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EVALUATION OF ANTI PYRETIC POTENTIAL OF METHANOLIC EXTRACT OF LAGENARIA SICERARIA SEED AGAINST BREWER'S YEAST INDUCED PYREXIA IN RABBIT.

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ABSTRACT

Fever, also known as pyrexia and febrile response, is defined as having a temperature above the normal range due to an increase in the body's temperature set-point. There is not a single agreed-upon upper limit for normal temperature with sources using values between 37.5 and 38.3 °C (99.5 and 100.9 °F). The increase in set-point triggers increased muscle contraction and causes a feeling of cold. This results in greater heat production and efforts to conserve heat. When the set-point temperature returns to normal, a person feels hot, becomes flushed, and may begin to sweat. Rarely a fever may trigger a febrile seizure. This is more common in young children. Fevers do not typically go higher than 41 to 42 °C (105.8 to 107.6 °F). Hyperpyrexia is a fever with an extreme elevation of body temperature greater than or equal to 41.5 °C (106.7 °F). Such a high temperature is considered a medical emergency as it may indicate a serious underlying condition or lead to significant side effects. The most common cause is an intracranial haemorrhage. Antipyretics from anti- 'against' and pyretic 'feverish') are substances that reduce fever. Antipyretics cause the hypothalamus to override a prostaglandin-induced increase in temperature. The body then works to lower the temperature, which results in a reduction in fever. The main aim and objective of my present research work was the phytochemical screening and evaluation of anti pyretic activity of Methanolic extract of Lagenaria siceraria seed against Brewer's yeast induced pyrexia in Rabbit. The effect of Methanolic extract of Seeds of Lagenaria siceraria significantly ($P<0.01$) reversed yeast induced pyrexia in rats throughout the observation period up to 6 h. From the above experimental data it was displayed that MELS shows significant reduction of elevated temperature as compared to control group, almost identical to that of aspirin treated group, hence the results indicateed that the test extract possessed significant antipyretic potential.

KEYWORDS: Pyrexia, Febrile response, Hyperpyrexia, Intracranial haemorrhage, Antipyretics etc.

INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or prevent in life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Nature has provided an excellent storehouse of remedies to cure all diseases. In Ancient days, almost all the medicines used were from natural sources, particularly from plants. Many traditional remedies employ herbal drug for the treatment of liver hepatic diseases.[1] *Lagenaria siceraria* (Cucurbitaceae) has great reputation in ayurvedic medicine for treatment of fever. So the present study was focused to evaluate the anti pyretic activity of Methanolic extract of *Lagenaria siceraria* seed against Brewer's yeast induced pyrexia in Rabbit.

Body temperature regulation: The stresses of physical exertion often are complicated by environmental thermal conditions. Performing in extreme heat or cold places a heavy burden on the mechanisms that regulate body temperature. Although these mechanisms are amazingly effective in regulating body temperature under normal conditions, mechanisms of thermoregulation can be inadequate when we are subjected to extreme heat or cold. Fortunately, our bodies are able to adapt to such environmental stresses with continued exposure over time, a process known as acclimation (which refers to a

short-term adaptation, e.g. days to weeks) or acclimatization (the proper term when we are referring to adaptations gained over long periods of time, e.g. months to years). Humans are homeothermic, which means that internal body temperature is physiologically regulated to keep it nearly constant even when environmental temperature changes. Although a person's temperature varies from day to day, and even from hour to hour, these fluctuations are usually no more than about 1.0 °C (1.8 °F). Only during prolonged heavy exercise, fever due to illness, or extreme conditions of heat or cold do body temperatures deviate from the normal range of 36.1 to 37.8 °C (97.0 – 100.0 °F). Body temperatures reflect a careful balance between heat production and heat loss.

Fever: The general manifestation of liver disease includes fever. This is often the result of infection and is caused by release of chemicals (pyrogens) from damaged tissue and cells involved in inflammation. Pyrogens act on the hypothalamus, which releases prostaglandins act on the hypothalamus thermostat to a higher temperature. The body responds by activating heat-promoting mechanism.[2] The general manifestation of liver disease includes fever .The fever is an elevation of body temperate above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus. A normal body temperature is ordinary maintained, despite environmental variation, through the ability of the thermoregulatory center to balance heat production by the tissues (muscle, liver) with heat dissipation. The balance is shifted to increase the core temperature. Hyperthermia is an elevation of body temperature above the hypothalamic set point due to insufficient heat dissipation. Whereas the normal temperature in human has been said to be 370C (98.60F), but in one large service of normal young adult, the morning oral temperature averaged 36.70 C, with standard devotion of 0.2^{o} C.

