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Certified that the synopsis entitled **"PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF ACTIVE CONSTITUENTS OF *TERMINALIA CATAPPA (LINN.)"*** is of the bonafide research work done by Shri Abdul Vahab A , research scholar for PhD programme *In Pharmaceutical Sciences in the faculty of Modern Medicine* under my direct supervision

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INTRODUCTION

Herbs and spices have a traditional history of use, with strong roles in cultural heritage, and in the appreciation of food and its links to health.[1]Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

Since the extracts of medicinal plants are abundant sources of multiple biomolecules and chemical entities, they can act synergistically to produce multiple effects at different sites of action. They can bind specifically to the proteins and enzymes at the binding sites of various receptors. Our aim is thus also to target such phytoconstituents responsible for a specific activity of a medicinal plant.

OBJECTIVE

In the present study, we have chosen a plant *Terminalia catappa (Linn)* which is popular as a medicinal plant. Literature survey of the plant revealed that the fruit and leaf of the plant is reported for the anti diabetic activity, anti-oxidant activity, hepatoprotective activity, anticancer activity, anti-inflammatory activity, analgesic and antimicrobial activity. The bark of *Terminalia arjuna* which is coming under Combretaceae family shows hepatoprotective activity, anti-oxidant activity[2].*Terminalia chebula is* reported to have cytotoxic activity[3]. Keeping this view in the mind, bark extracts of *Terminalia catappa* was selected for the pharmacological and phytochemical screening. There is no report available on the study of *Terminalia catappa Linn* bark extracts in the treatment of diabetes mellitus, hepatotoxicity, inflammation and analgesia. Hence, the present study was undertaken to evaluate the anti-diabetic,[4,5] hepatoprotective, anti-inflammatory and analgesic activity.

DISTRIBUTION

Terminalia catappa (Linn) is seen in warmer part of India and called as Indian almond. This species is globally distributed from Indo-Malaysia to Australia [6].Tropical almond

(*Terminalia catappa*) is a large, spreading tree now distributed throughout the tropics in coastal environments.

METHODOLOGY

PREPARATION OF THE EXTRACT

The bark of *Terminalia catappa* was collected from botanical garden, RIMSR, Puthuppally and washed thoroughly with distilled water and shade dried at room temperature. The dried samples were powdered and extracted by using Soxhlet apparatus [(7)] with alcohol and water. These extracts were subjected to phytochemical screening.

ACUTE TOXICITY STUDY

This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD).All the studies were approved by Internal Animal Ethics Committee no: 001/PHD/UCP/CVR/13dtd 14/5/13.The ethanolic and aqueous extract of *Terminalia catappa* was studied for acute toxicity at dose of 2000 mg/kg i.p. in female albino rats. The extracts were found devoid of mortality of the animals. Hence 2000 mg/kg was considered as LD_{50} cut off value. The screening doses selected for the studies as per OECD guidelines No. 423 (Annexure - 2D) were 1/5th of 2000 mg/kg 400 mg/kg [6].

ALPHA - AMYLASE INHIBITION ASSAY

Diabetes is a lifelong disorder, presently termed as life style diseases because it is markedly affected by day to day variations in diet, exercise, infection and stress. These factors have to be addressed on daily basis while managing diabetes and the patient is the person best equipped to deal with the situation. Hence, a thorough knowledge of the disease and how it alters normal body functions and the awareness of its acute and chronic complications is necessary .It enables the diabetic patient to take better care of him or herself.[7]

In –vitro determination of sugar lowering potential of the extracts were evaluated here. Standard drug used is acarbose.There was a dose dependant increase in inhibition seen in the alpha amylase enzyme. Concentration of 125µg/ml shows 47.86% of inhibition in ethanolic

extract and 43.26% in aqueous extract, whereas at 100µg/ml, the inhibition seen was 77.55% in ethanolic extract and 74.52% in aqueous extract. IC_{50} value of ethanolic extract is at 15µg/ml, aqueous extract shows IC_{50} at 21µg/ml and acarbose shows IC_{50} value at 10µg/ml.

ORAL GLUCOSE TOLERANCE TEST

The study was conducted in normal rats. Overnight fasted albino rats are divided into 4 groups with 6 animals in each group. Glucose (2g/kg) was to given 30 minutes after the administration of extract or standard drug glibenclamide as the case may be. The blood glucose level is estimated at 0, 30, 60, 90 and 120 minutes by using glucometer. The treatment groups, glibenclamide 5mg/kg, ethanolic extract 400mg/kg and aqueous extract 400mg/kg reduced the blood glucose level.

