, both legs were noted at 15, 30, 60, 120, 180, 240, 300, 360 minutes after carrageenan challenge. The percent difference in the right and left paw volumes of each animal of control and diclofenac-treated group were calculated and compared with the mean percent change in paw volume in control and drug treated animal and express as percent oedema inhibition by the drug.

Cotton pellet granuloma method:[26] The animals were weighed and numbered. The animals were randomized into different groups (vehicles or standard or test compound) according to the body weight. Each group consisted 6 rats. To maintain aseptic conditions hands were washed with disinfectant. Cotton pellets were prepared (each weighing 20 +/-1mg weight) by weighing the raw cotton on a weighing balance rolling then in a pellet shape . These pellets were sterilized in an oven (106KPa for 30 min). They were placed individually in a container. Applied 500 microlitres (10micrograms of drug/500microliters of acetone) of the vehicle or drug or standard solution to the respective pellets and allow them to dry at room temperature to achieve the constant weight. The animals were anaesthetized using ethyl ether, later on which were procured on the dissecting board. The back of animals was shaved. Shaved area were cleaned with 70% ethanol as disinfectant and given an incision with the help of surgical blade in the lumbar region of the rat. By using artery forceps, subcutaneous tunnels are formed to place sterilized cotton pellets

(treated with vehicle or standard or test compound) on both sides in the scapular region. The incision were stitched using sutures surgically, later it was wrapped with the help tissue adhesive. Allow the cotton pellets to remain in the animal body for 6 days. On 7th day animals were weighed again and sacrificed using chloroform anaesthesia; then the pellets were removed, with the help of forceps and scissors and weighed separately. These pellet weight refers to the wet weight of granuloma plus weight of the cotton implanted. Pellets were allowed to dry in an oven at 60^{0}C for 18 hours. The pellets were removed from the oven and weighed them again. This refers to the dry weight of the granuloma plus weight of the cotton implanted. The individual weights of the cotton pellet (20+/-1mg) were subtracted from the above mentioned weights. This gives the respect net wet or dry weights of granuloma which was compared with standard group.

Statistical Analysis: *In-Vitro* assays were performed in triplicate and results are expressed as Mean ± S.D. Later IC $_{50}$ values were calculated. In-Vivo experiments were performed and the calculations were processed by Tukeys test followed by one way ANOVA. All the calculations were done using Graphpad Prism software.

RESULTS:

Qualitative Phytochemical Screening: The qualitative phytochemical screening was taken on to know the presence of different phytochemical constituents in ethanolic extract of the root of *Caesalpinia digyna* by using different standard methods. The preliminary phytochemical screening of ethanolic extract revealed the highest amount of various medicinal active constituents like carbohydrates, alkaloids, flavonoids, glycosides, saponins, sterols and triterpenoids, tannins, phenols, proteins and amino acids and quinones respectively. Table No. 1

DISCUSSION:

The present research work takes account of the phytochemical investigation and pharmacological evaluation of root of *Caesalpinia digyna* for anti-inflammatory activity.

Phytochemical Screening: The different phytochemicals have been found like Alkaloids, Flavonoids, Glycosides, Saponins are accountable for its antiflammatory, antimicrobial activities. Steroids and triterpenoids are known to produce antibacterial effect and antianalgesic effect on central nervous system. The phenolic compounds like flavonoids and tannins are a major group of compounds which are known for primary antioxidants or free radical scavengers. These compounds were found in the root of ethanolic extract of *Caesalpinia digyna Rottler*.

Thin Layer Chromatography: The less polar compound moves higher up the plate (resulting in a higher R_f value). If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places, and all compounds on the TLC plate will move higher up the plate.

Pharmacological Evaluation: The RBC membrane stability test is based on the finding that a number of non-steroidal anti-inflammatory agents inhibit heat induced lysis of erythrocytes, presumably by stabilizing the membrane of the cell. The percentage protection of erythrocyte membrane by *Caesalpinia digyna* extracts were given in Figure 3. The erythrocyte membrane may be considered a model of the lysosomal membrane which plays an important role in inflammation. The compounds which prevent the lysis of membrane caused by the release of hydrolytic enzymes contained within the lysosomes may relieve some symptoms of inflammation. When the human RBC is subjected to hypotonic stress, the haemoglobin release from RBC, will be prevented by anti-inflammatory drugs because of the membrane stabilization. It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane, while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules. It was noted that ethanolic extract of root which showed positive tests for phenolics and flavonoids exhibited highest membrane stabilizing activity compared to that of standard diclofenac. In low dose the activity is low but increases the same with higher concentrations. The activities of the various fractions are comparable to that of diclofenac at the concentration of 50, 100, 150, 200, 250 and 300 µg/ ml. Hence anti inflammatory activity of the extracts was concentration dependent

