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Research Article

Investigation of Antiangiogenic Activity of *Cassia Fistula* Extract In Chick Embryo Chorioallantoic Membrane Model

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ABSTRACT

Plant which harbors curative elements or properties in one or more of its parts may be termed has medicinal plant. Plant based medicaments have been employed since the dawn of civilization for prolonging the life of man for combating against various body ailments. So medicinal plants like *Cassia Fistula* have their different pharmacological activities so they are cultivating in many places of India. *Cassia fistula* has been reported to have different pharmacological effects like Anti-inflammatory, Antifungal activity, Antibacterial Activity, Antihelminthic Activity along with these effects it also has Antiangiogenic effect. Which used in the treatment of cancer.

The study was conducted to investigate the scientific basis for the traditional use of *Cassia fistula* by determining its Antiangiogenic effect in chorioallantoic membrane assay (CAM assay).

Cassia fistula was evaluated for antiangiogenic activity by chorioallantoic membrane assay (CAM assay). Antiangiogenic effect was assessed using *Cassia fistula* induced hypotensive rat.

Antiangiogenic effect was evaluated in hypotensive rat, in comparison to control group with the standard (salicylic acid) and test (*Cassia fistula*) group showed significant activity.

Antiangiogenic activity was evaluated in CAM assay, in comparison to control group with the test group showed significant activity of less angiogenesis.

From the present study it can be concluded that ethanolic extract of *Cassia fistula* shown Antiangiogenic activity.

Key-words: *Cassia Fistula,* Antiangiogenic effect, chorioallantoic membrane assay, Chick Embryo, etc.

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Introduction:-

The World Health Organization (WHO) encourages the inclusion of herbal remedies that have been proven to be efficacious and safe, into primary health care. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy. Several chemotherapeutic agents have been developed in the modern system of medicines as a result of screening of medicine of the medicinal plants in various parts of the world. The isolation of biologically active alkaloids such as Atropine, Quinine, Serpentine, reserpine, narcotine, caffeine, nicotine, etc. are result the initial leads obtained from the traditional system of medicine. Explanation of chemical constituents of plants and pharmacological screening may provide us the basis for the developing the leads for the development of novel agents for curative purposes for treating various illness like Diabetes, Cancer, Sexually Transmitted diseases Neurological and immunological disorders etc. (1).

The Normal angiogenesis (formation of new blood vessels) occurs during fetal development to create the baby's circulatory system, and in the uterus during the menstrual cycle, as well as occurs around a wound or cut to help with healing. Tumor angiogenesis is the formation of new blood vessels that grow into the tumor, giving it nutrients and oxygen to assist its growth. Because new blood vessel growth plays a critical role in many disease conditions, including disorders that cause blindness, arthritis and cancer, angiogenesis inhibition is a "common denominator" approach to treating these diseases. Antiangiogenic drugs exert their beneficial effects in a number of ways: by disabling the agents that activate and promote cell growth, or by directly blocking the growing blood vessel cells. Angiogenesis inhibitory properties have been discovered in more than 300 substances, ranging from molecules produced naturally in animals and plants, such as green tea extract, to new chemicals synthesized in the laboratory. A number of medicines already approved by the U.S. Food and Drug Administration (FDA) have also been found to possess antiangiogenic properties, including celecoxib (Celebrex), bortezomib (Velcade), and interferon. Many inhibitors are currently being tested in clinical trials for a variety of diseases in human patients. (2)

Angiogenesis is a progressive, multistep physiological process by which new blood vessels are generated from pre-existing vasculature. Adult vasculature is maintained mostly in an angiostatic state that must be switched off to allow for new blood vessel formation. This angiogenic switch is a part of normal physiologic responses, for example to tissue injury, as well as a critical step in the pathology of tumor progression. It is commonly accepted that specific mechanisms underlining the angiogenic switch involve a selective remodeling of the extracellular matrix (ECM) by proteolytic enzymes and the induction, generation or release of angiogenic growth factors, which induce endothelium sprouting, followed by reorganization and formation of new blood vessels.

To analyze the mechanisms underlying normal and pathological angiogenesis, numerous in vivo angiogenic assays have been established employing different species of laboratory animals, including mammals (mouse, rat, hamster, and rabbit), birds (chicken and quail), and fish (mainly zebra fish). In this chapter, we will focus on major models of angiogenesis in the chick embryo. The use of chick embryo models for angiogenic studies is facilitated by the existence in avian species of a specialized respiratory tissue, named the Chorioallantoic membrane (CAM) that allows for gas exchange between the embryo and the atmosphere surrounding the egg and in effect performs the function of a lung during embryonic life (3).

In the chick embryo, the chorioallantois is formed between days 4 and 5 of development, when the outer mesodermal layer of the allantois fuses with the mesodermal lining of the chorion, and a network of blood vessels is gradually formed between the two layers. The central portion of the CAM is fully developed by day 8 to 10 at which time it becomes capable of sustaining tissue grafts, while the outskirts of the CAM are still developing and expanding until the CAM fully envelopes the embryo at day 12 of incubation. Histologically, the CAM consists of three germ layers, that is, ectoderm, mesoderm, and endoderm. The ectoderm faces the shell membrane and is underlined by the respiratory capillary plexus, which starts to form between days 5 and 6 of embryonic development by both angiogenesis and vasculogenesis (4). This capillary plexus is very dense and appears as a honeycomb network of tiny capillaries originating from terminal capillaries. The mesoderm of the chorioallantois is a collagen-rich embryonic connective tissue transversed by blood vessels belonging to the arteriolar and venous systems. The mesoderm is underlined with a thin endoderm layer, which separates the CAM from the allantoic cavity (3,5).

