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PHYTOCHEMICAL ANALYSIS AND ANTIPYRETIC ACTIVITY OF EXTRACT OF THE AEGLE MARMELOS LEAVES ON DIFFERENT MODELS

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ABSTRACT

The current treatment for fever is NSAIDs. However, NSAIDs cause damage to the liver and kidneys, inhibit COX-1, which affects the digestive system, and increase the risk of blood clots. As a result, efforts are being made to develop powerful antipyretic drugs from medicinal herbal plants. *Aegle marmelos* (Bael) is a popular medicinal plant in the Ayurveda and Siddha systems of medicine and folk medicines used to treat various disease and disorders including fever. The present study was undertaken to evaluate the antipyretic property of *Aegle marmelos* (L.) Correa leaves (Family: Rutaceae) on different induced pyrexia models in albino rats. The yeast induced pyrexia method was standardized first by injecting 12.5% yeast suspension (s.c) followed by recording the rectal temperature at regular intervals. It reveals that the ethanolic extract, at dose of 200 mg kg-1 body wt. and 400 mg kg-1 body weight, produced significant ($p<0.001$) reduction in elevated body temperature in a dose dependent manner. Followed by TAB vaccine induced pyrexia in rats. The antipyretic effect of extracts was comparable to that of Paracetamol (100 mg kg-1 body weight, p.o.), a standard antipyretic agent.

KEYWORDS: *Aegle marmelos*, Brewer's yeast, Paracetamol, Pyrexia, TAB vaccine.

INTRODUCTION

Plants are one of the important sources of medicine. The application of plants as medicine dates back to prehistoric period. Several indigenous drugs used in modern medicine have figured in ancient manuscripts such as Rigved, the Bible, and Quran. Over six thousand years ago, the ancient Chinese were the first to use the natural vegetation as medicine. Due to their affordability, accessibility, and relative lack of toxicity, plant-derived products are gradually becoming a viable substitute. Interactions between various ingredients occur, either increasing activity or decreasing the chance of negative consequences. This is one of the fundamental principles supporting herbal medicine. When substances work together to create an effect bigger than the total of their separate contributions, these interactions can be additive or really synergistic.[1]

"Health for all" is a dream and a goal to share and work for humanity. Unfortunately, there is no doubt that modern medicines are not suitable for the majority of the population in the future. As a result, other sources of knowledge have been used to gain overall health benefits. As a rule, alternative medicines and traditional medicines from plants are important, but they are not sufficient for use in diseases.[2]

Fever is an important and notable sign and symptom of many diseases that are caused in the human body. They are the result of any inflammatory condition, infection or any other disease occurs due to any external agents. When a person's body temperature rises above the normal range (36.5–37.5°C) due to an infection, tissue damage, malignancy, transplant rejection, or other inflammatory disease conditions, they are said to have a fever.1 Up to 75% of extremely sick patients experience fever or pyrexia.[3]

The pyrogenic exogenous and endogenous substances are necessary for the initiation, manifestation, and management of the febrile reaction. Exogenous pyrogens, including interleukin-1b, enhance the creation and release of internal pyrogens, which causes it (IL-1b). On the other hand, endogenous pyrogens relocate to the hypothalamic organum vasculosum of the lamina terminalis (OVLT), where they induce the production of prostaglandins E2 (PGE2). Due to this, the thermostatic set point rises, which results in a feverish reaction. White blood cells extravasate into inflamed areas as a result of the inflammatory reaction brought on by microbial tissue invasion, which also activates local vascular endothelial cells

and leukocytes. Activated leukocytes all produce pyrogenic cytokines, including IL-1b, Tumor necrosis factor (TNF), and IL-6. Endogenous pyrogens go through the bloodstream to the brain, where they induce the inducible cyclooxginous-2 (COX2) enzyme to increase the formation of PGE2 by vascular endothelial cells.[4]

E-prostanoid receptors are found in neurons in the pre-optic area of the anterior hypothalamus (POAH), the brain site of the primary thermoregulatory controller that compares and integrates central and peripheral thermal information. PGE2 then works by binding to the type 3 PGE2 receptor on glial cells, resulting in the production and release of cyclic adenosine monophosphate (cAMP) which acts as a neurotransmitter, activating thermo sensitive neurons to raise the thermostatic set point from normothermic to fever levels, signaling efferent nerves, particularly sympathetic fibers that innervate peripheral blood vessels.[5] It occurs in 19 to 30% of pediatric emergency visits, which creates tension among parents. Many medications used to treat fevers, such as NSAIDs, have a number of negative side effects, including ulcers, stomach perforations, bleedings in the stomach, and blockages. Non-steroidal anti-inflammatory drugs (NSAID) are used worldwide to treat inflammation, pain, and fever. However, they often produce significant side effects and are toxic to various organs of the body, causing problems such as kidney failure, allergic reactions, reduced auditory ability, and increased risk of hemorrhage due to interference with platelet function. Therefore, the development of novel compounds with analgesic and antipyretic activities without side effects are needed.

