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EVALUATION OF ANTIINFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *MANILKARA ZAPOTA* SEEDS IN RODENTS

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ABSTRACT

Manilkara zapota a naturally occuring seasonal plant belonging to the family sapotaceae. The fruits and seeds of this plant are very nutritious and they also have several medicinal properties. It is widely used as the immunity booster and prevent inflammation. It belongs to the nativity of the southern Mexico and also across Central America. The present study explains the anti inflammatory activity of ethanolic extract of the seeds of *Manilkara zapota* . The extract prepared was administered orally for 2 hours with 30 min interval in two doses (100 mg/kg and 200 mg/ kg). As the dose increases the extract successfully decreased the inflammation caused by the egg albumin (1 ml/kg). Anti inflammatory activity was examined by using the digital plethysmometer. The anti inflammatory activity is mainly due to presence of flavonoids These findings justify that traditional use of Manilkara zapota seeds can be beneficial to mankind.

Keywords: *Manilkara zapota* L, Sapotaceae, Anti-inflammatory, Flavonoid

INTRODUCTION

Inflammation is a natural immune response produced by body against several factors or agents which include pathogens, toxic substances and damaged cells. Due to these factors inflammation may be acute or chronic response which can lead to tissue damage **[1]**. The major signs of inflammation include redness, swelling,

heat, pain and disturbance of function. The former four signs can be seen only in acute inflammation but the latter can be observed in all types of inflammation **[2]**. Inflammation can also be defined as a protection given by our body's white blood cells and the substances produced by them against different types of infection caused by vast number of microorganisms. This inflammation is caused by the production of the prostaglandins which are produced by the COX-I and COX-II enzymes **[3]**.

However, in other conditions, such as arthritis, our body's immune system causes inflammation even if there are no external intruders to repel. Our immune system identifies our cells as invaders in certain autoimmune illnesses, attacking healthy tissues by assuming them as abnormal or contaminated.

Acute or chronic inflammation can be either transient or persistent. Inflammation that is acute disappears in a few days or hours. Even after the initial trigger has subsided, chronic inflammation can persist for months or even years. Chronic inflammation is related to the following conditions:

- Cancer
- Heart problems
- Diabetes
- Asthma
- Alzheimer's disease

The causes of inflammation include **[4]**

- Exogenous:
    1. Physical agents
    2. Biological agents
    3. Chemical agents
- Endogenous
    1. Enzyme activation
    2. Metabolic pr oducts
    3. Circulation disorders

The symptoms of inflammation include:

- Redness
- a swollen joint maybe warm-to-the-touch
- aching joints
- Joint rigidity
- a joint that isn't functioning optimally
- Frequently, you'll experience just a couple of these symptoms.

Additionally, inflammation can result in flu-like symptoms like:

- Fever Chills
- exhaustion / lack of energy
- Headaches
- reduced appetite
- muscle rigidity

The natural methods of treatment which include the materials that are obtained from the mother nature are generally preferred in the modern-day society where the adverse effects are very less in chance. So, the whole world again turned back to the olden ages where the people are dependent on the plants and animal sources.

Inflammation is the condition or the method initiated by our own immune system to act against the foreign particles entering the human body. As mentioned above the symptoms of the inflammation these may lead to the severe effects and may also lead to death sometimes.

Now a days anti-inflammatory agents are recognized as frequently used agents. They are available in both prescription and over the counter drugs as a non-steroidal medication that are recommended in a typical neurosurgical practice. Nonsteroidal anti-inflammatory drugs (NSAIDS) or Steroidal anti-inflammatory agents (SAIDS) are used to treat inflammation. Development of safe and effective drugs for anti-inflammatory therapy is necessary. Because, prolong use of the NSAIDS shows some adverse effects in the body such as Hepatotoxicity, Bleeding in gastrointestinal tract, Obesity, Sodium retention and Osteoporosis, these result in severe health problems **[5]**.

So, traditional medicines and natural products can act as great alternative to NSAIDS which show most of the side effects that result in severe health problems. These natural products offer a great a hope in identifying a leading group and helps in development of anti inflammatory drugs that treat inflammation. Anti-inflammatory activity is one of the most reported effects among the different biological activities that are published in the recent years **[6]**.