Until day 11 or 12 of chick embryo development, the blood vessel system of the CAM is highly angiogenic that is, undergoing maturation through a constant generation of new blood vessels as well as establishment of new blood vessel anastomoses. Therefore, between day 8 and day 10, the developing CAM vasculature is ready to

sprout in response to additional proangiogenic stimuli and, in turn, is very responsive to antiangiogenic factors. This feature renders the chick embryo CAM models well suited for experimental validation of pro- and anti-angiogenic compounds. The response of the CAM to angiogenic stimuli is relatively rapid and most assays require only 3 to 5 days. In addition, recent modifications of the originally described assays readily allow for quantitation of the angiogenic process, revolutionizing the use of CAM assays in angiogenesis studies. In addition, the chick embryo is naturally immunoincompetent until embryonic day 17, thus allowing for grafting of cells of different species origin, such as human tumor cells, and therefore providing a useful tool for analysis of the proangiogenic potential of test cells.

Angiogenesis disorders are threatening to mankind. No satisfactory remedy available at present, because therapy is not enough to cure such disorders and also this therapy is contraindicated. The greatest disadvantage in presently available potent synthetic drugs lies in their side effects like nausea, vomiting, constipation, ulcer, respiratory depression and hypertension, toxicity and reappearance of symptoms after discontinuation. Hence search for new Angiogenesis agents that retain therapeutic efficacy and yet devoid of adverse effects are justifiable.

There is much hope for finding active antiangiogenic compounds from indigenous plants as these are still used in therapy despite the progress in conventional chemistry and pharmacology in producing the effective drugs.

Taking all the above points into consideration in the course of study we have selected plant Cassia fistula a local plant belong to family Fabaceae only few scientific publications are available about Cassia fistula which made us to take up present investigation of this plant and represent the antiangiogenic activity of its ethanolic extract.

Materials and Methods:-

- Taking all the above points into consideration in the course of study we have selected plant *Cassia fistula* a local plant belong to family Fabaceae only few scientific publications are available about *Cassia fistula* which made us to take up present investigation of this plant and represent the antiangiogenic activity of its ethanolic extract.
- Salicylic acid - DNS Fine Chemicals, India
- Ketamine - Neon Laboratories, Mumbai
- Absolute alcohol - A.R.99.9%, Changshu Yangyuan Chemicals, China
- Formalin - Finar Chemicals, India

MATERIALS for CAM ASSAY:

- Chick embryo,
- Sterile plaster,
- Sterile glass rod.
- Petri dishes.

Experiments were conducted at Jayamukhi College of Pharmacy, Warangal. Permission for all procedures was granted from the Institutional Animal Ethical Committee (IAEC). Experiments were carried out with twelve female wistar rats weighing 180-200g, were obtained from animal house of Unisankyo, Hyderabad. Animals were housed in the animal house of Jayamukhi College of Pharmacy, Narsampet for experimental purpose.

Methodology

- The leaves and branches of *Cassia fistula* are collected and shade dried. Coarse powder is made from these dried leaves and subjected to extraction by using alcohol as solvent.
- Each crude extract obtained after evaporating the solvent subjected to preliminary phytochemical screening and these extracts are utilized for antiangiogenic activity.
- Stock solution of Cassia *fistula* under study was prepared freshly, on the day of experimentation using distilled water. The dose of formulation-200mg/kg, was selected for the study, the selected doses represent 1/10 of 2000mg/kg.
- Stock solution of salicylic acid under study was prepared freshly, on the day of experimentation using distilled water. The dose of formulation- 1.5mg/kg.
- Healthy weighing between 180-200g female wistar rats - the chosen experimental animals were maintained in our animal house (12:12 dark: light cycle), with adequate ventilation, hygienic conditions maintained on normal palliated diet and water ad libitum. A group of three animals were housed in

polypropylene cage of (47cm × 34cm × 20cm) on paddy husk bed and covered with stainless steel wire mesh with provision for water and feed.
- All experiments were performed in research lab all parameters of different tests were observed and recorded by person blind to treatment protocol.
- Data generated from various experimental procedures were photograph.
- Experimental protocols of animal experiments were duly approved by Institutional Animal ethics Committee, IAEC of Jayamukhi College of Pharmacy Narsampet (certificate attached).

Qualitative chemical tests were conducted for extract of Cassia *fistula* to identify the various phytoconstituents. The phytochemical investigation showed presence of Flavonoids, Saponins, carbohydrates, starch, gum, proteins, tannin and Phenolic compounds.

Pharmacological Investigation

- **Acute (Oral) Toxicity Study**

Acute toxicity dose of Ethanolic Extract of *Cassia fistula* was obtained from the previous articles as per OECD guidelines was studied. Dose of 2000mg/kg doesn't show any toxic symptoms, so according to guideline; it is considered as a LD50 cutoff value. Doses selected for pharmacological study of antiangiogenic effect are mentioned below. The dose of *Cassia fistula* extract was selected on the basis of earlier work on mortality studies. The dose that showed 100% survival without any abnormalities Ethanolic extract of *Cassia fistula*: 200mg/kg (1/10th of 2000mg/kg).

- **IC_{50} Value For Chick Embryo Chorioallantoic Membrane:**

The dose of *Cassia fistula* extract was selected on the basis of earlier work on mortality studies. The dose that showed 100% survival without any abnormalities on hatching was selected in each case. IC_{50} Value for *Cassia fistula* extract was 38µg/mL. As per demand, the drug was prepared further throughout the experimental period in water for injection (6).

Preparation of *Cassia fistula*: Based on the previous studies for the antiangiogenic activity dose of *Cassia fistula*, 200mg/kg b.w. orally was selected. As per demand, the drug was prepared further throughout the experimental period in water for injection.

Preparation of salicylic acid: Based on the previous studies the dose of salicylic acid, 1.5mg/kg body weight i.p. was selected. As per demand, the drug was prepared further throughout the experimental period in alcohol.