As a result, research into the discovery of new antipyretic agents that are safe, more effective, and less expensive, particularly from natural products, is encouraged. A large portion of the global healthcare industry is comprised of medicinal plants and herbal treatments. At least 80% of people now rely on herbal remedies and dietary supplements in some capacity for their basic healthcare, a dramatic growth in usage over the previous three decades.

Handling of human illnesses with traditional medicinal plants has been an integral part of traditional medicine for centuries nationwide. Significantly, herbal medicines play a valuable role in developed, as well as developing, countries in improving primary healthcare for the reason that they have effective biological and medicinal properties with easy accessibility and low costs.[6] Ethiopia is endowed with leftovers of traditional medicinal plants with wide diversity of active secondary phytoconstituents that are used for treating a variety of human ailments including fever, inflammation, and oxidative stress.[5, 6] *Aegle marmelos* (L.) Corr.,

(Rutaceae) is among the widely used traditional medicinal plants in Ethiopian folk medicine which is frequently testified for its potential on antipyretic potentials by traditional medicine practitioners in different parts of Ethiopia. The Bael tree is considered as a sacred tree by the Hindus. They offer its leaves to Lord Shiva during worship. The plant, popularly known as the Bael tree. Flavonoids, glycosides, tannins, alkaloids, triterpenes, steroids, and saponins are identified as phytoconstituents in A. marmelos extract.

Plant profile[7]

Taxonomical classification

Kingdom - Plantae

Subkingdom - Tracheobionta

Super division - Spermatophyta

Division - Magnoliophyta

Class - Magnoliopsida

Subclass - Rosidae

Order - Sapindales

Family - Rutaceae

Genus - Aegle

Species - *Aegle marmelos*

Figure no. 1: *Aegle marmelos* tree with leaves.

Vernacular names of *Aegle marmelos* L.

English - Wood/Stone apple, Bengal Quince, Indian Quince

French - Oranger du Malabar

Indonesian - Mojo tree

Latin - *Aegle marmelos*

Marathi - Kaveeth

Nepali - Bel, Gudu

Sanskrit - Shreephal, Bilva, Bilwa

Tamil - Vilva Maram, Vilva Pazham

Telugu - Maredu

MATERIALS AND METHODS

The aim of the present study is to evaluate the antipyretic activity of *Aegles marmellose* leaves ethanol extract in Brewer's yeast induces pyrexia in Albino mice.

Materials and Instruments- A rotary evaporator (Yamato, Japan), electronic balance (yamto), syringes with needles, and feeding tube were used with their respective function.

Drugs and Chemicals- Normal saline (H. R., Leuven, Belgium), distilled water, absolute methanol absolute ethanol (Indenta Chemicals, India), brewer's yeast (Titan Biotech Ltd., India), TAB vaccine and Paracetamol, obtained from the respective vendors, were used in the experiment.

Swiss albino mice (18-25 g) were obtained from the stock in breed colony. All chemicals with analytical grade were purchased from Merck, i.e. ferric chloride, hydrochloric acid, sodium hydroxide, sodium acetate, n-hexane, methanol, chloroform, sulfuric acid, glacial acetic acid, ethanol, ether, and Mayer, Dragendorff, and Bouchardat reagent.[8]

Collection- Leaves of *Aegle marmelos* were collected from Vangapally village around Swami Vivekananda institute of pharmaceutical sciences, Yadadri Bhongiri in the month of June 2023.

Shade drying- Leaves of *Aegels marmelose* were shade dried to avoid evaporation of volatile of active constituents.

