

INVITRO & INVIVO PHARMACOLOGICAL MODELS FOR THROMBOLYTIC ACTIVITY IN HYMENAEA MARTIANA AND GILLARDIA PULCHELLA ETHANOLIC EXTRACTS USING K-CARRAGEENAN INDUCED THROMBOSIS

Dr.Mehnoor Farheen***, Afrose Fatima**, Aqsa amtul* Ajmeri begum*

***Head of department – Pharmacology,
Shadan women's college of pharmacy- Khairtabad- 500004- Hyderabad.

Abstract:

The project is focused on the study of anti-thrombotic effects of the flower extract of Gillardia pulchella and bark extract of Hymenaea martiana using k-carrageenan induced thrombosis method. To evaluate the anti-thrombotic activity of ethanolic extracts of Hymenaea martiana and Gillardia pulchella, it is tested by phytochemical analysis, invitro and invivo models. Oral dosing of the plant extracts in different doses alleviates the thrombosis. This may be achieved by serum estimation of the haemoglobin, CRP, WBC, etc and histopathological study of the tail. The rats' tails were dissected out from one animal of each group for histopathological examination for the estimation of parameters like thrombus, congestion in the vein, disruption of cellular matter and epithelial morphology. When the microscopy of the tail was done, it was found that thrombosis was healed with the plant extracts when compared to standard drug (heparin) . The study asserts that further reasearh needs to be taken on the benefits of Hymenaea martiana and Gillardia pulchella in Thrombosis.

Keywords: Thrombosis, Hymenaea martiana, Gillardia pulchella, K-Carrageenan, Heparin

INTRODUCTION

INTRODUCTION OF THROMBOSIS

DEFINITION:

The phenomenon in which the supply of blood to the tissues is disturbed by the emergence of thrombus¹(clot) inside a blood vessel is called as "thrombosis". The chance of thrombosis occurrence can be increased by increase in gore coagulability, stoppage of the normal stream of blood & injured endothelial quilting of the bloodvessel.

SIGNS²:

Increment in the gore thickening:

Cancer, lack of water dispense to the body, pregnancy (increased pressure in pelvic area & legs), presence of a hypodermic cannula and hormone substitution remedy, all shoot up the blood clotting and causes instability in the body.

Stoppage of the normal blood flow:

This can happen when you see no proper bodily movement, when blood vessels collapse and when the nearby veins get blocked by some growth or an apparel.

Bruised endothelial quilting of the gore vessel:

It is due to Diabetes, puffing of fats in the ichor vessels, soaring of blood lining and trauma.

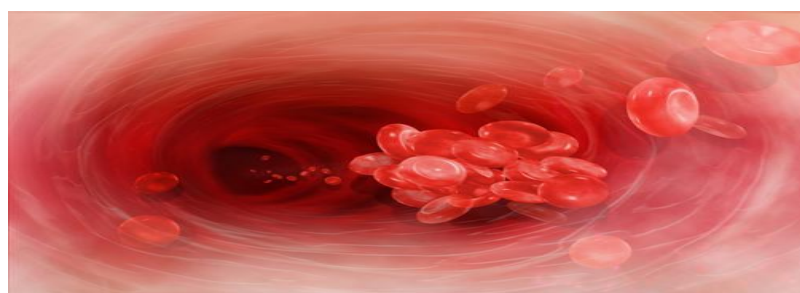


Fig.1.Obstruction of blood supply by thrombus.

EMBOLISM:

Embolism³ is an expression used to describe an “obstructed artery” which gets blocked by lump of blood or any other foreign material. This can also be referred to as “thrombus”. Some other elements blocking the artery are

- Lumps that embark on cancer.
- Liquor amnii formed during labour.
- Indwelling IV catheter break off.
- Fats released from the bone fractures.
- Pustule from a boil.

CATEGORIZATION OF EMBOLISM⁴:

- ❖ **PULMONARY EMBOLISM:** “Deep vein thrombosis” is the condition in which the clots are formed in the legs. There are chances that these lumps may wedge in the lungs. In critical conditions, this may cause death, but many a times it’s cut down to smaller particles by body itself.
- ❖ **RETINAL EMBOLISM:** The nano vessels of the blood in retina when gets occluded by the clots, this may cause an unexpected defect in an eye.
- ❖ **CEREBRUM EMBOLISM:** When thrombus enters the cerebrum, this leads to ischaemic attacks.
- ❖ **AMNIOTIC EMBOLISM:** The amniotic fluid during the pregnancy may get clotted and if this reaches the mother’s lungs, causes pulmonary embolism.
- ❖ **SEPTIC EMBOLISM:** The elements formed during the infections, when they reach the blood vessels may block the blood stream.
- ❖ **AIR EMBOLISM:** Snorkelers rising up hurriedly to the surface may get affected by air embolism, blocking the normal flow of blood.
- ❖ **FAT EMBOLISM:** Blood clots or air bubbles will be formed when there is entrance of adipose cells in the blood stream.

PATHOPHYSIOLOGY⁶:

The concomitants of thrombosis is given prudently by Virchow’s triad. It comprises of endothelial vandalization, hypercoagulability & arterial or venous gore stasis. Increased platelet activation, build up of cohesive molecules, an increment in the tissue factor of platelet & build up of pro-inflammatory cytokines takes place during destruction to the gore wall. Then there will be inflammation that is instigated by the cytokines that encourages interactivity linking WBC and cells of endothelium.

Finally, there will be start up in the clump formation owing to the emergence of cohesive molecules that is on account of the interactivity between the cells of endothelium and WBC.

Thrombosis usually crops up when the body’s endogenous anticoagulants like are ineffective. Anti platelet agents forms the basis in the therapy of thrombosis since platelets plays an imperative role in emergence of occlusion of arteries as opposed to occlusion of veins.

1. ENDOTHELIAL INJURY⁷:

- a) Curbs the platelet assembling by degenerating the adenosine diphosphate.
- b) Attachment of thrombomodulin to thrombin triggers the start up in zymogen.
- c) High affinity binding of the heparin to the antithrombin III and speeding up its neutralization.
- d) Arousing of the fibrin by different plasminogen activators.
- e) An adhesive glycoprotein called Von wille brand factor, helps in the thrombocytes union and subendothelium during the injury.

2. ROLE OF PLATELETS²:

Thrombocytes play a predominant role in in maintaining haemostasis and during vascular injury.

a) Platelet adherence:

The interactivity between the membrane receptors & the sucked up plasma proteins play a vital role in adherence of the platelets. Glycoprotein of blood along with some other important proteins on the membrane receptor is responsible for the platelets adherence.

b) Platelets starting up:

Thrombocyte triggering factors , ADP and cytoplasmic granules are released on adherence of platelets. Collagen receptors like GpIIb/IIIa will be switched on during switching on of platelets for forming a pseudopodal disposition to undergo delivering consequence.

c) Platelet aggregation:

The turned on platelets outstretch the pseudopods, clusters and gets accumulated, P2Y₁ & P2Y₁₂ also have a momentous task in accumulation of platelets. P2Y₁₂ responds to the ADP and increases significantly to complete the accumulation process of platelets.

3. ROLE OF COAGULATION SYSTEM:

The coagulation process when summed up results in cleavage of fibrinogen to fibrin monomers forming a crassamentum which stops the bleeding. It includes two pathways.

During the body tissue damage, some aspects and factor X are activated along with the calcium ions in the “intrinsic pathway”.

During the body tissue damage, thromboplastin when interacts with the factor VII switches on factor X in the “extrinsic pathway”. When the inbred and outward pathways merge to switch on factor X that forms a multiplex with factor Va , factor 3 in companionship of calcium ions, this then preceds to the switching on of nonua protein to ceramide which then converts fibrinogen to fibrin. Fibrin polymers then form inscrutable fibrin by the actuation of factor XIII. This whole process is voiced as “common pathway” since it includes both inbred and outward pathways.