Effect of *Cassia Fistula* On Chick Embryo

- **Chorioallantoic membrane:**

Effect of antiangiogenic with ethanolic extract of cassia fistula was studied in chick embryo using chorioallantoic membrane assay. Fertilized eggs were obtained from the hatcheries (Sri Venkateshwara hatchery, village: chengerla, Dist: karimnagar). The shells of fertilized eggs were disinfected and incubated in aseptic incubator and aseptic conditions were maintained throughout the period of experimental work. Treatment hours is between 96 to 120 hours, were selected according to the development of CAM. The eggs were grouped as per initiation hours of doses (100ng, 300ng, 600ng, 10µg, 30µg) six eggs of each concentration are grouped separately and incubated at 37.0°C temperature with relative humidity of 70-75%. The groups were maintained separately in the incubator. The treatment doses were initiated at hours stated above and development was continued for 168-hrs.i.e.on completions of CAM venation and capillary networking after the treatment (7).

- **Dose Administration by Window Method:**

On completion of scheduled period of incubations the windows were prepared in embryos under aseptic conditions and cassia fistula extract doses were spread on them in different embryos of experimental groups. Control group of embryos were maintained as normal. The windows made for administration were sealed with sterilized adhesive tapes and the embryos were immediately transferred to incubators to continue further incubation hrs adjusting the experimental time slot until completion of 168hrs. On 168hrs of incubation, the shells were removed and the embryo and yolk were gently removed into the glass plate which contains normal saline. The developed vasculature on CAM was imaged with a digital camera (8).

Effect of *cassia fistula* on rat aorta:

Wistar rats were divided into four groups containing three animals in each group. Rats are treated with *cassia fistula* extract, salicylic acid and both *cassia fistula* extract and salicylic acid in three groups and one group is treated as control. Drugs were induced for 12 days.

Group 1-was treated as control and administered with 1ml of normal saline (orally).
Group 2-was treated with ethanolic extract of *cassia fistula* (36.00mg/kg, orally).
Group 3- was treated with salicylic acid (0.27mg/kg intraperitoneal).
Group 4- was treated with Ethanolic extract of *cassia fistula* (18.00mg/kg orally) and salicylic acid (0.135mg/kg intraperitoneal).

Effect of *Cassia Fistula* On Rat Ecg

The ECG Analysis Module automatically detects PQRST onset, amplitude, and interval to assess heart function with default (species specific) or customized detection settings. The module's Beat Averaging feature reduces noise and movement artifact for easy data comparison before and after experimental intervention.
The module exports time, interval and amplitude data, as well as graphing:

1. QT/RR
2. QT/Time
3. RR/Time

For further analysis, noise-contaminated and abnormal waveforms can be identified with the Beat Classifier, which also allows exclusion of unwanted individual beats.

When saved, the data remain unaltered by any calculations, ensuring the crude ECG can be reanalyzed at any time. ECG analysis settings can also be saved and recalled at any time using the Analysis Manager.

Data Acquisition

AD Instruments Power Lab data acquisition systems include an analog-to-digital data acquisition unit and Lab Chart Pro software. Power Lab data acquisition units are ideal for capturing cardiovascular biopotentials, with 16-bit resolution on all gain ranges, hardware filters that eliminate environmental interference, and sampling speeds of up to 200 kHz per channel. Provided with the Power Lab unit, Lab Chart Pro software is an intuitive interface for controlling hardware and transducers, data acquisition and display options, and automating repetitious procedures (such as channel calculations). Lab Chart Pro also provides specialized software modules for analyzing discrete data sets. With Lab Chart Pro's ECG Analysis Module (provided), Power Lab data acquisition systems seamlessly detect and convert analog ECG to digital data, and provide a diverse range of powerful detection, measurement, display, analysis and extraction options.

ECG Settings

The ECG analysis Module analyzes real-time or saved ECG traces. For optimal acquisition and analysis of ECG, several default detection algorithms are available, which account for species-specific ECG cycle and beat ranges (such as S-T absence in rodents).
Default detection parameters are included for:

1. Human, Guinea pig, Pig, Rat, Dog, Mouse, Rabbit

Detection settings can be created for other species, and default detection settings are completely customizable.

ECG Beat Classification

The Beat Classification tool categorizes beats according to activity and isoelectric noise, and presents them graphically for effortless identification of QRS complex and RR interval variance.

This easy to use tool allows rapid removal of artifact generated by movement, electrical interference and baseline drift, as well as exclusion of unwanted individual beats, such as extrasystole and supraventricular arrhythmias.

ECG Averaging View For easy comparison of ECG before and after experimental intervention, as well as additional removal of artifact, the Averaging View displays the mean PQRST trace from a selected data block. Each averaged PQRST complex is automatically labelled (markers can be adjusted manually) and used to generate tabular data logs and graphs of individual and mean:

1. Start times
2. End times

3. P, Q, R, S and T amplitudes
4. PQRST interval times.

Results

Extraction of Plant Priciples

The plant of *cassia fistula* is collected and shade dried. Coarse powder is made from these dried plant and subjected to extraction. Extract are prepared by using solvent as ethanol with Soxhlet apparatus. The percentage yield, colour, consistency and solubility in water were noted in Table No. 1.

Table.No.1 Percentage yield, colour, consistency and solubility in water of extract

Plant	Extract	Percentage yield	Color	Consistency	Solubility in Water
Cassia fistula	Ethanol	2.46	Dark green	Sticky	Highly soluble

Preliminary Phytochemical Screening

The preliminary phytochemical evaluation of *cassia fistula* Plant revealed the presence of Flavonoids, carbohydrates, starch, gum, proteins, tannins, Saponins and Phenolic compounds. The results of phytochemical analysis were significant in ethanolic extract. This observation clearly indicates that most of the bioactive compounds of *Cassia fistula* are soluble in ethanol, hence we have selected ethanolic extract for our studies. The results of phytochemical analysis were shown in table No. 2.