Preparation of plants extract

The plant extract was prepared by cold maceration method, one part of plant material was soaked in nine parts of solvent.[8] Absolute Ethanol and absolute methanol (30%) were used as solvent. Plant powder (100 g) was soaked in solvent (900mL). The plant material was soaked for 7 days and shake vigorously for 10min twice daily. The flask was placed in

laboratory on room temperature (20°C). At the end, filtration of soaked plant material was done using numerous layers of muslin cloth for coarse filtration. Whatman #3 filter paper was used for filtration of the coarse filtrate. Rotary evaporator was used for evaporation of solvent under reduced pressure, and alcoholic and methanolic extract were placed in bottle on (20°C) temperature.[9]

Phytochemical screening

The chemical constituents of the Ethanolicextract of *Aegle marmelos* leaves (L.) Correa (EACL) and methanolic extract of *Aegle marmelos* (L.) Correa leaves (MACL) were identified by qualitative analysis and confirmed by thin layer chromatography for the presence of flavonoids, tannins, steroids and saponins.

Acute pharmacological studies

The acute pharmacological studies are carried out at Swami Vivekananda Institute of Pharmaceutical Sciences (Reg. No. 1983/PO/Re/S/17/CPCSEA) the form B was approved by IAEC members for the animal species, and further parameters of the study has been evaluated at college as per OECD guidelines 423 for 14 days. The dose for *Aegle marmelos* was found to be 300mg/kg.[10]

Acute toxicity studies

Acute oral toxicity studies was performed as per OECD-423 guidelines (acute toxic class method), with Ethanolic extract of *Aegle Marmelos L* using albino mice (n=6) of either sex, selected by random sampling for acute toxicity study.[11]

Pharmacological investigation

Anti-pyretic activity: The estimation of anti-pyretic efficacy of Ethanolic extract was carried out using Brewer's yeast and TAB vaccine induced pyrexia methods.

Brewer's yeast induced pyrexia

Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of brewer's yeast in normal saline. Only animals whose rectal increased by at least 1.0º C after subcutaneously injecting 10.0 ml/kg of Brewer’s yeast.[12]

TAB vaccine-induced pyrexia

This was studied in mice. The animals were maintained in the laboratory for 24 h prior to the experiment. The antipyretic activity was assessed by the method of TAB (Typhoid) vaccine-induced pyrexia with some modification.

The animals whose rectal temperature increased by at least 1.0ºC after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible tail thermostat probe coated with lubricant, and temperature was recorded using a digital telethermometer. The experimental animals were randomly divided into four groups containing six animals in each group. The control group (I) was orally administered 0.5ml saline while the standard group (II) was given 150 mg/kg Paracetamol and groups III and IV were prescribed dose I and II of methanol extract of test drugs, respectively.[13]

Group I: Normal Control (CMC)

Group II: Paracetamol (150mg/kg)

Group III: Test Drug I (Ethanolic extract leaf *Aegle marmelos*)

Group IV: Test Drug II (Ethanolic extract leaf *Aegle marmelos*)

The rectal temperature was recorded at time intervals of 1, 2, 3and 4 h after drug administration. Animals are rehabilitated with standards anti-pyretic drugs.

The antipyretic activity was assessed by the method of TAB (Typhoid) vaccine-induced pyrexia with some modification. In this method the mice were divided into groups, each group consisted of six animals. The control group was treated with 2 ml/kg of saline. The normal rectal temperature of a group of mice was recorded by a telethermometer at hourly intervals for a period of 4 h. TAB vaccine was administered intravenously into the mice at a dose of 0.5 ml/mice. C. magna was administered orally in doses of 100 and 200 mg/kg, 60 min after TAB vaccine when there was significant pyrexia. Subsequently, the rectal temperature was recorded every 30 min up to 3 h. Paracetamol 100 mg/kg (orally) was used for comparison.[14-16]

Statistical analysis: The significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's multiple comparison tests using Graphat Instat Software (San Diego, CA, USA) $p < 0.05$

accepted as significant. The experimental results are represented as mean ± SEM (standard error mean). Student's t-test was used.[17-18]

RESULTS AND DISCUSSION

Preliminary phytochemical

The early phytochemical analysis of the ethanolic extracts suggests that alkaloids, carbohydrates, glycosides, phytosterol, saponins, tannins, proteins, flavonoids, essential oils, vitamins and diterpenes are among the phytoconstituents present.

Acute toxicity studies In the acute toxicity studies the extracts of METC did not showed any toxic symptoms or mortality up to the dose level of 5000mg/kg, body weight in rats, and hence the extracts was considered to be safe and non toxic for further pharmacological screening.

Table no. 1: Acute toxicity studies of *aegle marmelos* leaves.