MECHANISM OF COAGULATION SYSTEM⁹:

There are 5 mechanisms that keep a tight rein on switching on of thrombocytes and agglomeration

- ✚ Activation of “protein C” when thrombomodulin binds to proteins and degradation of FVa & FVIIIa by this activated form along with some cofactors.
- ✚ Degradation of the serine proteases by antithrombin & this action of “antithrombin” gets activated constantly in the sight of heparin sulphate.
- ✚ Curbing of the action of “tissue factor” by lipoprotein associated coagulation inhibitor.
- ✚ Curbing of the fibrin formation by “plasmin” which proteolytically cleaves fibrin into fibrin degradation products.
- ✚ Switching on of the thrombocytes and agglomeration cascade is reticent by the release of granules by “prostacyclin” which synthesizes cAMP.

4. ALTERED BLOOD FLOW:

Rapidly moving central stream of gore consists of erythrocytes & white cells. The slow moving stream adjoining the border of the central stream consists of platelets. The periphery of the endothelial veneer is with no cell plasma.

Instability in circumvolution embark thrombosis. Slowing down of blood leads to moving of hemocytes and platelets to the periphery forming a pavement. Continuous release of oxygen is perceived in normal flow of gore. Instability in circumvolution may harm the endothelium by more and more deposition of platelets and the fibrin. This sort of instability in the gore may embark formation of “arterial and cardiac thrombi”.

5. HYPERCOAGULABILITY OF THE BLOOD¹⁰:

- ✓ Augmentation in the ferritin, prothrombin, factor VIIa, VIIIa & Xa (coagulation factors).
- ✓ Augmentation in the platelets and their increased tenacity.
- ✓ Decline in the agglomeration inhibitors like antithrombin III.

Hypercoagulability may be heightened by the propelling age, smoking, usage of the oral contraceptives and obesity.

COMPLICATIONS:

Manifold factors that impose the danger of thrombosis. Some will be inborn, some are acquired, some will be natural and some will be weave of these. This disease can be more harmful when it is imputable to the hazardous agents. Hazard makers can be preowned for the clinical purposes inspite of the certitude that they can't shed light on pathophysiology.

Strong threat factors:

- ✚ Splintering of the buttocks
- ✚ Major surgeries
- ✚ Injury in the spinal cord
- ✚ Major trauma
- ✚ Replacement of the knee/hip

Moderate threat factors:

- ✚ Thrombophilia
- ✚ Venous thrombo embolism
- ✚ Paralysis
- ✚ Gestation/postpartum
- ✚ Bump by sarcoma
- ✚ Cardiopathy /respiratory failure
- ✚ Arthoscopic knee surgery

Weak threat factors:

- ✚ Obesity
- ✚ Immobility
- ✚ Long distance travel
- ✚ Laparoscopic surgery
- ✚ Varicose veins
- ✚ Pregnancy

MEDICATION DURING THROMBOSIS:

Classification of drugs used in Thromboembolism

Drug Class	Prototype	Action	Effect
Anticoagulant Parenteral	Heparin	Inactivation of clotting Factors	Prevent venous Thrombosis
Anticoagulant Oral	Warfarin	Decrease synthesis of Clotting factors	Prevent venous Thrombosis
Antiplatelet drugs	Aspirin	Decrease platelet aggregation	Prevent arterial Thrombosis
Thrombolytic Drugs	Streptokinase	Fibinolysis	Breakdown of thrombi

Fig.2. Drugs utilized in thromboembolism

✚ **ANTI COAGULANTS:** Curbs the clotting in the vessels of gore as seen in seen thrombosis.

These type of medications are prescribed to mankind who are precarious of getting attacked with the clots. Eg: Apoplexy, Coronary infarction, etc.

✚ **ANTI PLATELETS:** Curbs the emigration & assembling of the platelets as seen in crassamentum of arteries.

These type of medications are prescribed to mankind who are precarious to conglomeration by the platelets. Eg: Cramp of chest, choking, poor circulation, etc.

✚ **FIBRINOLYTICS:** Disintegrates the clots after they have been formed as seen in crassamentum of veins.

These type of medications are prescribed to the people whose gore vessels get jammed up. Eg: During extreme exercises, bacterial infection, malignancy, etc.

PLANT PROFILE:

HYMENAEA MARTIANA:



Fig.3. Hymenaea martiana

GENERAL FACTS:

PHYLOGENETIC CLASSIFICATION:

- Kingdom: Plantae- Plants
- Sub kingdom: Tracheophytes
- Division: Angiosperms
- Class: Eudicots
- Order: Fabales
- Family: Fabaceae

- Genus: Hymenaea
- Species: Martiana

SYNONYMS:

Cynometra martiana Baill
Hymenaea sellowiana Hayne

VERNACULAR NAMES:

English:Hyacinth bean, lablab
Hindi: bhatvas, sem
Kannada: avare, baele, balli, holadavare, maniavare
Malyalam: amara, avara
Marathi: anvare, kadavebaala, paote, parvate
Sanskrit: nispavah
Tamil: avarapai
Telugu: adaviccikudu, aslanda, annapa, chikkudu, anumulu

MORPHOLOGICAL TRAITS:

Hymenaea martiana grows in torrid environment. It is signalized by trunk, leaves that are bifoliated & a resinous discharge. The flowers in this vegetation are chalky in colour, arranged in axillary and extreme conoid. The fruits in the flora are xyloid with brown carapace and the seeds inside it are surrounded by yellow flesh and these seeds are not released by the fruit after maturation (indehiscent). The apotheosis of the tree ranges from 10-20 meters with a trunk thickness of about 70 centimeters. The fruits from these vegetation play an eminent role in sustenance and the bark and cotyledon of the plant are considered as therapeutic.

BIOLOGICAL CONSTITUENTS:

Leaves of the cotyledon when undergone maceration showed the presence of flavonoids, saponins, anthracene derivatives and naphthoquinones.

While the drupe after the maceration manifest the presence of anthracene derivatives, monoterpenes, flavonoids, diterpenes, naphthoquinones and saponins. Whereas the seeds after maceration manifest the existence of bioflavonoids and anthracenes.

TRADITIONAL USES:

This vegetation is preowned during multiple chaos like anemia, asthma, sore throat, pulmonary weakness, dysentery, diarrhoea, cough, intestinal colic, etc. It is employed therapy during viral and kidney disorders.

MEDICINAL USES:

As Antioxidant, antifungal, antibacterial, antispasmodic, hepatoprotective, vermifuge, diuretic, expectorant, fortifying agent, etc.

GILLARDIA PULCHELLA:



Fig.4. Gillardia pulchella

PHYLOGENETIC CLASSIFICATION:

- Kingdom: Plantae- Plants
- Subkingdom: Tracheophytes
- Division: Angiosperms
- Class: Eudicots
- Order: Asterales
- Family: Asteraceae
- Genus: Gillardia
- Species: Pulchella

SYNONYMS:

Calonnea pulcherimma Buc'hoz

Gillardia bicolor Lam.

Gillardia drummondii

Gillardia lobata Buckley

Gillardia neomexicana A. Nelson

Gillardia picta D. Don

Gillardia scabrosa Buckley

Gillardia villosa Rydb

COMMON NAMES:

Taptavrana, Blanket flower, Fire wheel

MORPHOLOGICAL TRAITS:

The fanning out stem of *Gillardia pulchella* is shaggy and is upright and has an apothecium of 60cm. Cotyledons of this vegetation are mostly elemental and alternate and is 6-8cm long with the fringes being smooth to coarsely denticulated. The stem of this flora is often found shaggy near the base and leaves are often located in this area of the vegetation. The pirouette of this vegetation has a daisy like blossoming and is 4-6 cm in thickness and is intensely red, orange or yellow in colour surrounded by 20 strap shaped fleurons. The outer ray florets of the plant is often yellow in colour and the central disc florets of the flower is more red-violet in colour

BIOLOGICAL CONSTITUENTS:

The building blocks seen in the vegetation were Phytol (7.58%), n-hexadecanoic acid (26.90%), methyl ester (6.73%) and cyclopropaneoctanoic acid.