Table No.2. Preliminary phytochemical screening of *cassia fistula*

Phytochemical constituents	Ethanolic extract
Alkaloids	+
Glycosides	++
Carbohydrates	+
Flavonoids	+++
Saponins	+++
Tannins	+++
Steroids	++
Proteins	+
Fats and oils	-
Starch	++
Gums	+++
Phenolic compounds	+++

'+' indicates the presence of compounds; '-' indicates the absence of compounds; '++' indicates more clarity; '+++' indicates better response.

Acute Toxicity Studies

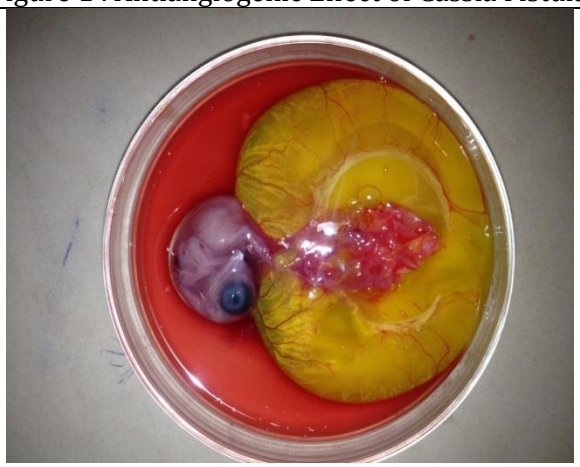
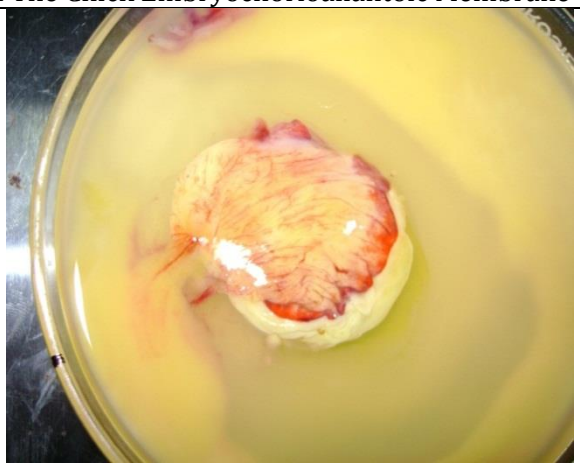
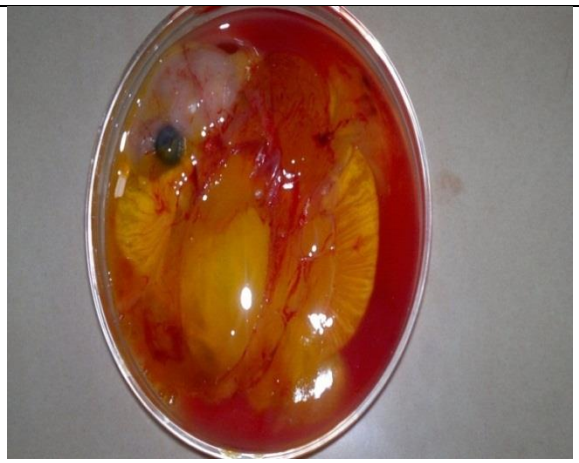
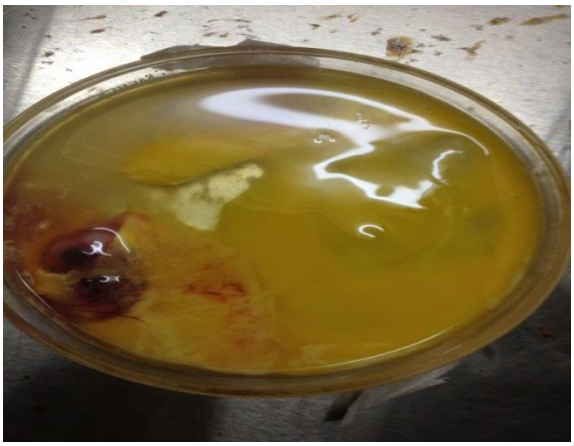
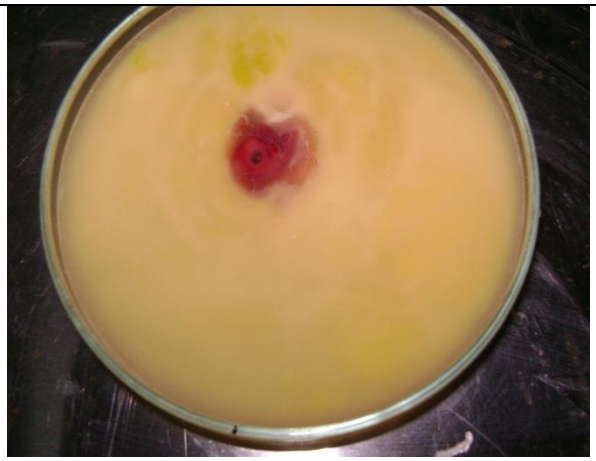
Acute toxicity dose of Ethanolic Extract of *Cassia fistula* was obtained from the previous articles as per OECD guidelines was studied. Dose of 2000mg/kg doesn't show any toxic symptoms, so according to guideline; it is considered as a LD50 cut off value. The dose of *Cassia fistula* extract was selected on the basis of earlier work on mortality studies. The dose that showed 100% survival without any abnormalities
Ethanolic extract of *Cassia fistula*: 200mg/kg (1/10th of 2000mg/kg).