Manilkara zapota L. is most commonly known as sapota and chiku belongs to the family sapotaceae which is frequently used as immune booster and also in case of inflammation **[7]**. It is indigenous to the southern Mexico and also across Central America **[8]**. Different parts of the plant commonly treat coughs and colds and possess diuretic, antidiarrheal, antibiotic, antihyperglycemic, and hypocholesterolemic effects **[9]**.

MATERIALS AND METHODS

Plant material

Fresh fruits of *Manilkara zapota* are purchased from the local shops and street vendors in Guntur belonging to the state of Andhra Pradesh. Then the seeds are separated from the fruits and they are taxonomically identified by Dr. P. Satyanarayana Raju garu M.Sc., M.Phil., Ph.D from the Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, AP on 17thoctober 2022.

These freshly collected seeds are then made into smaller particles and then dried in the sunshade. They are allowed to dry for about 7 days and then milled into coarse powder with the help of mechanical grinder and then seived with 40 mesh sieve to obtain fine uniform powder and preserved in an indisputable container.

Preparation of extract [10]

Soxhlation is the method employed in the extraction of *Manilkara zapota* Linn (EEMZ), at room temperature and ethanol as the solvent. The dried powder of the seeds of *Manilkara zapota* Linn. (400g) was extracted with 50% ethanol (EtOH). The obtained extract is first filtered and then dried in aseptic hood at room temperature to make it concentrated. The extract obtained is treated with petroleum ether for the separation of the lipid layer and and defattying is done by using the seperating funnel. Thus obtained extract is used for further studies.

Animals

Wistar rats of either sex were used for this experiment. Animals were brought from the animal house of Chalapathi Institute of Pharmaceutical Sciences. Wistar rats weighing 150-250g were opted for this study. These animals have their freedom for the food and water and they were encased in natural light dark cycle (12h each). The wheat flour kneaded with minute amount of water and also refined oil from vegetables is the food fed to the animals. These rats are accustomed for atleast 4 to 5 days to the laboratory conditions in advance to the studies performed. The procedure designed to carry out the experiment was approved by the Institutional Animal Ethics Committee, and the laboratory animals were observed and soothed as per the guidance of CPCSEA, Ministry of Forests and Environment (Proposal number 18/IAEC/CLPT/CPCSEA).

Preliminary phytochemical screening [11]

Some chemical tests are performed to observe that different phytochemical constituents exist or not in the EEMZ, viz., alkaloid (Mayer's test), flavanoids (Ferric chloride test), phenols (Lead acetate test), Glycosides and sterols (Salkowski test), saponins (Frothing test), proteins and amino acids (Ninhydrin test) as per the norms of the standard methods.

DRUGS

The drugs that are used for the study are Diclofenac sodium (Dr.Reddy's laboratories, Uttarakhand).

VEHICLE

Plant extract (EEMZ) was dissolved in water and given to the rats through oral route. Diclofenac sodium is solubulised in water and injected i.p. Volume of oral administration and i.p. injection was 1 ml/kg of rats.

Drug treatment

In the studies that are performed, the rats were divided into 5 different batches for evaluating of the anti-inflammatory. Every group consists of 5 animals. Group I is considered as control and is administered only with saline. Group II is named as negative control which is treated with inducing agent egg albumin. Group III is

represented as standard which is treated with egg albumin and standard drug Diclofenac sodium in water. Group IV and V are named as test I and test II where the rats are administered with the egg albumin and also the test extract EEMZ of doses 100mg/kg and 200mg/kg.

A day before the conduction of the study the rats are fasted. The very next day the rats are treated with the egg albumin for inducing the inflammation except the group that is considered as the control and the control group is treated with saline. After a time period of 1hour the group III is treated with the standard drug that is Diclofenac sodium (10 mg /kg). Group IV and V are treated with EEMZ at a dose of 100mg/kg and 200mg/kg. The paw volume of the rats is considered as the parameter for the measuring of inflammation. Paw volume is measured at a regular time intervals of 0 min, 30 min, 60 min, 90 min, 120 min by using the digital plethysmometer. The percentage inhibition of paw volume is measured. The results that are obtained are statistically analysed by the two way ANOVA.