MEDICINAL USES:

It is used chiefly as a therapy for sore eyes, sore nipples of mothers (douched in tea made from the vegetation) & it's powdered root is preowned for the skin disorders. The breed is used as a decorative during occasions, used for gardening purposes and is believed that this species brings good luck.

AIM AND OBJECTIVE**AIM :**

To scout the thrombolytic activity/effect of *Hymenaea martiana* and *Gillardia pulchella*.

OBJECTIVES:

- Collection and authentication of plants
- Extraction of the vegetation extricate
- Phytochemical screening
- Exploring of fibrinolytic pursuit in the animal models

MATERIALS AND APPROACH:**CHEMICALS:**

Toxicant: K-Carrageenan, ethanol as an extractor, Standard drug: Heparin(low molecular wt), anaesthetic: diluted formalin , distilled water/saline.

PLANT MATERIAL:

The woof of *Hymenaea martiana* and flowers of *Gillardia pulchella* were collected, recognized and then authenticated by the botanist Dr.Madhava Chetty, Assistant professor of Botany in pharmacognosy department at Sri Venkateshwara college in Tirupathi.

PREPARATION OF EXTRACTS:

(BY MACERATION):

The unrefined powder of *Hymenaea martiana* bark and the crude powder of *Gillardia pulchella* flowers were bought and then they were colesced with 500ml of ethanol each in distinct ceramic containers and the mixture was packed airtight through aluminium foil. The filterates of these plants were taken out in different bowls and were kept aside for the evaporation process to occur in these mixtures until these mixtures became thick and creamy.

EXPERIMENTAL DESIGN:

EXPERIMENTAL ANIMALS:

The experimental scrutiny on the animals was conducted at Shadan women's college of pharmacy, Khairtabad, Hyderabad. Fifty four albino wistar rats of about 8-12 weeks of epoch were captured from VAB Bio Sciences, Musheerabad, Hyderabad(282/PO/Bt/S/2000 CPCSEA) and these animals were acclimitized for about 14 days in the animal enclosure of Shadan Women's college of pharmacy. The animals obtained were properly housed and kept in well aerated cages made of wire mesh conserved at a temperature of 25°C and normal 12 hr light/dark cycle. These animals had free admittance to food (agro diet) and water. All the experimental procedures on the animals were performed keeping in mind the CPCSEA guidelines and standard procedures were followed for the utilization and care of animals.

ACUTE ORAL TOXICITY STUDIES:

The OECD guideline No. 425 was manoeuvred for performing acute oral toxicity studies of *Hymenaea martiana* and *Gillardia pulchella* on 5 animals (Albino wistar rats) for each plant extract. These creatures were orally administered extricates of the plant(500 mg/kg was manoeuvred as a starting dose till 2000mg/kg). These creatures were then distinctly observed carefully for the first 4 hours. These creatures were keenly observed for about 14 days time period and they were observed at regular intervals especially for pioneer first 48 hours.

EXPERIMENTAL GROUPS:

Sno.	GROUPS	AGE OF ANIMALS	DOSAGE SCHEDULE
1.	Normal control	8-12 weeks	Normal saline
2.	Thrombotic control	8-12 weeks	K-Carrageenan(20mg/kg B.W subplantar injection)
3.	Standard control	8-12 weeks	K-Carrageenan(20mg/kg B.W subplantar injection)+ Std heparin 50 IU (I.P)
4.	Plant-I(200mg/kg)	8-12 weeks	K-Carrageenan + 200mg/kg of plant-I ethanolic Extract (p.o)
5.	Plant-I(400mg/kg)	8-12 weeks	K-Carrageenan + 400mg/kg of Plant-I ethanolic Extract (p.o)
6.	Plant-II(200mg/kg)	8-12 weeks	K-Carrageenan + 200mg/kg of plant-II ethanolic extract(p.o)
7.	Plant-II(400mg/kg)	8-12 weeks	K-Carrageenan + 400mg/kg of Plant-II ethanolic extract(p.o)

8.	Plant I+ Plant II (200mg/kg)	8-12 weeks	K-Carrageenan + 200mg/kg of Plant-I ethanolic extract(p.o)+ 200mg/kg of Plant-II ethanolic extract(p.o)
9.	Plant I+ Plant II (400mg/kg)	8-12 weeks	K-Carrageenan + 400mg/kg of Plant-I ethanolic extract(p.o)+ 400mg/kg of Plant-II ethanolic extract(p.o)

Thrombotic control: K-Carrageenan, **Std. drug:** Heparin
Plant-I: *Hymenaea martiana*, **Plant-II:** *Gillardia pulchella*

MODEL OF ANIMAL USED:

RAT TAIL THROMBOSIS MODEL INDUCED BY K-CARRAGEENAN:

K-Carrageenan induced rat tail thrombosis template was manouvred to produce ictus in the rats to test the thrombolytic pursuit of the vegetation *Hymenaea martiana* & *Gillardia pulchella* invivo. The k-Carrageenan was first liquified in the saline and was disposed to rats through the subplantar region in their right hind paw. Consignments of toxicant used was 20mg/kg B.W of the rat. After this, the thrombus occurred in the tails were measured in centimeters with the succour of a scale and the whole process was photographed at 24th, 48th & 72nd hr after the carrageenan injection.

The creatures were put through to the respective treatment in accordance with allotted group for 30 days after the ictus in the tails of the rats became visible.

The rats were slaughtered at the epilogue of the experiment and the blood samples were sent to the research laboratory for the assessment of the haematological parameter after the blood illustrative were taken through the retro orbital puncture separating the serum.

COLLECTION OF SAMPLES:

COLLECTION OF BLOOD SAMPLES:

The venipuncture acquired from the rats through the retroorbital plexus were collected in proper bottles and were sent to the laboratory for the serum estimation of haemoglobin, CRP, WBC, etc. The extent of blood obtained from the creatures was upto 5ml which was done with the help of capillary tubes.

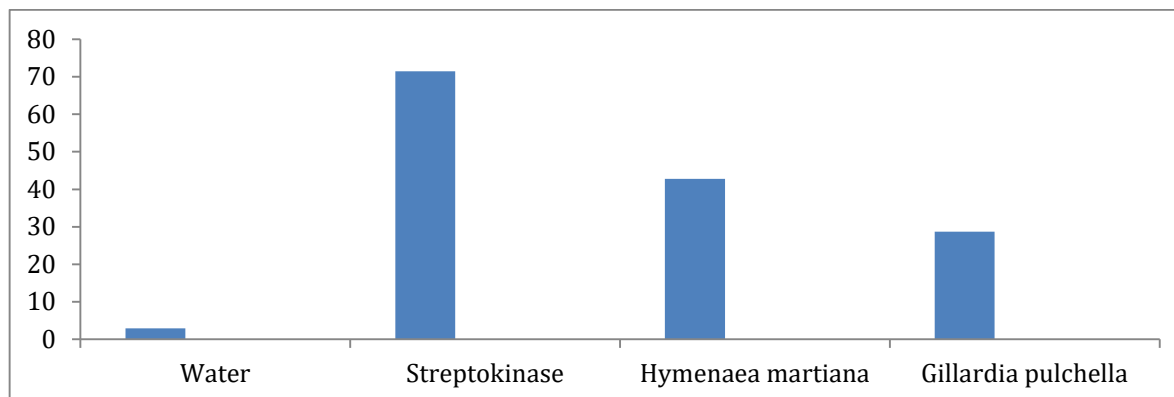


Fig.5. Collected blood samples of rats.

INVITRO METHODS:

- 1) **CLOT LYSIS TEST:** The 100µL extracts of each fraction of *Hymenaea martiana* and *Gillardia pulchella* were added to the clot containing tubes. 100µL Streptokinase was added to clot of standard tube and 100µL of water was added to the clot of blank tube, those were used as positive and negative controls respectively. Then all the tubes were incubated at 37C for 90 min and weighed again for getting the weight variation among the pre weight and final weight that was achieved for clot lysis.

