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Phytochemical Screening and In Vivo Antipyretic Activity of the Hydroalcoholic Leaves Extract of Acacia Catechu

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ABSTRACT: The aim of the study was to evaluate the antipyretic activity of hydroalcoholic leaf extract of Acacia catechu using Brewer's yeast-induced pyrexia model in wister albino rats. The hydroalcoholic leaf extract at a dose of 100mg/kg & 200 mg/kg were evaluated for antipyretic activity. The extract of Acacia catechu plant showed a significant ($P < 0.01$) dose dependent antipyretic effect in yeast induced elevation of rectal temperature in experimental rats when compared with the standard paracetamol. So It can be recommended for further studies. The extracts were also phytochemically screened for alkaloids, tannins, saponins, flavonoids, terpenoids, quinones and anthraquinones and quantitative analysis of total phenolic, flavonoids and alkaloid were determined by the well-known test protocol available in the literature. 24 albino rats weighing 180-220gm were used. They were divided in to four groups of six rats each. Group one serve as control (n=6) and was given normal saline, group two serves as standard group (n=6) was given 150mg/kg of paracetamol, while groups three and four serves as test groups were treated with 100mg/kg & 200 mg/kg (n=6) of plant extract respectively. A 15% suspension of 10ml/kg of brewer's yeast was injected subcutaneously to induce fever in all the experimental animals. After 24hrs, the rectal temperature was taken and the animals were administered Acacia catechu (100mg/kg & 200 mg/kg) and paracetamol (standard group, 150mg/kg) orally. The body temperature of the rats was measured rectally over a period of 4hours. Acacia catechu (100mg/kg & 200 mg/kg) significantly reduced yeast induced pyrexia when compared with the group two (paracetamol, 150mg/kg) Thus, this experiment shows that the antipyretic effect of Acacia catechu is dose dependent and the effect is as a result of the flavonoid component of the extract.

Key words: Acacia catechu, Hydroalcoholic extract, antipyretic activity, Brewer's yeast-induced.

I. INTRODUCTION

Pyrexia

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [Chattopadhyay D et al., 2005]. Normally the infected or damaged tissue initiates the enhanced formation of pro inflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [Spacer CB et al., 1994].

As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vaso constriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [Veugelers PJ et al 1997]. Drugs having anti- inflammatory activity generally possess antipyretic activity (e.g) non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that prostaglandin (PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit prostaglandin synthesis could help to explain their antipyretic activity.

Fever is one of the most common presenting signs of illness in office-based primary care pediatric practice, accounting for 19% to 30% of visits [Eskerud et al., 1992 and Baucher et al., 2001]. Infants and young children are particularly susceptible to fever because of their small body size, high ratio of body surface area to weight, and

low amount of subcutaneous fat. Although most experts consider fever a beneficial physiologic response to the infectious process, it can lead to patient irritability and stress as well as high parental anxiety [Guton H et al., 1997]. Therefore, physicians usually prefer to prescribe antipyretic agents in addition to nonpharmacologic, physical fever-reducing modalities [Baraff et al., 1993].

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature [Rajani G. P. et al 2011]. Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, & inability to concentrate. This increase in set point triggers increased muscle tone & shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected person's comfort [Duraisankar M. et al 2012].

According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine [Gupta M. et al 2008]. Due to poor hygiene practices and malnutrition, children in developing countries frequently suffer from various forms of infections which present as fevers. These fevers are often accompanied by aches and pains which all lead to morbidity and mortality [Ighodaro Igbe et al., 2009].

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE2 within the hypothalamic region [Rajani G. P. et al 2011].

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High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV [Veugelers PJ et al., 1997]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis [Cheng L et al., 2005]. 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. Natural COX-2 inhibitors have lower selectivity with fewer side effects [Cheng L et al., 2005].

The pathogenesis of fever:

Many of the mediators underlying pyrexia have been described in recent years (Figure 1). The critical "endogenous pyrogens" involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to affect a fever response [Luheshi GN et al., 1998]. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacteria outer membrane lipo polysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1b [Dinarello CA et al., 1999].