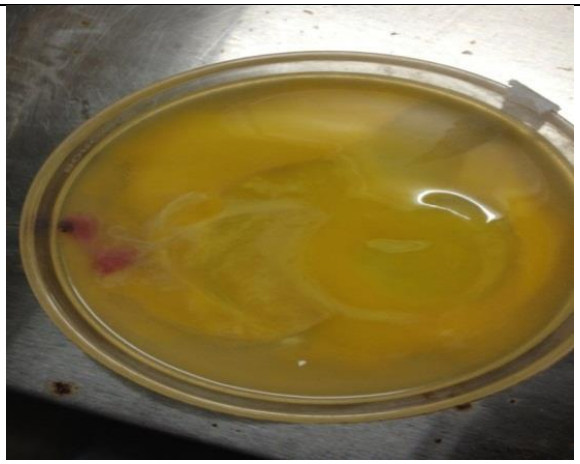
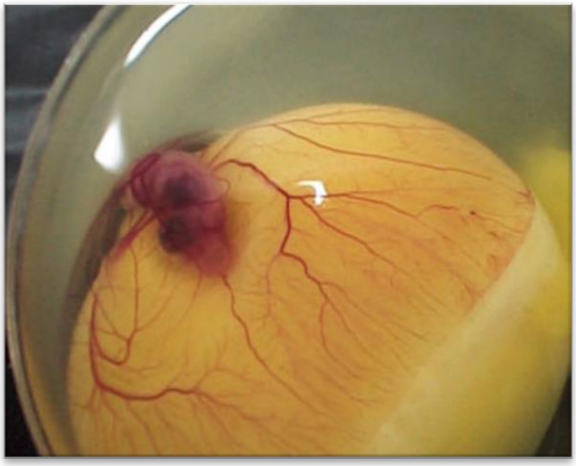
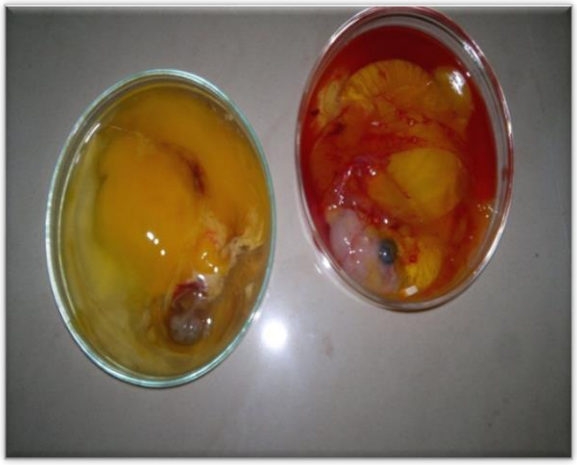
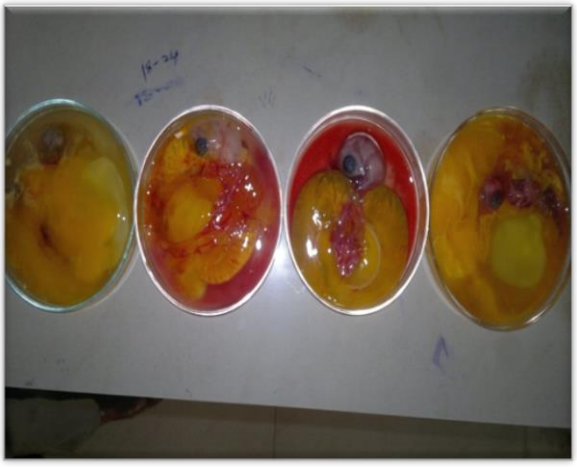
The dose of *Cassia fistula* extract was selected on the basis of earlier work on mortality studies. The dose that showed 100% survival without any abnormalities on hatching was selected in each case. IC_{50} Value for *Cassia fistula* extract was 38µg/mL (6).

Antiangiogenic Effect of *CASSIA FISTULA* Extract in the Chick Embryochorioallantoic Membrane

Effect of antiangiogenic with ethanolic extract of cassia fistula was studied in chick embryo using chorioallantoic membrane assay. After period of incubation, embryos are taken to laminar air flow chamber and they are maintained in aseptic area conditions then the shells were removed and the embryo and yolk were gently poured into the glass plate which contains normal saline. Then the developed vasculature on CAM was imaged with a digital camera and these are the following images.

Figure 1 : Antiangiogenic Effect of Cassia Fistula Extract In The Chick Embryochorioallantoic Membrane

 **CONTROL**	 **CASSIA FISTULA** (100 ng)
 **CONTROL**	 **CASSIA FISTULA (300ng)**
 **CONTROL**	 **CASSIA FISTULA (600ng)**

 **CONTROL**	 **CASSIA FISTULA (10µg)**
 **CONTROL**	 **CASSIA FISTULA (30µg)**
 **COMPARING TEST WITH CONTROL AND SIZE OF CHICK**	 **COMPARING TEST WITH CONTROL AND SIZE OF CHICK**

From the above images ethanolic extract of *Cassia fistula* shows the effect of antiangiogenesis by different doses of drug it exhibit difference in formation of blood vessels. It results the antiangiogenic activity.

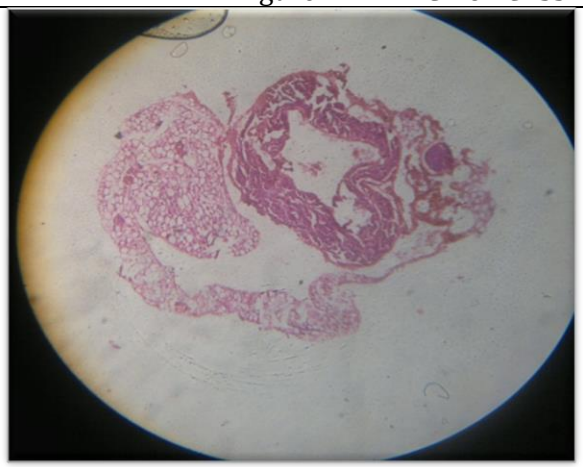
Chick Cam Model:

In this method for evaluation of antiangiogenic activity employs the effect of drug on blood vessels formation at different concentrations of *Cassia fistula* was studied in egg using chick CAM model. At maximum concentration formation of blood vessel is low comparing with the minimum concentration it produces high blood vessel formation and these are compared with the control group, concentrations used in this method are as follows (100ng, 300ng, 600ng, 10µg, 30µg). However formation of blood vessel in test group were found to be less significant when compared with control group. The results are presented in figures from Fig. No 1. In figures test group compared with control group, in these figures chick of different sizes are observed in control group the size of chick was 1.5±0.12cms and it contains blood whereas test group the size of chick was 0.5±0.23cms and no blood was observed.