Pathogenesis of fever: The pathogenesis of fever is cased in toxins from bacteria such as endotoxin act on monocytes, macrophages, and Kupffer cells to produce different Cytokines that at as endogenous pyrogens (EPs).There is good evidence that IL1β, IL-6, β-1FN, γ-IFN and TNF-α can act independently to produce fever. These cytokines are polypeptides, and it is unlikely that circumventricular organs activate the preoptic are of the hypothalamus. Cytokines are also produced by cells in the CNS these may act directly on the thermoregulatory centers. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles (Mahesh S.Paschapur., 2009). The fever produced by cytokines is probably due to local release of prostaglandins produces fever .in addition, the antipyretic effect of aspirin exerted directly on the hypothalamus And aspirin inhibits prostaglandin receptors –EP_1, EP_2, EP_3 and $EP_4$and knockout of the EP_3 receptor impairs the febrile response to PGE_2, IL-1β and bacterial lipopolysaccharide (LPS).[4]

Scope and objective of the present studies: In Ancient days, almost all the medicines used were from natural sources, particularly from plants. Plants continue to be an important source of new drugs even today. The importance of botanical, Chemical and pharmacological evaluation of plant-derived agents used in the treatment of human ailments has been increasingly recognized in the last decades. Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active multitude of Chemical compounds.[5]

A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage. Modern allopathic medicine does not hold promise to cure liver diseases perfectly. Hence, it need for an agent which could protect against the liver damage until no correct medicine is available from synthetic drugs. Modern research is now focusing greater attention on the generation of scientific validation of herbal drugs based on their folklore claim. In this modern era, a large Indian population still relies on the traditional system of medicine which is mostly plant based, hence it is considered necessary to have experimental evidence and validation.[6]

The traditional systems of medicine like Ayurveda, Siddha, and Unani have a major role in the treatment of liver ailments. In the recent years, importance is being given to ayurvedic polyherbal formulations due to their effective therapeutic action and lack of side effects. The gourd family consists of a number of bioactive plants, which have been used extensively from ancient time for their therapeutic values. Lagenaria siceraria member of gourds and is traditionally used for treatment of various disorders. The plant, Lagenaria siceraria (Molina) standley (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout the India. Since time immemorial the fruit is used as immunosuppressant, diuretic[7], cardio-tonic, cardio-protective[8] and nutritive agent. The fruit is also reported to have good source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene. It is also reported to contain Cucurbitacins, fibres, and polyphenol. Two sterols namely campesterol and sitosterol have been identified and isolated from the petroleum ether fraction of methanol extract of Lagenaria siceraria fruits, which is reported to possess antihepatotoxic activity.[9] The fruit has been reported to possess antioxidant activity, hypolipidemic and triton-induced hyperlipidemic rats. HPLC analysis of methanol

extract from plant shows the presence of flavones-c glycosides. Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of Lagenaria siceraria possesses immunoprotective, antitumor, anti HIV and antiproliferative properties.[10] The present investigation has, therefore, been designed to study the anti pyretic activity of Methanolic extract of Lagenaria siceraria seed against Brewer's yeast induced pyrexia in Rabbit.[11]

PLANT PROFILE

Scientific classification[12]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Lagenaria
Species	Lagenaria siceraria

Distributión & Description: Plant is considered to be of African and Asian origin. L. siceraria is one of the popular vegetable, grown almost all the year round, particularly in frost free areas. It can be cultivate in all kinds of soil, but thrives best in heavily manure loams. It required warm humid climate or plenty of watering when grown during dry weather. Seeds may be sown in nursery beds and seedlings transplanted when they have put forth 2-3 leaves. It is sown from the middle of October to the middle of March and the later crop, from the beginning of March to the Middle of July. Vines are allowed to trail on the ground or trained over walls, trees, or other support; trailing over give high yield of fruit.[12]