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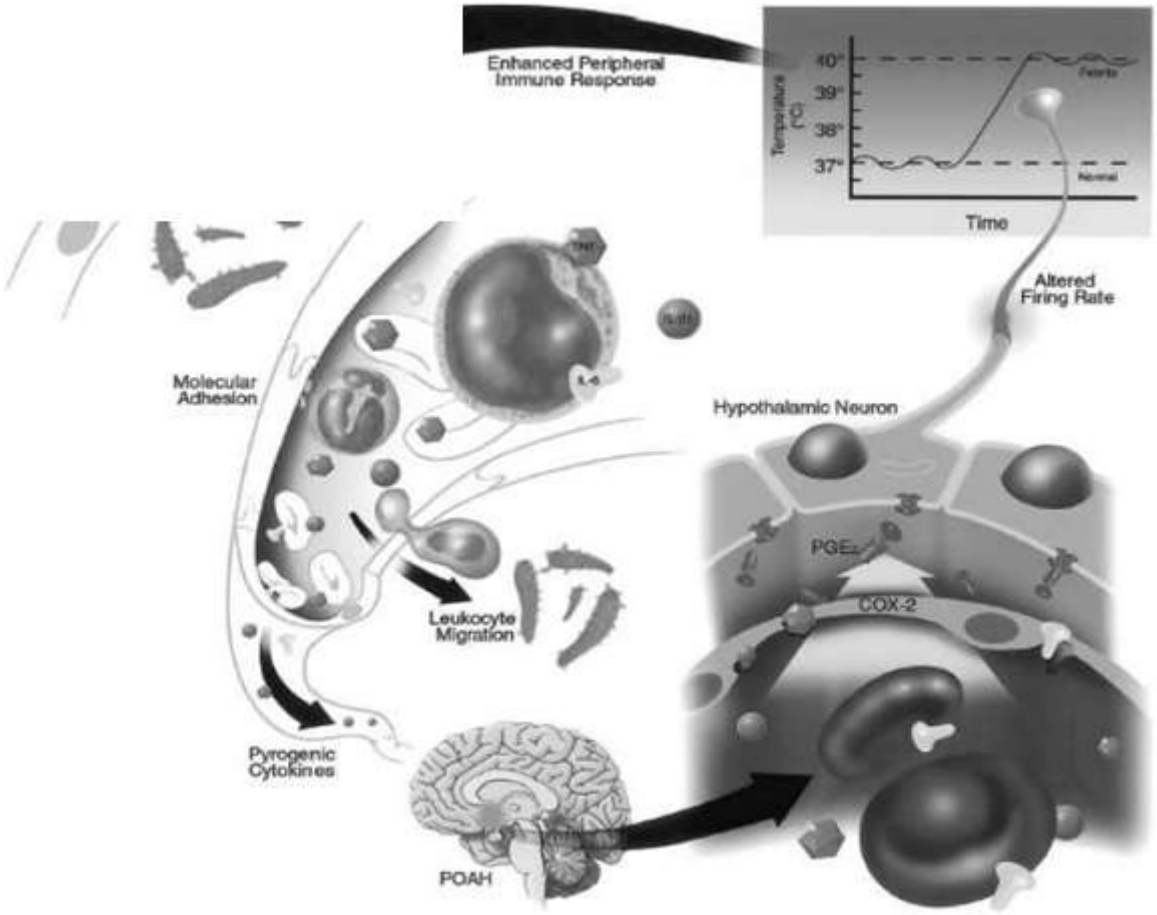

Figure 1.1: Fever generation after infection.

Microbial tissue invasion sparks an inflammatory response and activates local vascular endothelial cells and leukocytes. The extravasation of white blood cells into inflamed areas depends on a multistep interaction with endothelial cells regulated by a variety of cytokines, chemokines, and adhesion molecules. Activated leukocytes release the pyrogenic cytokines interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6). Hematogenous dissemination (depicted here) allows these endogenous pyrogens to stimulate vascular endothelial cell production of prostaglandin E2 (PGE2) within the central nervous system. Peripheral inflammatory signals may also travel along neural connections (such as the vagus nerve) to trigger central nervous system PGE2 production. Neurons within the preoptic area of the anterior hypothalamus (POAH) bearing specific E-prostanoid receptors orchestrate the febrile response after the PGE2 signal. PGE2 alters the firing rate of these neurons, resulting in an elevated thermoregulatory set point. The febrile set point body temperature is reached through the regulated evocation of behavioral and physiologic changes aimed at enhancing heat production and reducing heat dissipation. Fever is believed to augment the peripheral and systemic inflammatory response to infection in part by modulating the expression of inflammatory cytokines and enhancing leukocyte function [Biren N. Shah et al., 2010].

Antipyretics

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature [Periyasamy Gomathi et al., 2011].

Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase,

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resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α [Adesokan A.A et al., 2008] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain [Germain Taiwe et al., 2011].

Crude drugs are derived from natural sources like plants, animals and minerals. It is important that they should be properly identified and characterized for their physical and chemical characteristics and their quality should be enforced.

Materials and methods Plant material

Leaves of Acacia catechu was collected from Vindhya herbals Bhopal (M.P.) in the month of September, 2019.

Chemical reagents

Paracetamol (Dr Reddy's Laboratory, Hyderabad, India), yeast extract powder, carboxymethyl cellulose 5% as a suspending agent (HiMedia Laboratories Pvt Ltd, Mumbai, India), All other chemical used in this study purchased from SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction of plant material

Dried powdered leaves of Acacia catechu has been extracted with hydroalcoholic using maceration process for 48 hrs. After complete extraction the solvent was evaporated and concentrated to dry residue. % yield was calculated for each extract after drying under vacuum (Budholiya et al., 2019).