Table 1: Phytochemical Investigation of ethanolic extract of *Caesalpinia digyna* root

S.no		Chemical tests	Ethanolic extract
1.		**Tests for carbohydrates**	
		a) Molisch's test (general test)	Positive
		b) Caramelisation	Positive
		c) Bromine water test	Positive
		d) Borntrager's test	Negative
	A	**Tests for reducing sugars**	
		Fehling's test and Benedicts test	Positive
		Test for hexose sugars	Positive
	B	a) Selwinoff's test	Negative
2.		**Tests for alkaloids**	
		a) Dragendorff's test	Positive
		b) Wagner's test	Positive
		c) Mayer's test	Positive
		d) Hager's test	Positive
		e) Tannic acid test	Positive
3.		**Tests for flavonoids**	
	A	Tests for anthocyanins	Positive
		Sulphuric acid and Sodium hydroxide test	Negative
		Tests for flavonones	Positive
	B	Sulphuric acid and Sodium hydroxide test	Positive
		Millon's reagent test	Positive
		Ferric chloride test	Positive
	C	Alkaline reagent test	Positive
	D	Lead acetate test	Positive
	E	Ammonia test	Positive
	F	Shinoda test	
4.		**Tests for glycosides**	
	A	**Tests for cardiac glycosides**	
		a) Keller Killani test	Positive
		b) Legal's test and Baljet's test	Positive
		c) Bromine water test	Positive
		Tests for anthraquinones glycosides	
	B	Borntrager's test and Modified Borntrager's	Positive
5.		**Tests for saponins**	
		a) Foam test	Negative
		b) Haemolysis test	Positive
6.		**Tests for sterols and triterpenoids**	
		Libermann buchard test and Salkowski test	Positive
7.		**Tests for tannins**	
		a) Ferric chloride test and Gelatin test	Positive
		b) Lead acetate test	Positive
		c) Match stick test	Positive
		d) Alkaline reagent test	Negative
8.		**Tests for phenols:** Ferric chloride test, Zinc hydrochloride test and Shinoda test	Positive
9.		**Tests for proteins and amino acids**	
		a) Millons test	Positive
		b) Ninhydrin test	Negative
		c) Biuret test	Positive
10.		**Test for quinones :** Hydrochloric acid test	Positive

Percentage Yield: The percentage yield of ethanolic solvent extract of root of *Caesalpinia digyna* is 8.75 w/w. Table No.2

Table 2: Percentage yield of EECD

Solvent	Weight of extracts (gm)		Percentage yield (% w/w)
	Practical yield	Theoretical yield	
Ethanol	7	80	8.75

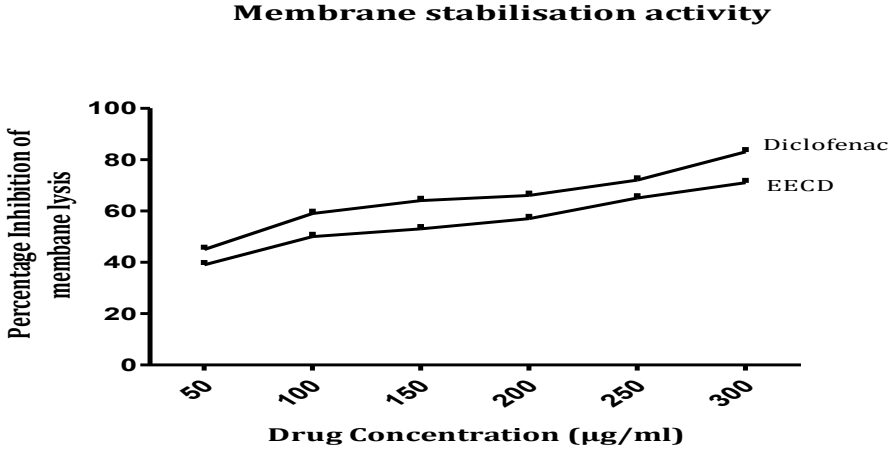
Thin Layer Chromatography: TLC was performed and R_f values for sample 1 was found to be 0.63 and R_f value for sample 2 was found to be 0.72