DIGITAL PLETHYSMOMETER [12]

The paw volume was calculated using the Digital plethysmometer. It comprises of a water cell mounted on a clear acrylic stand. This water cell has an exit with a stopcock and an entrance where the paw is dipped. When a paw is dipped into the water cell, an electronic display that is attached to the water cell displays the amount of water that is expended, expressed in millilitres, and taken as the volume of the paw. The animal's paw is placed inside a specific water cell that contains water and changes resistance when the paw is submerged in it. This resistance change has a precision of 0.1 ml, and it is calibrated in ml and presented on the readout in ml.

Figure 1: DIGITAL PLETHYSMOMETER

RESULTS

Preliminary phytochemical screening

The studies show that the extract contains the phytochemical constituents such as alkaloids, glycosides, flavonoids, phenols by performing them using the standard methods.

Digital plethysmometer

Measuring the paw volume (using digital plethysmometer)

Paw volume of the rats are measured which determines inflammation caused in them. EEMZ (100mg/kg and 200mg/kg) that is administered orally have exhibited its activity on inflammation caused by egg albumin on the mark with that of the standard diclofenac sodium(100mg/kg). The inflammation caused by the egg albumin(1mg/kg) have elevated the paw volume which was significantly decreased in case of the standard Diclofenac sodium(10mg/kg) and EEMZ (200mg/kg p.o.). ($p<0.0001$) indicates the decrease in the paw volume which preferentially lowered the inflammation induced by the egg albumin (1ml/kg i.p.).

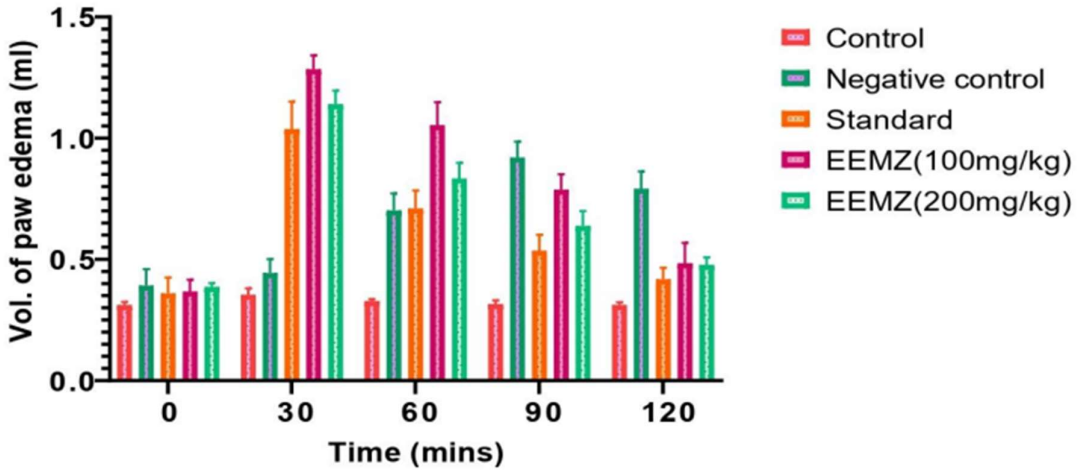

Figure 2: Data represent Mean ± SEM, (n=5). Two way ANOVA followed by Tukey's multiple comparisons test. Significance was shown at P<0.0001 when compared EEMZ (100mg/kg) and EEMZ (100 mg/kg) with Standard. ****Denotes P<0.0001 which is compared with Standard of rats. (ns) denotes non significant figures when compared with standard

DISCUSSION

EEMZ have a prominent effect at dose of 200 mg/kg which is very near to the standard Diclofenac sodium 10 mg/kg which is brought about by the flavonoids present in the sample. This study indicates that EEMZ possess the anti inflammatory activity equal with the levels of standard which is very helpful to the human race. Digital plethysmometer is the standard method for the evaluation of inflammation.

CONCLUSION

The ethanolic extract of *Manilkara zapota* seeds have exhibited the remarkable anti inflammatory activity which is examined by

the help of Digital plethysmometer. The further explorations of EEMZ can disclose the mechanism involved in this anti inflammatory activity.

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