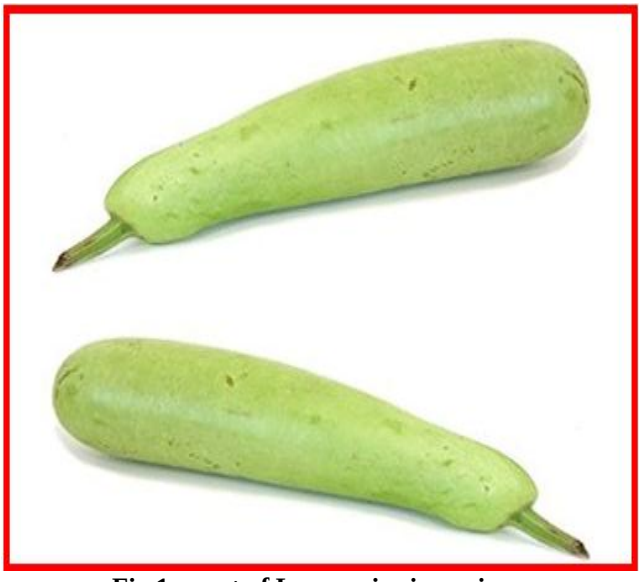

Fig 1: part of Lagenaria siceraria.

Fig 2: seeds of Lagenaria siceraria.

Properties and uses: The Bark is acrid, bitter, Pungent, alterative, aphrodisiac, tumors, bleeding piles and astringent, thermogenic, menstrual disorders, digestive used as refrigerant, anti inflammatory, aphrodisiac, emollient, hemorrhoids, hepatopathy, ulcer, and tumors. Leaves are astringent, anti inflammatory, tonic, ulcer, cooling agent, thermogenic, dysentery, seeds are used as ophthalmic, rubefacient, skin disease, ringworm, epilepsy, Fever, anti Inflammation, diuretic.[12]

EXPERIMENTAL WORK

Plant material collection and authentification: The Seeds of Abrus precatorius lam was collected from the areas around Nalgonda, AP, India in the month of Jan-2016. The plant were identified and authenticated by Dr. S. Baburaj, Botanist, Department of Botany, Thyagarajar College of arts and science, Madurai. L. siceraria fruits were collected from the local farms of Nalgonda District, Telongana, in the month of Jan-February. The collected seeds were air-dried under the shade in laboratory for 7-12 days. After complete drying, seeds were powdered and extracted thoroughly with light petroleum ether (40-60 °C) in a Soxhlet extractor to yield clear oil. On preliminary phytochemical screening, the Methanol extract showed positive for the presence of Carbohydrate, Flavonide, Tannin, saponins, triterpenoids and glycosides.

Drugs and Chemicals: The standard drug aspirin purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Experimental animals: The experiment was carried out on albino rabbits. They were 12 -14 months old, of both sexes, weighing between 1.5 and 1.6 kg. All rabbits were inbred. All the rabbits were kept in air conditioned animal house located in the KP Lab, Hyderabad. These animals were given grass, bread, maize, wheat grains and

were given water ad libitum. The experimental protocol and all the procedures were approved by Institutional animal ethical committee (IAEC) of KP Lab (KPL\IAEC\2016\36).

Methodology for extraction: Powdered crude drug (250g of the fresh air-dried) of Lagenaria siceraria seeds were extracted successively with petroleum ether 60-80% first to de-fat (as the seeds consists of high percentage of oil, fats). Again the final product (extract) was extracted with Methanol (60-80%) in soxhlet extraction apparatus for about 36 hours. The colorless solvent in the siphon tube was taken as the termination of extraction. The Methanolic extract was air dried at room temperature, weighed and percentage yield was calculated. Extract was preserved in a refrigerator till future use.[13]

Preliminary phytochemical screening of the Methanolic extract of *Lagenaria siceraria* [13].