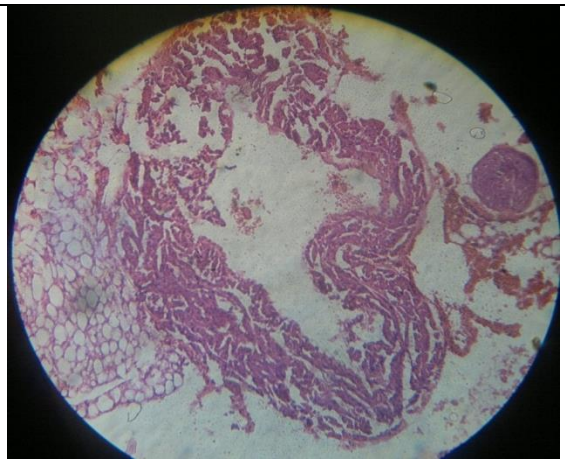
Effect of *Cassia Fistula* on Rat ECG and Aorta:

Wistar rats were divided into four groups containing three animals in each group are treated with drug for 12 days. Then first rats are anesthetized with the Ketamine and then, rats are dissected. Before the process of dissection ECG of each rat was taken by using power lab AD instrument. And then rats are dissected, aorta of rats are collected and stored in a 1% formalin solution. Then collected aorta of every rat sent to the lab for microtome preparation. And these are the following images of microtome of rat aorta.

Figure 2 : EFFECT OF *CASSIA FISTULA* ON RAT ECG and AORTA



CONTROL RAT AORTA (4X)



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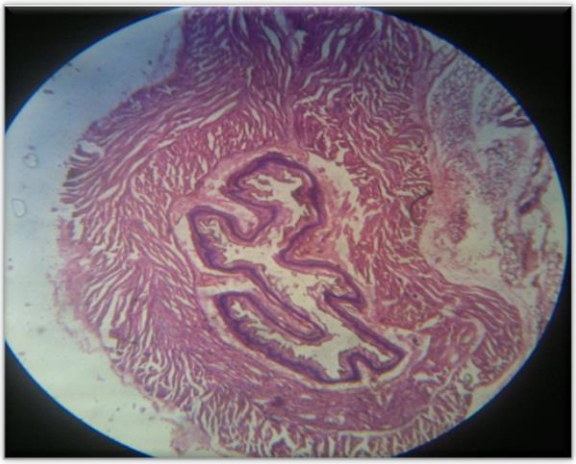
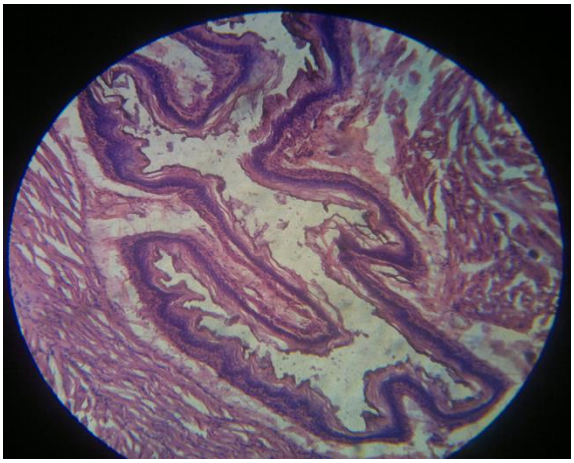
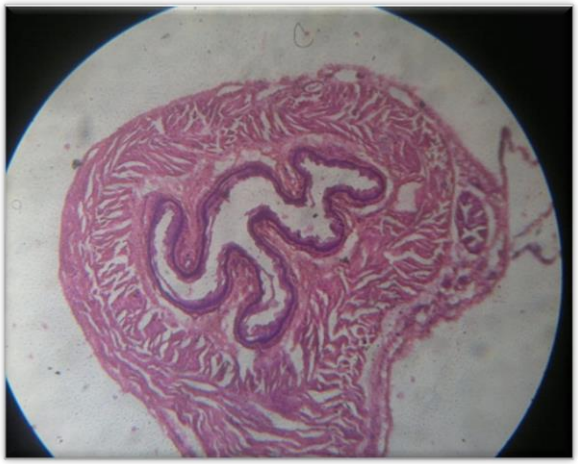


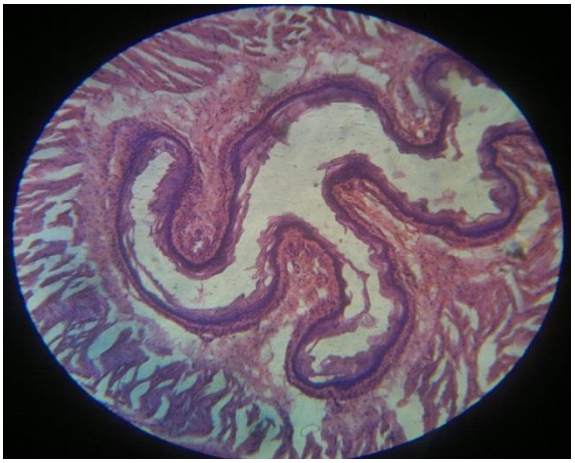
CASSIA FISTULA(4X)



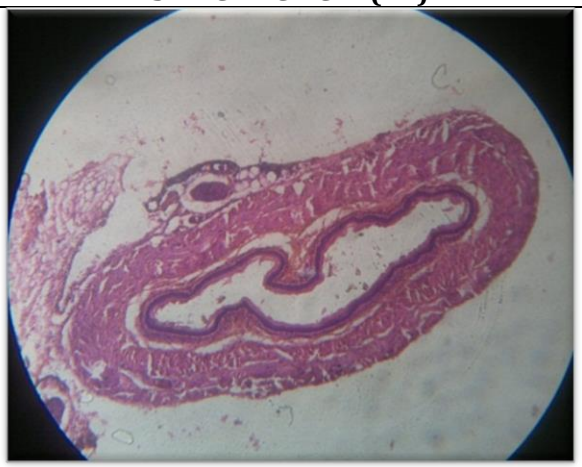
CASSIA FISTULA(10X)