Anti-inflammatory Activity: Membrane stabilizing activity

Table 3: Effect of EECD on membrane stabilization activity

Membrane stabilizing activity		
Drug Conc. (µg/ml)	**Percentage of membrane stability of EECD**	**Percentage of membrane stability of Diclofenac**
50	39.85±1.10	44.98±0.83
100	48.75±1.13	58.17±0.84
150	53.12±0.85	63.32±0.63
200	57.57±0.63	66.08±0.87
250	66.02±0.99	71.14±0.76
300	71.67±1.32	82.62±0.97
Values are expressed as Mean ± S.D; n=3; IC_{50} = 155.0±2.01 (Diclofenac), IC_{50} = 177.5±2.95 (EECD)		

Figure 3: Effect of EECD on membrane stabilization activity

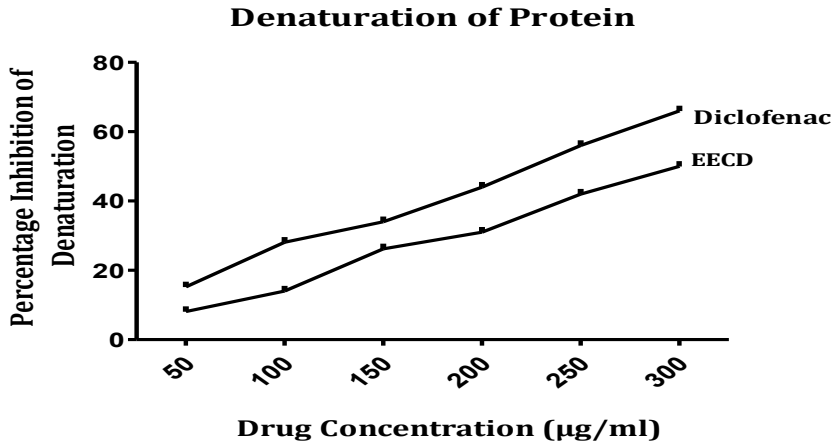


Protein Denaturation Activity:

Table No. 4: Effect of EECD on inhibition of protein denaturation activity

Protein denaturation activity		
Drug Conc. (µg/ml)	**Percentage inhibition of denaturation of protein of EECD**	**Percentage inhibition of denaturation of protein of Diclofenac**
50	7.94±0.13	14.58±0.60
100	13.53±0.45	28.48±0.72
150	25.76±0.54	32.48±1.53
200	30.72±0.58	43.73±0.59
250	42.52±1.24	55.99±1.22
300	51.51±1.63	65.84±1.59
Values are expressed as Mean ± S.D; n=3 IC_{50} = 223.0±5.71 (Diclofenac); IC_{50} = 302.6±8.23 (EECD)		

Figure 4: Effect of EECD on inhibition of protein denaturation activity



***In-Vivo* Methods: Carrageenan induced rat paw edema method:**

Table No. 5: Effect of EECD on inhibition of carrageenan induced paw edema in rats

Groups	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	0.73 ± 0.05	1.36 ± 0.08	1.66 ± 0.06	1.86 ± 0.06	1.87 ± 0.06	1.91 ± 0.07	1.87 ± 0.06
Standard	0.72 ± 0.07	1.02 ± 0.08 *** 25.6	1.03 ± 0.07*** 43.5	103. ± 0.06*** 51	0.76 ± 0.04*** 59.4	0.68 ± 0.04*** 65.8	0.62 ± 0.05*** 68
EECD 200mg	0.72 ± 0.09	1.23 ± 0.05 * 12.5	1.23 ± 0.05*** 26.7	1.34 ± 0.07*** 28	1.20 ± 0.1*** 40	1.02 ± 0.03*** 43	1.04 ± 0.08*** 49
EECD 400mg	0.71 ± 0.02	1.21 ± 0.05 * 14	1.02 ± 0.05*** 38	1.17 ± 0.05*** 41	0.94 ± 0.0.3*** 52	0.87 ± 0.08*** 53	0.77 ± 0.048*** 62
Values are expressed as Mean ± S.D; n = 6; one way ANOVA followed by Tukey's test * = P<0.05; ***=P<0.001 As compared to control group.							