EXTRACTS/DRUGS	MEAN±S.D (%CLOT LYSIS)
Negative control	2.96±0.63%
Positive control	71.43±3.46%
Hymenaea martiana	42.77±6.14%
Gillardia pulchella	28.71±2.08%



Clot lysis percentage

HISTOPATHOLOGICAL ANALYSIS:

For this analysis, animals were anaesthetized using diluted formalin on the 29th day of the demonstration in a dissecting chamber and then the tails were extricated from the dismembered creatures. These tails were then sent to the laboratory for the approximation of parameters like thrombus, congestion in the vein, disruption of cellular matter and epithelial morphology.

PARAMETERS FOR EVALUATION:

- ❖ Length of the ictus.
- ❖ Bleeding time.
- ❖ Clotting time.
- ❖ Haemoglobin levels.
- ❖ Leukocyte count.
- ❖ Platelet count.
- ❖ C-reactive protein (CRP) levels.
- ❖ Histopathological analysis of the scut.

RESULTS:**YIELD OF THE EXTRICATE:**

Percentage of extract=wt. of dry sample/wt. of sample x 100

Plant-I Extract: $132 / 500 \times 100 = 26.4\%$

Plant-II Extract: $120/500 \times 100 = 24\%$

ACUTE ORAL TOXICITY:

Hymenaea martiana and Gillardia pulchella deemed to be safe at all doses and neither mortality nor behavioural changes was encountered at a drench of 2000 mg/kg of the ethyl alcohol extracts when it was administered orally. Therefore, 200mg/kg was taken as a therapeutic dose.

PHYTOCHEMICAL SCREENING:

S. No	PHYTOCONSTITUENTS	TESTS	Plant-I Hymenaea martiana	Plant-II Gillardia pulchella
1.	Alkaloids	Dragendroff's test	++	++
		Hager's test	++	+
		Mayer's test	++	+

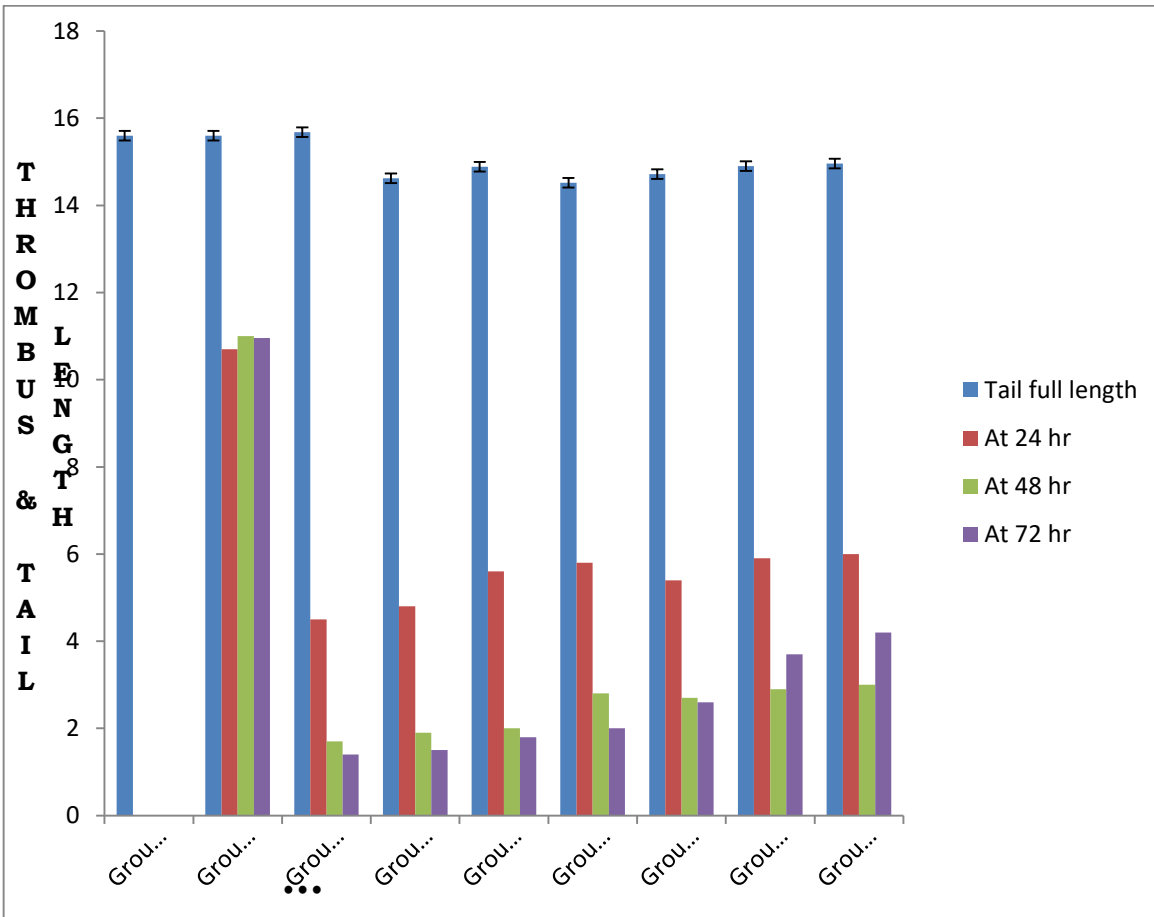
		Wagner's test	++	++
2.	Carbohydrates	Barfoed's test	+	+
		Molish's test	-	+
		Seliwanoff's test	+	++
3.	Reducing sugars	Benedict's test	++	+
		Fehling's test	++	+
4.	Flavanoids	Alkaline reagent test	++	+
		Shinoda test	++	+
		Ferric chloride test	++	+
5.	Glycosides	Borntrager's test	++	+
		Legal's test	++	+
		10% NaOH test	++	+
6.	Tannins	Bromine water test	+	-
7.	Phenols	Iodine test	++	+
		Lead acetate test	++	+
8.	Sterols	Ferric chloride test	++	+
		Salkowski's test	+	++
		Liebermann burchard's test	+	+
9.	Saponins	Foam test	++	++
10.	Coumarins	NaOH test	++	++
11.	Terpenoids		-	++