Plant constituents Test	Methanolic Extract
1.**Test for Alkaloids**	
a) Dragendroff's test	+
b) Mayer's test	+
c) Hager's test	+
d) Wagner's test	+
2. Test for Carbohydrate	
a) Molisch's test	
b) Barfoed's test	-
c) Fehling's test	-
d) Benedict's test	-
3.Test for Flavonoids	
a)Lead acetate test	+
b) Shinoda test	+
c) Sodium hydroxide test	+
4. Test for Fixed oils	
a) Spot's test	-
b) Saponification's test	-
5. Test for Phytosterols	
a)Salkowski test	+
b)Liebermann-Burchard test	+
6.Test for Glycosides	
a) Legal's test	+
b) Borntrager's test	+
7.Test for Proteins	
a) Millon's test	-
b) Biuret's test	-
8.Test for Tannins	
a) 5%Fecl3 solution	-
b) Lead Acetate test	-
c) Bromine water	-
9. Test for Triterpenoids	
a) Salkowaski test	-
b) Libermann-Burchard test	-
10.Test for Amino acids	
a) Ninhydrin test	-

"+" indicates presence and "-" indicates absence

Evaluation of Antipyretic activity[14]: Albino Rabbit of strain weighing between (1.5-2 kg) were divided into four groups of 3 animals each. Fever in rabbits was induced by injecting of 20% w/v Brewer's yeast suspension, in the marginal ear vein of the rabbits at the concentration of 0.01 ml per kg body weight. The animals having temperature were divided into five groups having five animals in each group and treated orally as follows: Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast suspension (10ml/kg) into the animal's dorsum region. 18hr after (0hr) the injection, the rectal temperature of each rat was measured using a Digital Telethermometer. Only rats that showed an increase in temperature of at least 0.70C were used for experiments. Methanol of Lagenaria siceraria (150 mg/kg and 250 mg/kg, p.o.), Aspirin (300mg/kg p.o.) or vehicle were administered orally and the temperature was measured at 1, 2, 3, 5, and 6h after treatment.

Group I (Control) : Normal saline (1ml/kg, p.o).
Group II (Standard treated) : Standard Aspirin (300mg/kg, p.o).
Group III (MELS Dose-1) : MELP (150mg/kg, p.o).
Group IV (MELS Dose-2) :MELP (250 mg/kg, p.o).

RESULTS AND DISCUSSION

Phytochemical screening: The results of preliminary phytochemical analysis of Methanolic extract of Lagenaria siceraria Seeds showed the presence of various phytochemical constituents like Carbohydrates, Phytosterols, glycosides, flavonoids, Alkaloids etc.

Antipyretic Activity: The effect of Methanolic extract of Seeds of Lagenaria siceraria significantly ($P<0.01$) reversed yeast induced pyrexia in rats throughout the observation period up to 6 h. The reference drug aspirin also suppressed fever induced by yeast in rats (Table-1).

Table 3: Effect of Methanolic extract of Lagenaria siceraria seeds on Brewer´s yeast induced fever in rabbit.

Groups	Treatment (Dose)	Rectal Temperature (^{0}C) at time(hr)					
		-18[a]	0[b]	1	3	5	6
I	Control (1ml/kg, p.o)	36.73±0.15	37.49±0.08	37.71±0.11	37.40±0.08	37.36±0.12	37.46±0.13
II	Aspirin (300mg/kg, p.o)	36.75±0.10	37.58±0.24	36.48±0.11*	36.0±0.09*	35.94±0.48*	36.02±0.09*
III	MELS Dose -1 (150mg/kg. p.o)	36.46±0.45	37.56±0.11	36.94±0.16*	37.72±0.16*	37.08±0.22*	36.95±0.25*
IV	MELS Dose -2 (250mg/kg, p.o)	36.66±0.08	38.21±0.53	36.72±0.13*	36.69±0.48*	36.83±0.12*	36.69±0.09*

The values are Mean ± S.E.M of 3 observations, *P<0.01When compared to control,
a- Temperature just before yeast injection and
b- Change in temp following yeast injection.

CONCLUSION

From the above experimental data it was displayed that MELS shows significant reduction (P<0.01) of elevated temperature as compared to control group, almost identical to that of aspirin treated group. Hence the results indicate that the test extract possessed significant antipyretic potential. The phytochemical studies revealed the presence of flavonoids, glycosides, Sterols, Alkaloids in the MELS, these may be responsible for its pharmacological activities.

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