Cotton Pellet Granuloma Method

Table No. 7: Effect of EECD on inhibition of cotton pellet granuloma in rats

Groups (n=6)	**Wet weight (mg)**	**Dry weight (mg)**
Control	504.8 ± 0.43	220.3 ± 0.57*
Standard	161.4 ± 0.91*	71.52 ± 0.84* 69
EECD 200mg	231.0 ± 1.83*	103.5 ± 0.78* 53.4
EECD 400mg	192.0 ± 0.87*	87.02 ± 0.62* 60
Values are expressed as Mean ± S.D; one way ANOVA followed by Tukey's test * = P<0.05		

Fig. No. 5: Effect of EECD on inhibition of Carrageenan induced paw Edema in rats

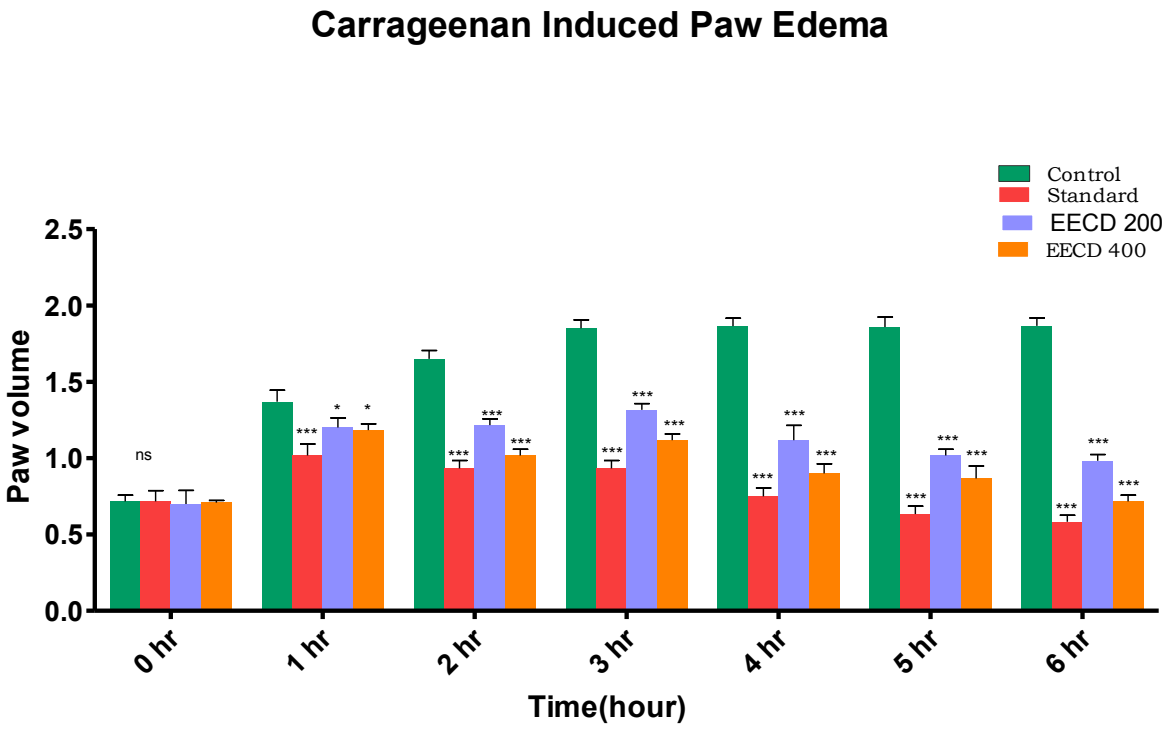
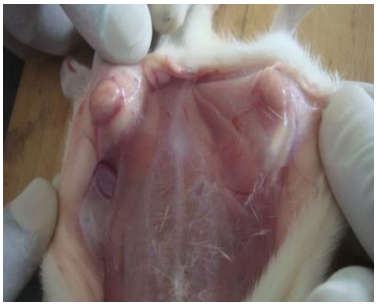


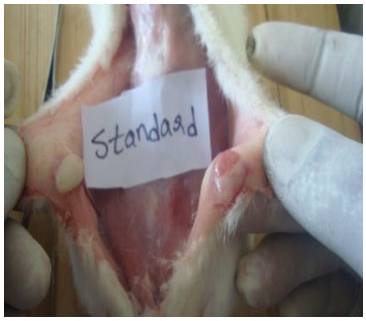
Figure 6: Photographs of cotton pellets exposed in animals after surgery