ALLOXAN INDUCED DIABETES

Diabetes was induced in albino rats by using alloxan monohydrate 120 mg/kg at a dose administered intraperitonially. After 72 hours of injection, the blood glucose level was noted on 1st, 7th, 14th and 21st days by using glucometer. The treatment groups, glibenclamide 5mg/kg, ethanolic extract 400 mg/kg and aqueous extract 400 mg/kg reduced the blood glucose level. The body weight is also noted on 1st, 7th, 14th and 21st days.

LIVER DISORDERS CAUSED BY DRUG OR TOXINS

Liver is a vital organ which plays a major role in metabolism and excretion of xenobiotics from the body. Liver cell injury caused by various toxic chemicals like certain antibiotics, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA) etc., excessive alcohol consumption and microbes is well-studied.[10]A vast majority of pharmacological and chemical agents are capable of causing liver injury. Hepatotoxins are of two major groups, namely, direct and indirect hepatotoxins. Direct hepatotoxic agents damage the membrane of hepatocytes directly resulting in interference of the cell metabolism. The indirect hepatotoxins cause hepatic injury as a result of selective interference with metabolic pathways or selective binding to / or alteration of a specific component.

ISONIAZID INDUCED HEPATIC DAMAGE IN RATS

Albino rats of either sex weighing between 150-200 gms were selected and were divided into 6 groups of each containing six animals. Group A (control) rats were fed with standard diet and were administered with 1%CMC (1ml/kg p.o.) once daily for a period of 21 days. In group B (Isoniazid control), rats were treated with isoniazid (54 mg/kg p.o.) once daily for a period of 21 days. Group C rats were treated with standard drug silymarin (50 mg/kg p.o.) and received isoniazid (54 mg/kg p.o.) 1 hour after administration of standard drug once daily for a period of 21days. Group D and E rats were treated with aqueous extract and alcoholic extract at (400 mg/kg p.o.) and received isoniazid (54 mg/kg p.o.) 1 hour after administration of aqueous extract and alcoholic extract once daily for a period of 21 days. At the end of the experiment, rats were sacrificed and blood was collected for the separation of serum, which was subjected to biochemical parameters. This was observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT, ALP and bilirubin when compared to normal control. [10]

ANTI INFLAMMATORY ACTIVITY

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection or physical trauma) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair.

Anti inflammatory activity was carried out by measuring paw edema using plethysmograph. Albino rats of either sex weighing between 140-180gm were used. The treatment protocol was performed using 4 groups of animals with 6 animals in each group. Group I -0.2ml of normal saline by oral route, Group II- diclofenac by oral route, Group III & IV-400mg/kg of aqueous and alcoholic extract by oral route.

Inflammation was induced by injection of carrageenan[11] to the plantar surface of hind paws. Standard drug and extracts were administered orally 30 minutes before the injection of carrageenan.

ANALGESIC ACTIVITY

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Clinically, pain can be considered as superficial, deep non –visceral pain from muscles, joints, ligaments and bones, visceral pain, referred pain, psychogenic pain[12].

In this study the rats were subjected to noxious stimuli by placing the tip of the tail on radiant heat source by using radiant analgesiometer. [(13)] Treatment protocol consisted of 4 groups of animals with 6 animals in each group. Group I – received normal saline, Group II – received diclofenac sodium orally and served as reference**,** Group III & IV - received aqueous and ethanolic extracts.

.In the present study, aqueous and alcoholic extracts of the bark of *Terminalia catappa* shows anti-diabetic [(8, 9)], hepatoprotective, anti-inflammatory and analgesic activity [(13)] which was found to be comparable to that of *Terminalia arjuna*.

DOCKING STUDIES

The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site. In general, there are two aims of docking studies: accurate structural modeling and correct prediction of activity. However, the identification of molecular features that are responsible for specific biological recognition, or the prediction of compound modifications that improve potency, are much more focused on capturing energetic than entropic effects**.**[14]

Glide scores of designed derivatives were arrived at from docking with prostaglandin receptor with PDB ID IRY8, with PPR-γ receptor with PDB ID-IZEO, with α-amylase receptor with PDB ID-4GQR, with glutathione peroxidase receptor with PDB ID-2F8A. Images were taken for ligand interactions of compounds with highest glide score docked to the receptors and standard drugs. ADME profile of the analogues was conducted using Qikprop.

LC-MS and heavy metal limit studies were conducted as per standard procedures laid down.