(+) indicates presence, (-) indicates absence

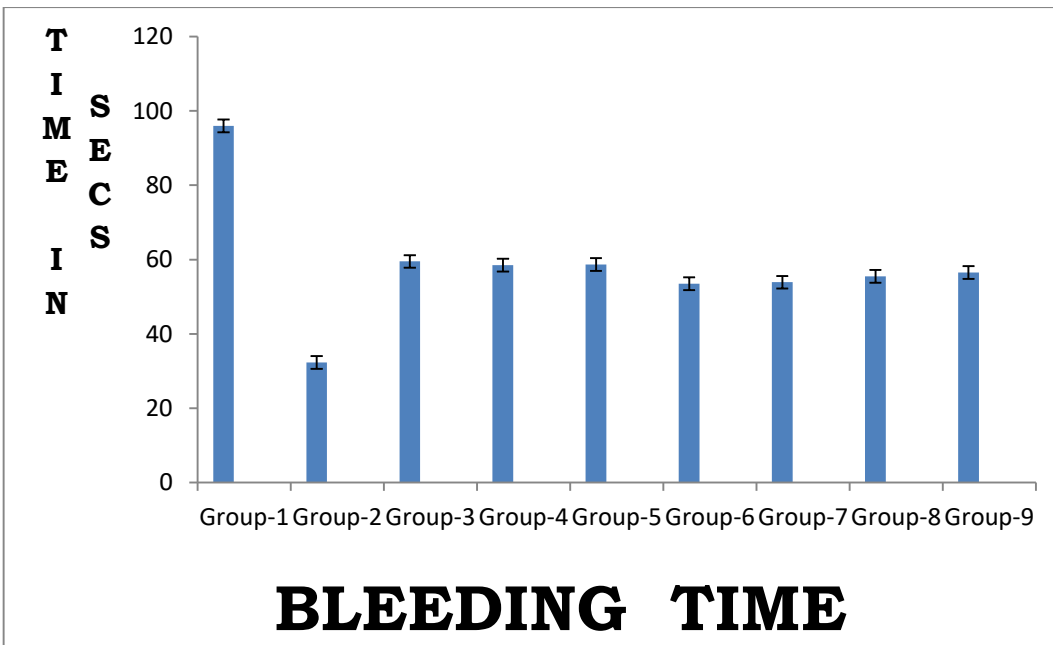


Fig.6. Phytochemical investigation of the extracts

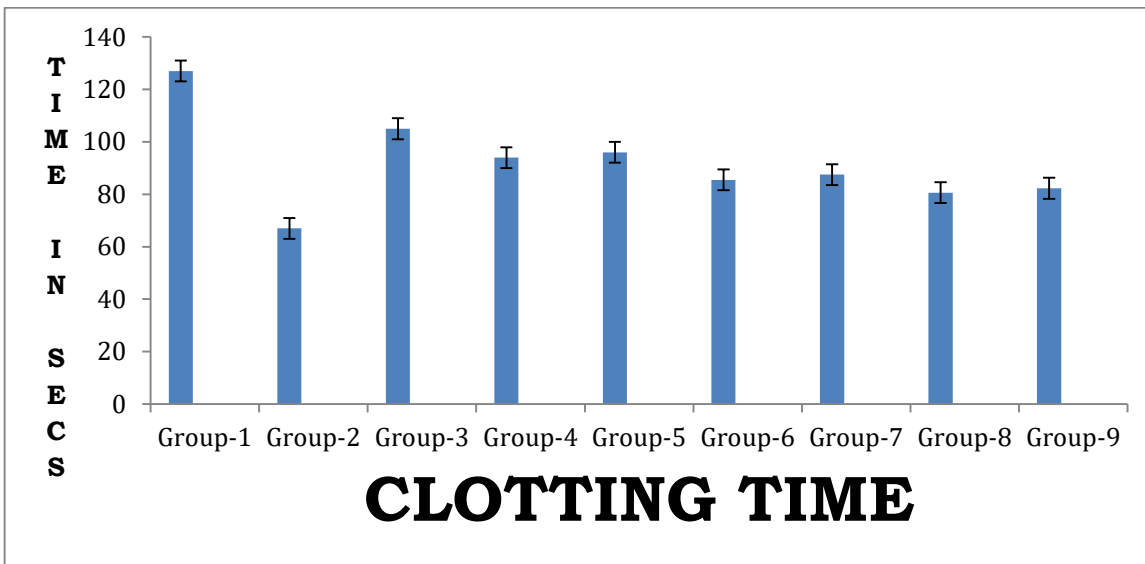
A) EXTENT OF THROMBUS IN TAIL:



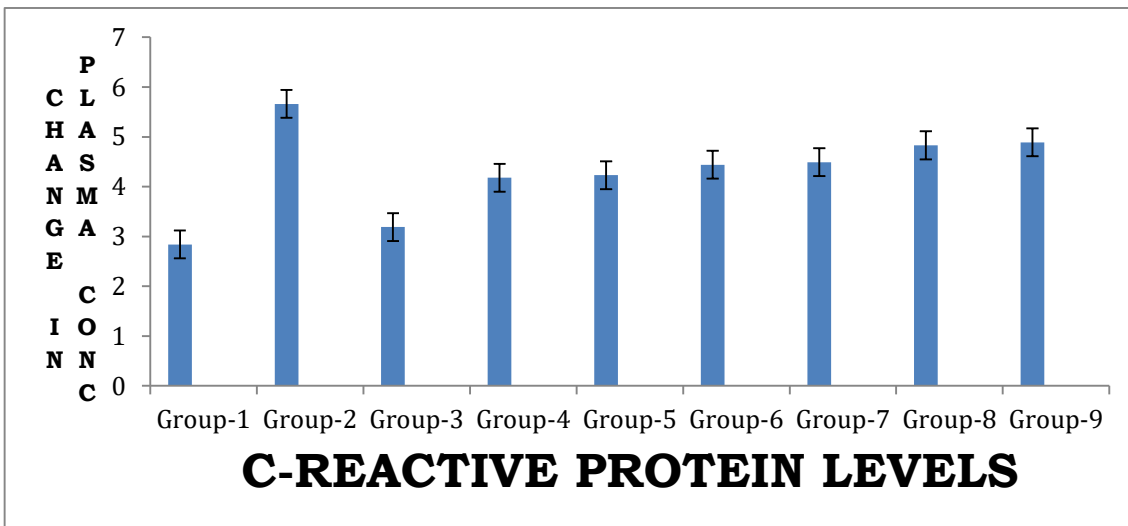
B) BLEEDING TIME METHOD:



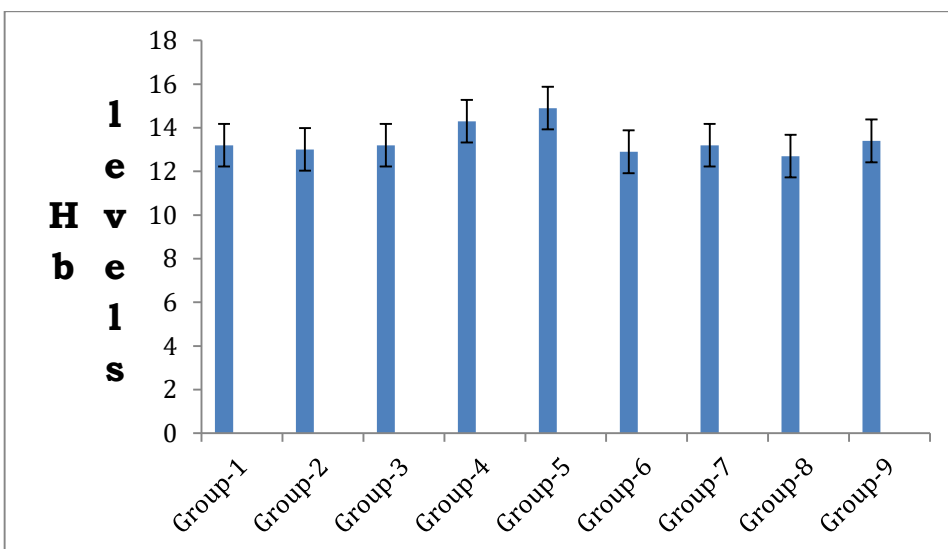
C) BLOOD CLOTTING TIME:



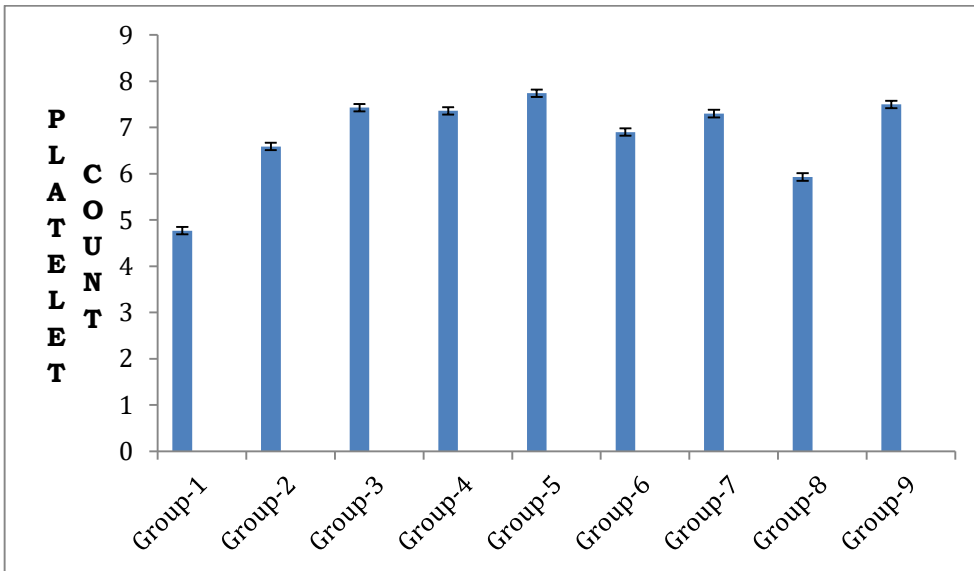
D) C- REACTIVE PROTEIN LEVELS:



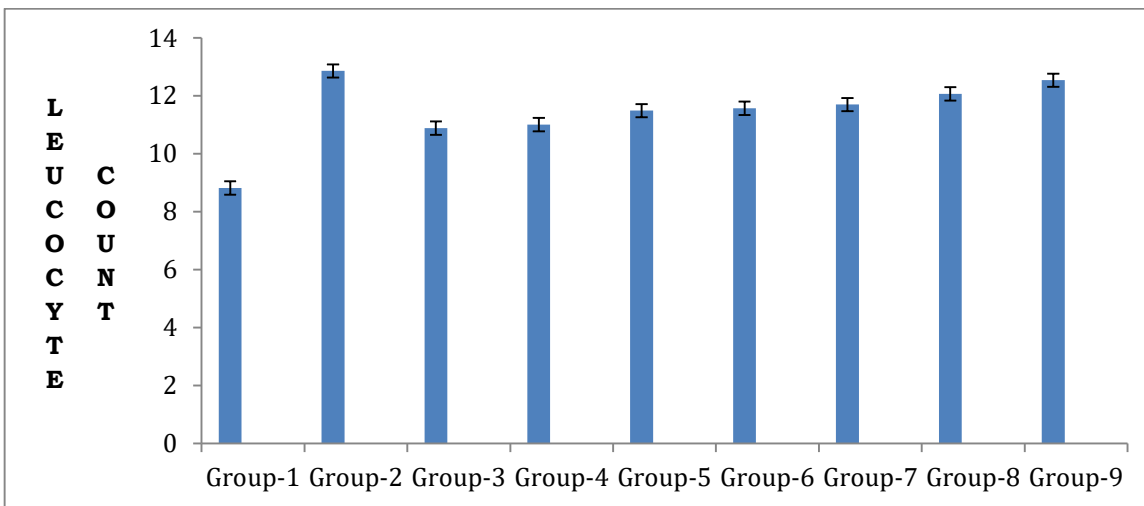
E) HAEMOGLOBIN LEVELS:



F) PLATELET COUNT:



G) LEUKOCYTE COUNT:



HISTOPATHOLOGICAL RESULTS OF THE EXPERIMENTAL ANIMAL:

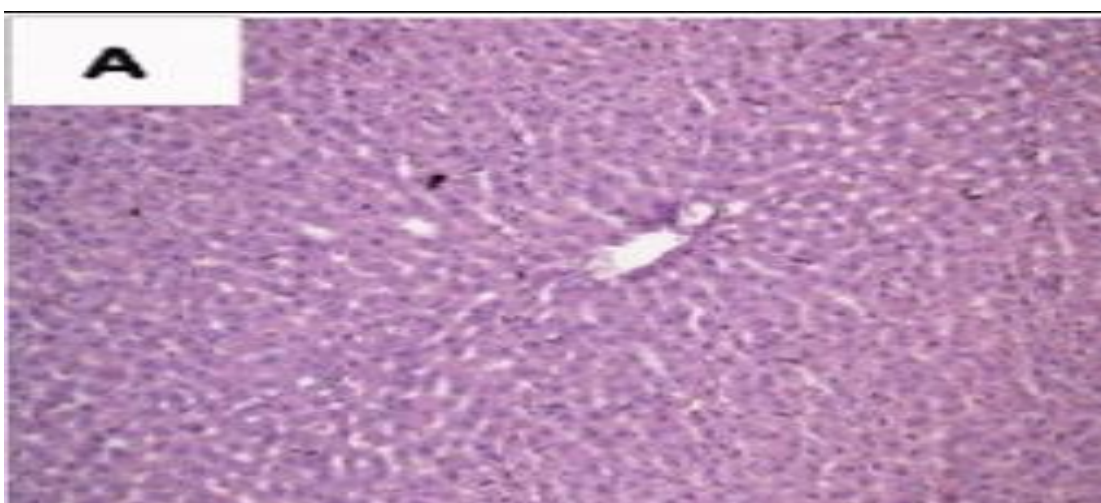


Fig.7. Normal histopathology of rat's tail.

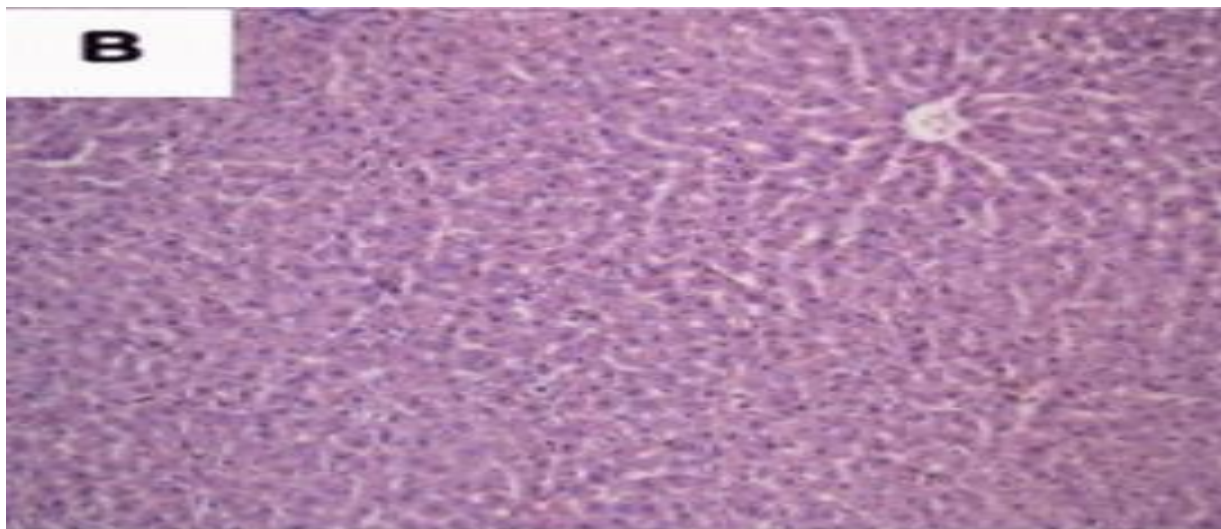


Fig.8.Histopathology of tail with standard drug.

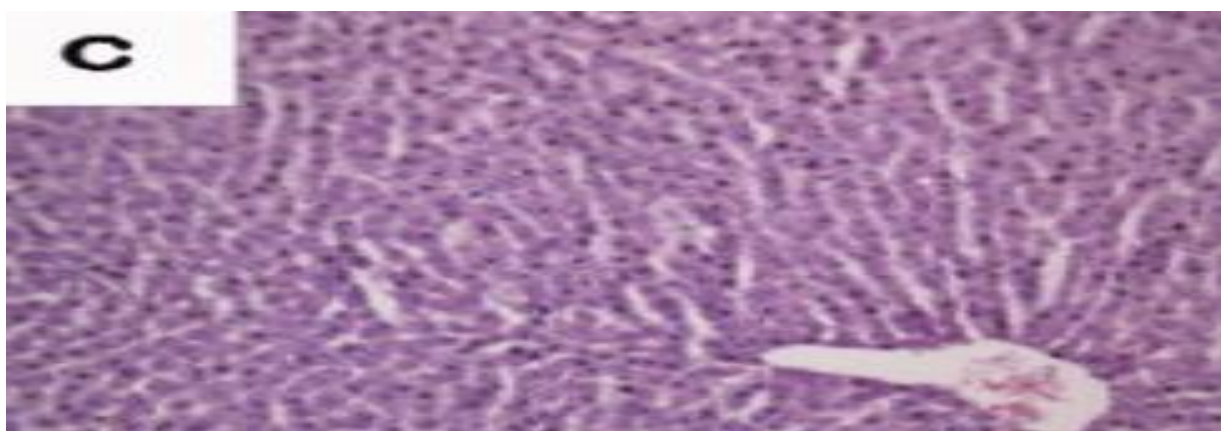


Fig.9. Histopathology of tail with toxic drug.