Sl. no	Treatment	Signs of Toxicity	Onset of toxicity	Weight variation	Duration of observation
1	EEAM (2000mg/kg)	Observed	After 20 hrs	5g	14 days
2	EEAM (5000mg/kg)	Observed	After 20 hrs	5g	14 days
3	EEAM (2000mg/kg)	Observed	After 20 hrs	5-10g	Till animals are alive

Antipyretic activity

Brewer's Yeast Induced Pyrexia in Rats. The anti-pyretic activity of Ethanolic leaves extract of *Aegle marmellos* against yeast induced pyrexia is shown in Table 2. The ethanolic leaves extract *Aegle marmellos* at a doses of 200 and 400 mg/kg showed significant effect against Brewer's yeast induced pyrexia method. There was a progressive dose dependent reduction in the temperature of rats treated with the extract. The reduction caused by the extract was significant when compared to control.

Table no. 2: Anti-Pyretic Activity of Ethanolic Extract of *Aegle marmelos* on Brewer's Yeast Induced Pyrexia in Rats.

Treatment	Rectal temperature (°C)				
	18 h after yeast administration	Temperature after treatment			
		1h	2h	3h	4h
Group-I Control	38.1±0.1	38.4±0.2	38.0±0.1	38.3±0.2	38.2±0.1

Group-II Negative control	40.2±0.1	40.4±0.3	40.1±0.2	39.8±0.1	39.1±0.3
Group-III Paracetamol	39.8±0.2	37.9±0.2	38.2±0.2	38.0±0.1	38.4±0.2
Group-IV (200mg/kg)	40.2±0.1	39.4±0.3	39.1±0.1	39.0±0.2	38.9±0.3
Group-V (400mg/kg)	40.1±0.1	38.8±0.1	38.5±0.2	38.4±0.1	38.5±0.2

Values were mean ± SEM, (n=6), **P<0.01 vs control. Data were analyzed by using One-way ANOVA followed by Dennett's test.

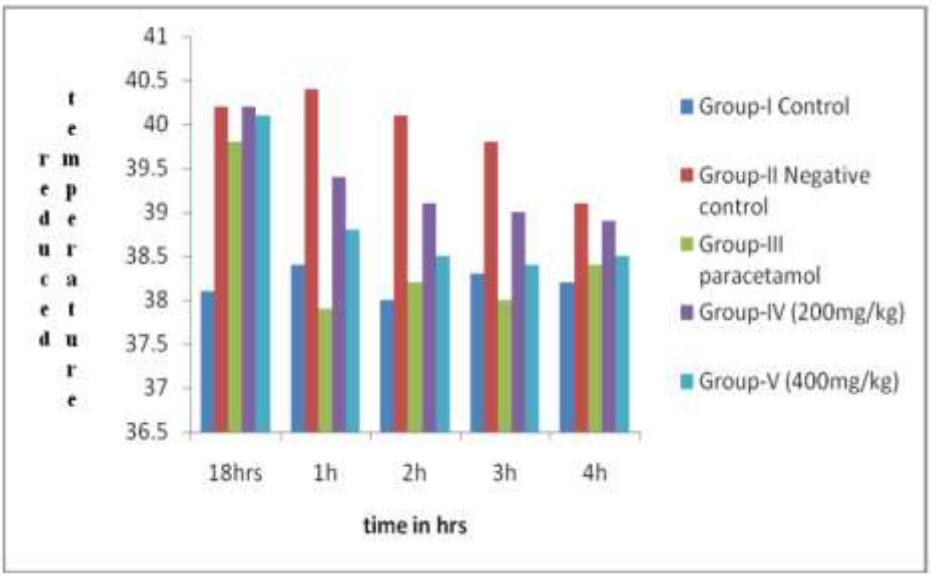

Figure no. 2: Anti-Pyretic Activity of Ethanolic Leaves Extract of *Aegle marmelos* (L) Roxb, On Brewer's Yeast Induced Pyrexia in Rats.

TAB vaccine-induced fever, the fever was significantly reduced and the body temperature was normalized by administration of 200 and 400mg/kg dose intraperitoneally. However, 100mg/kg dose of extract had no effect on the rectal temperature of mice (Not shown). The response in higher doses was almost comparable to that of Paracetamol.

Table no. 3: Antipyretic effect of alcoholic extract of *A. marmelos* on TAB vaccine induced hyperpyrexia in rats.