PIC NO. 1



Animals treated with Extract

PIC NO. 2



Animals treated with Standard

PIC NO. 3

Photograph of dry cotton pellets after surgical removal according to group

The test of inhibition of protein denaturation is a suitable method for assessing the anti-inflammatory activity. Indeed, it was verified that the denaturation of proteins causes inflammation and chronic diseases such as rheumatoid arthritis. The ethanolic extracts (showing most potent anti-inflammatory activity) contains mainly flavones and flavonoids, triterpenoids and phenolic compounds which may be responsible for this activity. The extracts tested 50, 100, 150, 200, 250 and 300 µg/ ml showed an efficiency to inhibit the denaturation of proteins led by the heat (Figure 4). The maximum percentage inhibition is at 300 µg/ ml which was compared with that of inhibition obtained by standard drug at 300 µg/ ml. A suitable animal model, Carrageenan-induced rat paw edema is for screening anti-inflammatory drugs and assessing the anti-edematous effect of the natural products. It is a strong chemical used for the release of inflammatory and pro-inflammatory mediators such as prostaglandins, histamine, leukotrienes, bradykinin, TNF- α, etc. The course of acute inflammation is usually biphasic. Phase 1 involves the release of serotonin and histamine from mast cells as well as kinins after the injection of phlorgistic agent in the first 1-2 hours. While the phase-2 is mediated by release of prostaglandins, COX and LOX products in 2-3 hours. Phase 2 is sensitive to both the steroidal and non-steroidal anti-inflammatory drugs. Two important types of inflammatory mediators, prostaglandins (especially prostaglandin E_2) is main mediator to cause the acute inflammation and leukotriene B_4 is the mediator of leukocyte activation in the inflammatory event. Although, the Cox and Lox pathways play a key role in the inflammatory process, the inhibition of cox is more effective in inhibiting carrageenan-induced inflammation than Lox inhibitors. From the results, the paw volume reduced significantly as compared to the control group. Oral (200 mg/kg) dose of EECD also significantly inhibited (***$p<0.01$) rat paw volume when compared to control. Higher dose of EECD reveals inhibition of paw edema(***$p<0.001$). Percentage inhibition of granuloma formation exhibited by higher dose of EECD extract is around 61% which is almost near to the standard inhibition of 68% where as lower dose of EECD extract exhibits only 53.4%, which shows dose dependent anti inflammatory activity.

CONCLUSION:

The phytochemical investigation reveals the presence of constituents Flavonoids, carbohydrates, flavones, phenols in the extract may be responsible for the anti inflammatory activity. The present study throws light on the effect of the plant *Caesalpinia digyna* Rottler in reducing the inflammation that has been induced in vitro by heat induced lysis of membranes of R.B.Cs, denaturation of protein as well as in vivo by carrageenan induced paw edema method, cotton pellet induced granuloma method. The results of phytochemical screening components of ethanolic extract of *Caesalpinia digyna* root might be accountable for the effective antioxidant capacity which leads to its effectiveness against inflammation. Membrane stabilization activity clearly revealed that concurrent administration of ethanolic extract of *Caesalpinia digyna* root significantly attenuated the lysis and changes in R.B.C membranes and prevented it till certain extent. According to the Inhibition of protein denaturation activity *in-vitro* results it can be stated that ethanolic extract of *Caesalpinia digyna* had offered protective effect against limiting protein denaturation process and white blood cells migration properties. Therefore the, extract leads to effective R.B.C membrane stabilization and protein inhibiting denaturation both contributing to invitro anti inflammatory activity. Carrageenan induced paw edema in rats suggests that ethanolic extract of *Caesalpinia digyna* had been effective against the formation of edema which in higher dose was almost equivalent to standard diclofenac. Prevention in formation of Cotton pellet granuloma in rats also proves its potency to prevent inflammatory reactions in vivo. Hence, the ethanolic extract of *Caesalpinia digyna* root can be advocated as anti-inflammatory agent. Further studies are required which compounds its accountabillity for the anti-inflammatory activity. The efficacy of *Caesalpinia digyna* for curing or alleviating inflammation may be a light for developing a potential herbal medicine for the future.

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