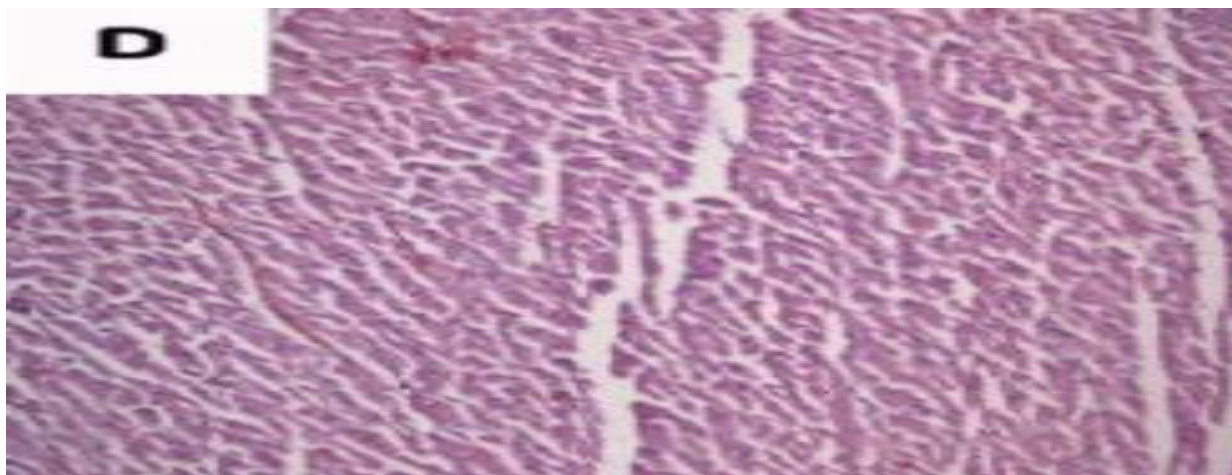


Fig.10. Histopathology of tail when treated with *Hymenaea martiana*.

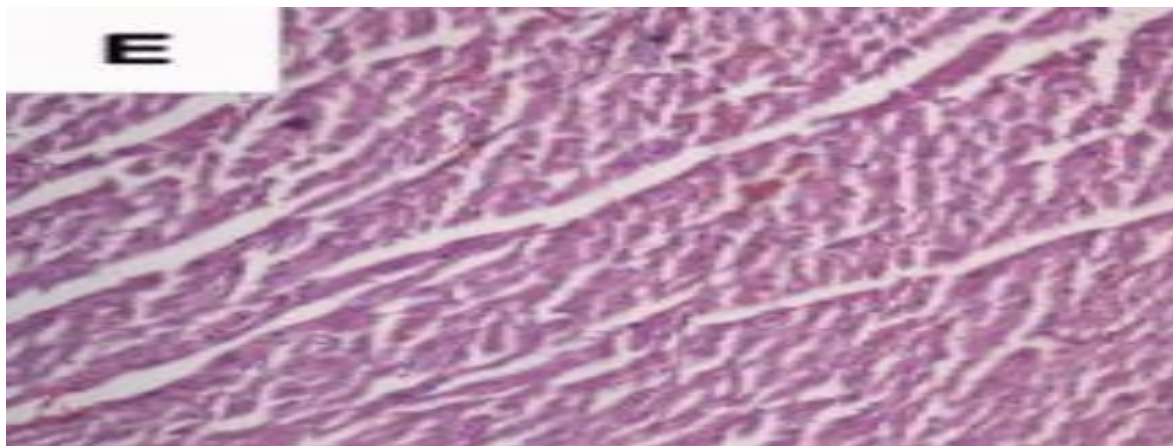


Fig.11. Histopathology of the tail when treated with Gillardia pulchella.

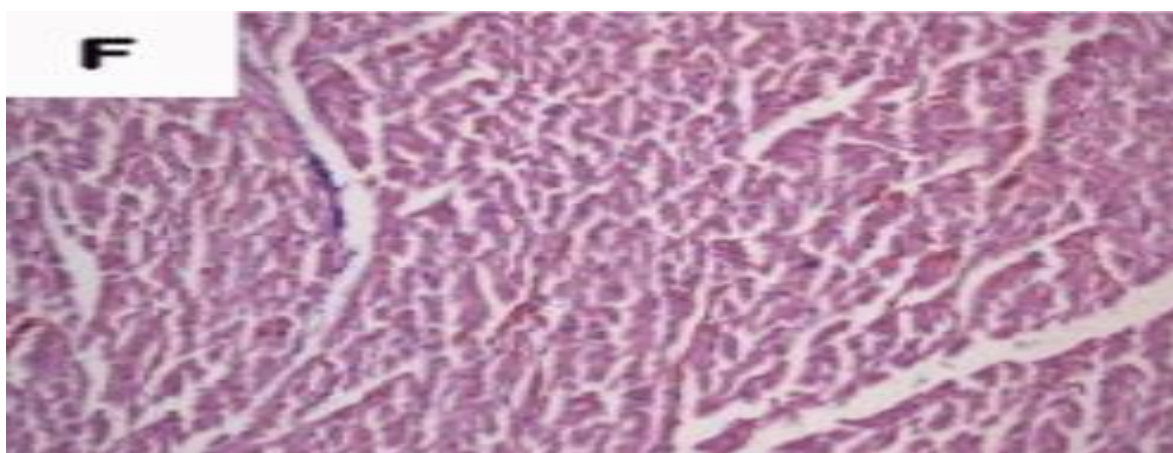


Fig.12. Histopathology of tail when combination of drugs was given.

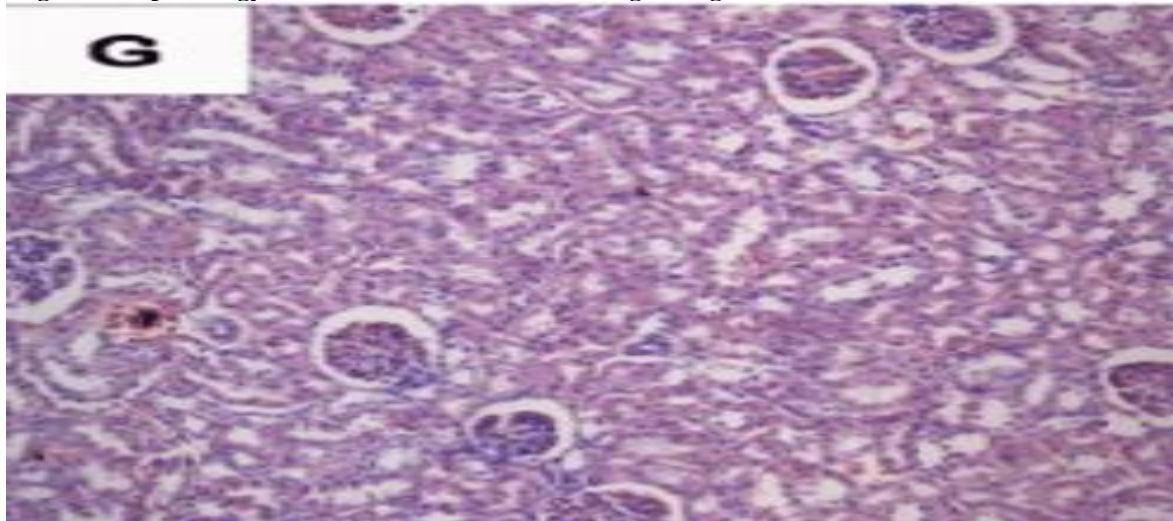


Fig.13. Dropping of details in cellular structure.