Treatment & Dose	Basal °C	°C 2 hrs after TAB Vaccine	° C after treatment with drugs/hr			
			1st hr	2nd hr	3rd hr	4th hr
Group-I Control	35.2±0.13	38.28±0.30	37.71±0.10	37.33±0.15	37.26±0.31	37.11±0.07
Group-II (200mg/kg)	35.6±0.21	38.06±0.34	38.01±0.28	37.75±0.18	37.26±0.48	36.16±0.11*

Group-III (400mg/kg)	35.92±0.18	38.25±0.38	38.12±0.36	37.88±0.79	36.91±0.24*	35.96±0.39**
Group-IV Paracetamol	35.3±0.14	38.01±0.29	37.96±0.45	37.15±0.13	36.91±0.24*	35.45±0.23**

Values are expressed as mean ± SEM of six animal per group (n=6). * p<0.05, **p<0.001

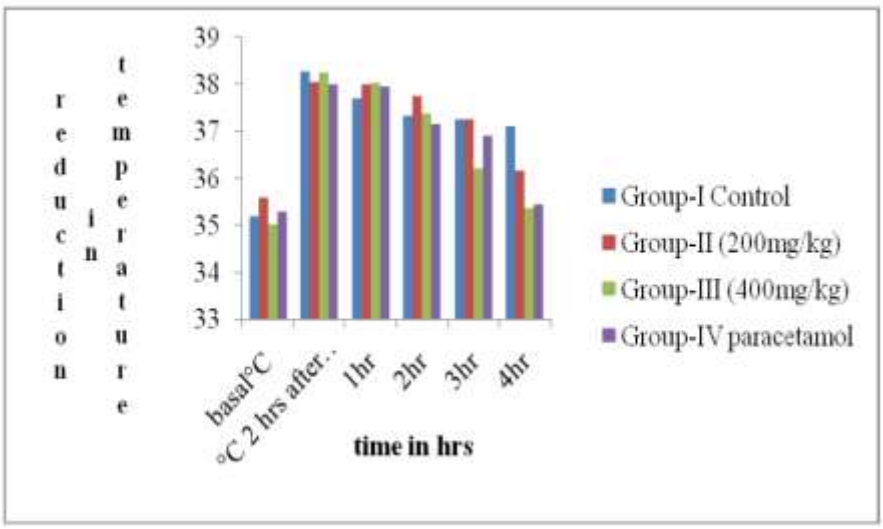

Figure no. 3: Anti-Pyretic Activity of Ethanolic Leaves Extract of *Aegle marmelos* (L) Roxb, On TAB vaccine Induced Pyrexia in Rats.

Antipyretic drugs such as acetylsalicylic acid reduce body temperature by inhibiting the synthesis of prostaglandin in hypothalamus. Similarly, paracetamol gives antipyretic effect by inhibiting the cyclooxygenase (COX) iso-enzyme in brain. Non-steroidal anti-inflammatory drugs (NSAIDs) like acetylsalicylic acid exert their antipyretic action by inhibiting prostaglandin synthesis (E-type) in the hypothalamus. As a result, elevated plasma prostaglandin level, as observed in fever is suppressed. Paracetamol, the reference antipyretic drug used in this study, also has same effect by a selective action on a specific cyclooxygenase (COX) iso-enzyme in the central nervous system (CNS). It might be likely to conclude that the *Aegle marmelos* extract prevents the prostaglandins synthesis. Plant extract exhibited significant reduction in the rectal temperature of mice as compared to negative and positive control groups. The results obtained reveal the significant antipyretic effect of the ethanolic extract of *Aegle marmelos.*

The oral administration of *Aegle marmelos* significantly attenuated rectal temperature of yeast induced albino rats. Thus it can be postulated that *Aegle marmelos*, contained pharmacologically active principles that interfere with the release of prostaglandins. After three hours of the test period, the ethanolic leaves extract of *Aegle marmelos* produced

appreciable antipyretic activity against brewer's yeast and TAB vaccine induced pyrexia in albino rat. It was revealed that the extract showed dose dependent antipyretic activity.

CONCLUSION

According to the findings of this study, the plant extract is found to be safe for mice and it can be concluded that the experimental plant extract and its solvent fraction possessed antipyretic activity. The study's overall findings suggest that *Aegle marmelos* leaf extracts could be used as a new source for the development of new plant-based antipyretic agents. The results obtained demonstrate the significant antipyretic activity of the ethanol extract of *Aegle marmelos* leaves on a yeast-induced pyrexia model and TAB induced pyrexia model.

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