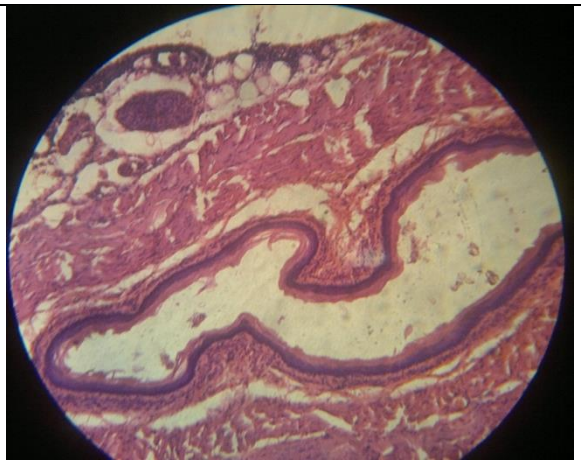
SALICYLIC ACID (4X)



SALICYLIC ACID (10X)



CASSIA FISTULA+ SALICYLIC ACID (4X)



CASSIA FISTULA+ SALICYLIC ACID (10X)

Rat Aorta Model:

In this method for evaluation of activity employs the effect of drug on rat aorta it consists of control, test and standard groups. An increase decreases in the size of wall and lumen of aorta shows the effect of drug. It wall consists of following layers outer to inner, they are tunica adventitia is an outer layer, then middle layer tunica media (which is a strong muscle layer), and inner most layer towards lumen is tunica intima and is lined with endothelium towards lumen.

Group 1: In control (1ml of normal saline) group no change is observed in the layers of aortic wall (tunica adventitial, medial and intimal layers) and also in the size of lumen, it resemblance normal aorta blood vessel.
Group 2: Where as in test (*Cassia fistula* of36.00mg) group change is observed compare to control group. That is (thick tunica adventitial, medial and intimal layers) are observed, and size of lumen decreases (constriction).
Group 3: In this (salicylic acid of 0.27mg) group change is observed compare to control group. That is (slight thick tunica adventitial and intimal layers, thick medial layer) are observed, and lumen size increases (dilation).
Group 4: In these (*cassia fistula* of 18.00mg and salicylic acid of 0.135mg) group slight change is observed in both lumen and wall of the aorta compare to test and standard groups, these group resemblances as like control group but its not up to control group (normal tunica adventitial layer and thick medial and intimal layers) are observed.
Size of different layers of aorta are compared in test (*Cassia fistula*) with salicylic acid treated group the following changes are observed they are constricted aorta in test due to thick tunica adventitial, medial and intimal layers but dilated aorta in salicylic acid treated due to slight thick of tunica adventitial and intimal layers and thick medial layer are observed.
The results are presented in figures from Fig. No 2.

RAT ECG:

In rat ECG we observed that heart rate of *cassia fistula* treated rat was increased compared with the salicylic acid treated. The changes observed in ECG are the following interval between the spatial maximum of the P wave and the onset of the QRS complex decreased while the magnitude of the P wave increased. The direction of the P vectors did not change. This pattern corresponds to the electrocardiographic manifestations of predominant right atrial overload. No significant changes in the QRS duration were observed. Also the magnitude and spatial orientation of the maximum QRS vectors remained constant. The interval between the QRS onset and the maximum spatial magnitude of the T wave shortened. The terminal QRS vectors and the ST vectors gradually shifted toward the right, and superiorly. The T magnitude lessened. In the first minute of the recovery period the P and T magnitudes markedly increased.

Discussion:-

Herbal drugs play an important role in healthcare programs in treatment of various diseases including angiogenicdisorders. An attempt has been made here to evaluate Cassia fistula Antiangiogenic activity and its effect on blood vessels. (9)

Chemical Constituentcassia fistula areanthraquinones, fistulic acid, rhein, rheinglucoside, sennosides A and B, phlobaphenes, emodin, chrysophanic acid, fistuacacidin, lupeol, beta-sitosterol and hexacosanol (9). The ripe pods and leaves of cassia fistula contains several anthraquinones, both in aglycone and glycoside forms such as rhein, aloe-emodin (10). Emodin inhibits vascular endothelial growth factor A induced angiogenesis, by blocking receptor 2 (KDR/FLK 1) phosphorylation. It is an active component in Cassia fistula as a tyrosine kinase inhibitor. The emodin, constituent of Cassia fistula also present in Rhubarb (Rheum palmatum). The study of rhubarb anthraquinone, emodin capable of inhibiting cellular proliferation, induction of apoptosis and prevention of metastasis (11). The revaluation of Cassia fistula using CAM assay suggests that several bioactive anthraquinone of Cassia fistula possessing promising anticancer/antiproliferation/ antiangiogenesisphytoconstituents. Rhein is anthraquinone derivative of rhubarb, inhibits proliferation of various cancer cells. Which is also present as a chief constituent in Cassia fistula research study involving rhein showed apoptosis and cell cycle arrest in human hepato cellular carcinoma BEL-7402 cells (12).

Vascular tone refers to the degree of constriction experienced by a blood vessel relative to its maximally dilated state. All arterial and venous vessels under basal conditions exhibit some degree of smooth muscle contraction that determines the diameter, and hence tone, of the vessel.