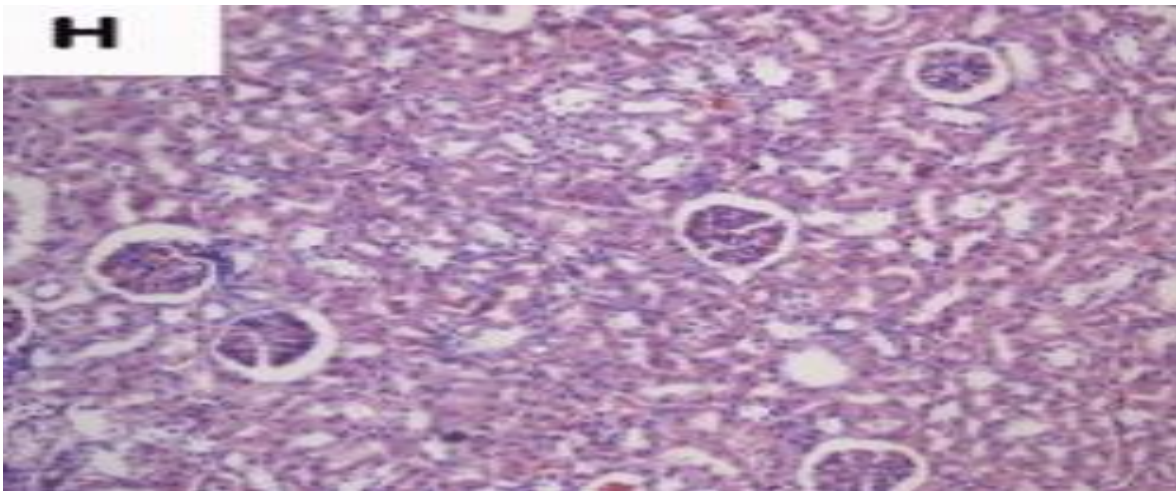


Fig.14. Severe swelling in the tail.

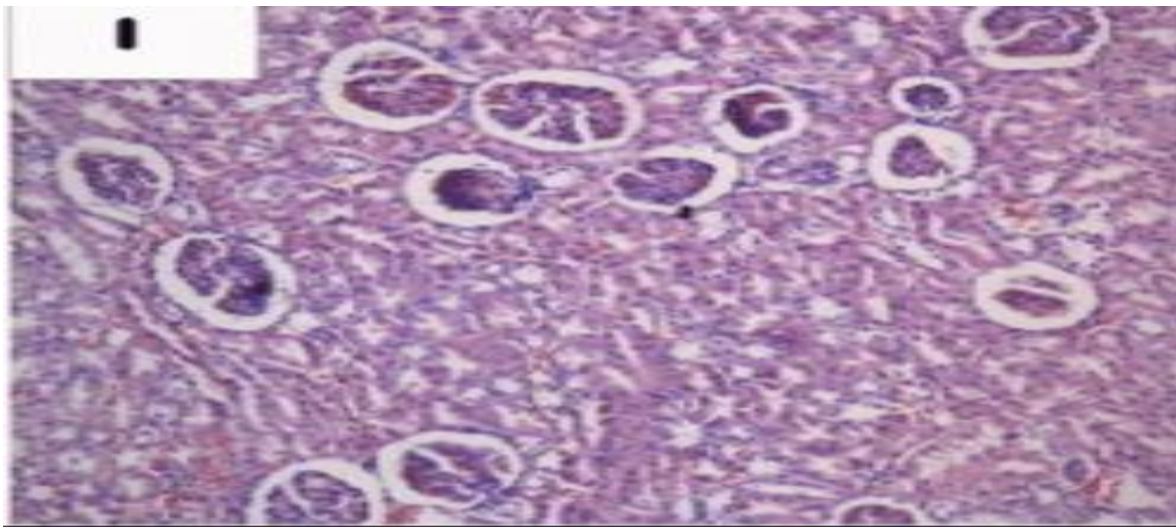


Fig.15. Fat accumulation in the tail.

DISCUSSION:

This demonstration was crised to see the shielding effect of *Hymenaea martiana* and *Gillardia pulchella* on k- Carrageenan induced animal model. This model is very useful in knowing antithrombotic sequel of the drugs who are just at their research stage. To boot, this model is simple and non intraoperative and also the researchers can observe the developing apoplexy in the tails through the naked eye in a time dependant manner.

In this trial, the toxicant used is K-Carrageenan type-I to persuade ictus in the tails of the rats which is given through the subplantar region at 20mg/kg B.W. The sort of carrageenan used in the enquiry is robust and comes from the family of sulphated polysaccharides and hasone sulphate group. The carrageenan was specified to the rats in the right hind paw by first weighing it and dissolving it in saline. After this, red wine coloured thrombus is spotted in the tails which advances with time. Since the thrombus launched in the tails is distinct and is distinguished from the normal border, it is undemanding to observe the time it first occurred, enlarging processs, progressing span and the stretch of thrombus formed can be smoothly visible with the naked eye. In this experiment of ours, the k-carrageenan was reckoned to be very effective since the ictus was accomplished in every single group of the animals under study.

The quality medication utilized for the demonstration was heparin (LMW) at 50 I.U dose for comparing the efficiency of the vegetation.

In this, the ethanolic extricates of both the vegetation were prepared through the maceration process. Then the creatures were split up into 9 batches, each accomodating 6 rats. The prepared extracts were given orally in four doses. At first an amount of 200mg/kg B.W of the plant extricates were given separately, followed by an increased dose of 400mg/kg B.W.of the extract. Then, in the end, a combination of both the extricates were given at 200mg/kg and 400mg/kg B.W after the arrival of the ictus in the tails of the rats following carrageenan injection for about 29 days.

CONCLUSION:

The consequence of the trial shows the extensive antithrombotic efficiency of the vegetation like *Hymenaea martiana* & *Gillardia pulchella*. For the first time we came to know that the meld of these herbs can play a sweeping role in reducing thrombosis. Exact mechanism of the extracts is indistinct. But from the literature, we come to know that flavonoids in *Hymenaea martiana* and n-hexadecanoic acid in *Gillardia pulchella* can be an essential part in thrombolytic activity since they are found to reduce inflammation and ultimately clotting of the gore. Nevertheless, an advanced study is requisite to get the exact mechanism of the plants.

REFERENCES:

- 1) Furie B, Furie B C. Mechanism of thrombus formation. *New England journal of medicine*.359(9):pg 938-949
- 2) Jameson JL. Pulmonary thromboembolism and deep vein thrombosis. In *harrison's manual of medicine* 20th ed. Mc.Graw Hill; April 5,2020
- 3) Rosendaal FR, Rentsma PH. Genetics of venous thrombosis. *J Thromb Haemost*.2009;1:301-4
- 4) Fatemeh Moheimani. Venous thromboembolism: classification, risk factors, Diagnosis and management. *Hindawi*. 17th oct 2011; vol 201
- 5) Aufderheide TP. Peripheral arteriovascular disease. In: Walls RM, Hockberger RS, Gausche-Hill M, eds. *Rosen's Emergency medicine: concepts and clinical practice*.9th ed.Philadelphia, PA:Elsevier;2018:chap 77
- 6) Prentice C.R.M. Pathogenesis of thrombosis. *Karger*. April 24,2009; 20: pg 50-59
- 7) Piero O.Bonetti. Endothelial dysfunction. Free access. 12 Dec 2002; 23: pg 168-175
- 8) Milka koupenona. Thrombosis and platelets: an update. *European heart journal*. 14th march 2017, vol 38, issue 11, pg 785-791
- 9) Stephanie A. Smith. How it all starts: invitation of the clotting cascade. *Critical review in biochemistry and molecular biology*. 28th may 2015; 50(4): pg 326-336
- 10) Amiram Eldor, Eliezer. The hypercoagulable state in thrombosis. *Blood* (2002) 99 (1): pg 36-43.