DISCUSSION

- The aqueous and alcoholic extract of *Terminalia catappa* showed anti diabetic, hepatoprotective activity, anti-inflammatory, analgesic activity.
- The prepared extracts were subjected to preliminary phytochemical studies. The aqueous and ethanolic extract revealed that the presence of tannins/phenols, carbohydrates, flavanoids, saponins.
- .In the present study, aqueous and alcoholic extracts of the bark of *Terminalia catappa* shows anti-diabetic, hepatoprotective, anti-inflammatory and analgesic activity which is comparable to that of *Terminalia arjuna* and *Terminalia chebula.*
- The fruit and leaf extract of *Terminalia catappa* has been reported to show anti-diabetic[8, 9,] analgesic and anti-oxidant activities[14].
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- Present study showed that EETC and AETC produce good α amylase enzyme inhibitory activity suggests a potential use in the management of diabetes.
- In OGTT the EETC 400mg/kg, AETC 400mg/kg dose showed promising results. This investigation showed that both extract shows glucose lowering effect mediated by an enhanced insulin secretion.
- Administration of EETC 400 mg/kg, AETC 400 mg/kg dose to alloxan induced diabetic rats showed a reduction in blood glucose levels at a high level of significance($p<0.001$). The extract showed hypoglycemic activity which may be due to insulin release from available β cells or its release from the bound form.
- The treated group with the EETC 400 mg/kg, AETC 400 mg/kg dose showed a significant increase in body weight as compared to the diabetic control group ($p< 0.001$). It may be due to its protective effect on controlling the glucose levels and improvement in the insulin secretion.
- Alloxan induced rats showed an increase in TC, TG, LDL, VLDL levels.HDL levels are decreased. The treated group showed an increased level of HDL, and reduced VLDL, TC, and TG values in a significant manner. This reduced VLDL, TC, and TG may be

indicating a lowering of insulin resistance, there by inhibiting hormone sensitive lipase responsible for FFAs

- The histopathological investigations showed a supportive evidence for this study. It showed an increase in presence of number of β cells and increased vasculation in Alloxan destructed pancreas probably due to the presence of stable cells. The extract treatment may also mobilize the progenitor cells into the cell repairing mechanism. These supportive evidences suggest that the plant possess good antidiabetic activity.

- Liver participates in a variety of metabolic activities perhaps by virtue of activity of number of enzymes and thus may be self exposed to too many toxicants, chemicals and drugs which could injure it.

- In present study isoniazid is used as hepatotoxicant to induce liver damage. The animals treated with isoniazid had markedly elevated biochemical parameters bilirubin, SGPT, SGOT and ALP when compared to normal control. Pre-treatment with silymarin, ethanolic extract and aqueous extract had showed good protection against isoniazid induced toxicity to liver.

- The administration of EETC 400 mg/kg, AETC 400 mg/kg dose to carrageenan induced paw edema rats showed a reduction in inflammation. The extract showed good anti-inflammatory activity too.
- The AETC 400 mg/kg and EETC 400mg/kg dose were administered to rats and subjected to noxious stimuli (pain) by using analgesiometer. The extracts showed analgesic activity.
- In the present study, aqueous and alcoholic extracts of the bark of *Terminalia catappa* shows anti-diabetic, hepatoprotective, anti-inflammatory and analgesic activity which is comparable to that of *Terminalia arjuna* and *Terminalia chebula.*
- Docking studies were carried out against various targets like prostaglandin receptor, PPR-γ receptor, α-amylase receptor and glutathione peroxidise receptor using Schrodinger Soft ware. Of the 5 compounds docked, leucocyanidin, catechin and ellagic acid showed highest glide scores.
- The compound leucocyanidin was screened for its analgesic and anti-inflammatory, ellagic acid for its anti-diabetic and gallic acid for its hepatoprotective activity using Osiris Property Explorer and PASS online Software.

- Heavy metals are being spoken out very widely in the global scenario, due to the recent episodes of a few Indian Ayurvedic formulations which have been found to have heavy metals more than that of the permissible level as advised by W.H.O. and F.A.O. of U.S.A. To ensure the safety of drug on long term consumption, studies were conducted to determine the levels of toxic elements like Cadmium (Cd), Lead (Pb), Mercury (Hg), and Arsenic (As). The studies indicated that these heavy metals were well within the limits thereby ensuring the safety of the study.
- LC-MS studies were conducted to confirm the presence of active molecules in the EETC.This lead us to the belief that these molecules act on the target receptors to produce the desired pharmacological effects.

SUMMARY AND CONCLUSION

- The present studies showed that *Terminalia catappa* bark extract EETC and AETC possess significant antidiabetic, hepatoprotective, anti-inflammatory and analgesic activity in albino rats.
- The presence of phytochemical constituents like flavanoids and tannins are supportive of these pharmacological activities depicted by the plant.
- Multiple chemical entities present in the extracts act synergistically to produce multiple effects at different sites of action.
- These Biomolecules bind specifically to the proteins and enzymes at the binding sites of various receptors to produce the biological effect shown.
- The specific binding of various phytochemical constituents to the specific receptor proteins responsible for a particular set of pharmacological activities shed light on probable mechanism of action too.
- This way we could target the phytoconstituents responsible for a specific activity of a medicinal plant.