Basal vascular tone differs among organs. Those organs having a large vasodilatory capacity (e.g., myocardium, skeletal muscle, skin, splanchnic circulation) have high vascular tone, whereas organs having relatively low vasodilatory capacity (e.g., cerebral and renal circulations) have low vascular tone.In Varicose VeinsDilated, lengthened and tortuous veins seen mainly in the legs but spermatic, esophageal and hemorrhoidal veins may be affected due to lack or incompetence of valves within the veins.Pilex(Himalaya Co, India) which contains extract of cassia fistula and other phytochemicals increases the venous tone and helps in venous drainage. Pilex contains Cassia fistula (Indian laburnum, Aragvadha) that is used in the treatment of varicose veins. It helps in shrinking engorged veinsand has a profound anti-inflammatory activity.(http://www.himalayaonline)

Vascular tone is determined by many different competing vasoconstrictor and vasodilator influences acting on the blood vessel. These influences can be separated into extrinsic factors that originate from outside of the organ or tissue, in which the blood vessel is located, and intrinsic factors that originate from the vessel itself or the surrounding tissue. The primary function of extrinsic factors is to regulate arterial blood pressure by altering systemic vascular resistance, whereas intrinsic mechanisms are important for local blood flow regulation within an organ. Vascular tone at any given time is determined by the balance of competing vasoconstrictor and vasodilator influences.

In general, extrinsic factors (neurohumoral) such as sympathetic nerves and circulating angiotensin II increase vascular tone (i.e., cause vasoconstriction); however, some circulating factors (e.g., atrial natriuretic peptide) decrease vascular tone.

Intrinsic factors include:

- Myogenic mechanisms (originating from vascular smooth muscle), which increase tone.
- Endothelial factors such as nitric oxide and endothelin can either decrease or increase tone, respectively.
- Local hormones/chemical substances (e.g., arachidonic acid metabolites, histamine and bradykinin can either increase or decrease tone.
- Metabolic by-products or hypoxia,which generally decrease tone.

The mechanisms by which the above influences either constrict or relax blood vessels involve a variety of signal transduction mechanisms that ultimately influence the interaction between actin and myosin in the smooth muscle (13).

Salicylates dilate blood vessels through inhibiting PYK2-mediated RhoA/Rho-kinase activation and thus lower blood pressure. It has been shown that high doses of salicylates,including aspirin and sodium salicylate, dilate blood vessels in vivo, probably through direct effect on vascular smooth muscle (14).
Vascular smooth muscle contraction is determined by the phosphorylation level of the myosin light chain (MLC), which can be achieved through activating MLC kinase (MLCK) and/or inhibiting MLC phosphatase (MLCP) (15). Rho kinase inhibitors decrease blood pressure in hypertensive animals, but not in normotensive animals (16).

Dilated blood vessel are observed in transverse section of aortic vessel following administration of salicylic acid causing systemic vasodilation as observed in microscopic pictures of aorta, the following treatment with Cassia fistula resulted in normalization of microscopic characters of thickened aortic tunica adventitial, medial and intimal layers with minimal size of lumen that causing systemic vasoconstriction of aorta.
The mechanism behind this an increase in the tone of aortic wall due to activation of PKY-2 mediated RhoA/Rho-kinase activation cassia fistula may be involved in the activation of PKY-2 mediated RhoA/Rho-kinase.FurtherECG studies given impression that following treatment with cassia fistulashows normalization of heart rateindicating ethanolic extract ofcassia fistula acting on the blood vasculature by increasing the tone of the blood vessel.

CONCLUSION:-
The present study was aimed to expose the antiangiogenic activity of ethanol extract of *Cassia fistula* plant in different models like chick embryo chorioallantoic membrane and rat aorta. *Cassia fistula* plant extract exhibited the presence of antiangiogenic activity from the above following results by using these methods like chick embryo chorioallantoic membrane and rat aorta. Ethanolic extract of *Cassia fistula*produced antiangiogenic activity in chick chorioallantoic membrane model. The following effect was observed when comparing test drug (treated cassia fistula) with the control, different concentrations of drug it exhibit difference in the formation of blood vessels. Even in smallest dose of *Cassia fistula*(100ng) produced antiangiogenicin chick chorioallantoic membrane model. So we conclude that the extract of *Cassia fistula* is can be used to treat diseases causing through angiogenesis. The present study indicates salicylate induced hypotension and is reduced by following treatment with ethanolic extract of *Cassia fistula.*
The probable mechanism of vasoconstriction is increase in the tone of blood vasculature may be through activating PYK-2 mediated RhoA/Rho-kinase activation, offering an insight into unique property such as effect of Cassia*fistula*on antiangiogenic and toning up of blood vasculature.
The mechanism involved in antiangiogenic is not fully understood. This work suggests that, isolating the active components responsible for the antiangiogenic activity of *Cassia fistula* and further studies to reveal the exact mechanisms of action responsible for the antiangiogenic activity of *Cassia fistula* plant. *Cassia fistula* plant shows not only antiangiogenic activity it also had several activities like Antioxidant, Antihelmintic activity, Antibacterial activity, Antifungal activity, Anti-inflammatory, Anti fertility activity, Anti-leishmaniatic activity, Antimicrobial activity, Antiparasitic activity, Antipyretic activity etc along these it had Antitumor activity which is responsible to treat against cancer same as the above study which act on angiogenesis.
The antioxidant potential of *Cassia fistula* can broaden its therapeutic applications towards the prevention of degenerative diseases, free radical scavenging activity, reducing power and lipid peroxidation are the basis for assessing their preventive role against free radical effect and will enrich the national food composition database antioxidants from *Cassia fistula* being safe.
Nature is still a perfect source for healthpromotion and for the supplementation of safe drugs. Development of natural products from plant (leaves and branches) of *Cassia fistula* being safe, eveready and economic can replace synthetic drugs with natural to act against the angiogenesis mediated diseases.
The present work suggests that it requires isolating and characterizing theactive components responsible for the antiangiogenic activity and furtherstudies to reveal the exact mechanism of action responsible for the antiangiogenic activity of *Cassia fistula* plant.

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