Preliminary screening for phytoconstituents

The freshly prepared hydroalcoholic extracts of leaves of Acacia catechu were qualitatively tested for the presence of phytochemicals by using standard procedures (Khandelwal, 2005; Kokate, 1994).

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC, TFC and total alkaloids are determined. Extracts obtained from leaves of Acacia catechu plant material of subjected to estimate the presence of TPC, TFC and total alkaloids by standard procedure.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al., 2011. A volume of 2 ml of extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al., 2011. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Experimental animals

Swiss albino rats of either sex (180-220 gm) were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water . The animals were kept on fasting overnight prior to the experimentation. They are maintained at room temperature under suitable nutritional and environmental conditions throughout the experiment and all the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee of College of Pharmacy, Sri Satya Sai University of Technology and Medical sciences, Sehore constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

The acute toxicity was performed according to OECD guidelines no. 423. The selected female albino rats were used for toxicity studies. The animals were divided into four groups

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of three in each. The animals were fasted overnight prior to the acute experimental procedure. hydroalcoholic extract of Acacia catechu. leaves was given orally to rats at the graded doses like 100, 300, 1000 and 2000 mg/kg body weight. Immediately, after dosing. The behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any.

Yeast-induced hyperpyrexia in rats

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into four groups of six animals and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (Saccharomyces cerevisiae) in the back below the nape of the rat. In brief, the rats were allowed to remain quiet in the cage for sometimes. A thermistor probe was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous (s.c.) injection of 10 ml/kg of 15% w/v Brewer's yeast suspended in 0.5% w/v methyl cellulose solution and the animals were returned to their housing cages. Twenty-four hour after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the hydroalcoholic extract of Acacia catechu leaves were administered orally at doses of 200 and 400 mg/kg to the treatment control groups animals, the normal control group received distilled water and standard control groups animals received 150mg/kg of paracetamol. Pre-drug control temperatures of all the rats were recorded at 24h immediately before the extract or paracetamol administration and again at 1h interval up to 4h after yeast injection (Mondal et al., 2016). The followings are group distribution.

Group	No. of animals in each group	Treatment/Dose
Group I Normal control	6	Brewer's yeast suspension (10 ml/kg b.w., s.c.)
Group II Standard Control	6	Brewer's yeast suspension (10 ml/kg b.w., s.c.) + Paracetamol (150 mg/kg p.o.)
Group III Treatment Group	6	Brewer's yeast suspension (10 ml/kg b.w., s.c.) + Acacia catechu hydroalcoholic extract at a dose of 100 mg/kg p.o.
Group IV Treatment Group	6	Brewer's yeast suspension (10 ml/kg b.w., s.c.) + Acacia catechu hydroalcoholic extract at a dose of 200 mg/kg p.o.

Statistical analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

II. RESULTS AND DISCUSSIONS

The crude extracts so obtained after the hot continuous extraction, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using hydro alcohol as solvents are depicted in the Table 1. Preliminary phytochemical screening of leaves of Acacia catechu extracts revealed the presence of various components such as phenolic compounds, flavonoids and saponins and the results are summarized in table 2.

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Table 1 Yield of hydroalcoholic extract

S.No.	Solvents	Acacia catechu
1	Ethanol:Water(70:30)	4.95%

Table 2 Result of Phytochemical screening of Ethanolic extracts of Acacia catechu

S. no.	Constituents	Ethanolic extracts of Acacia catechu
1.	**Alkaloids** Dragendroff's test Wagner's test Mayer's test Hager's test	 -ve -ve -ve -ve
2.	**Glycosides** General glycosides test	 -ve
3.	**Flavonoids** Lead acetate test Shinoda test	 +ve +ve
5.	**Tannins and Phenolics** 5% $fecl_3$ test	 +ve
6.	**Amino acids** Ninhydrin test	 -ve
7.	**Cabohydrates** Molichs test	 +ve
8.	**Diterpines**	-ve

Quantitative phytochemical assay was performed by calculating total phenolic content (TPC), total flavonoid content (TFC) and total alkaloids content. The TPC was calculated with respect to gallic acid (standard) and the TPC in ethanolic extract was found to be 1.02mg/g equivalent to gallic acid. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y = 0.06X+0.019$, $R^2= 0.999$, where X is the absorbance and Y is the quercetin equivalent (QE). Results were shown in table 3 and fig 2-3.

Table 3.Total phenolic and flavonoid content of Acacia catechu

S.No.	Extract	Total phenol (GAE) (mg/100mg)	Total flavono id (QE) (mg/100mg)
1.	Hydroalcoholic	1.985	0.869

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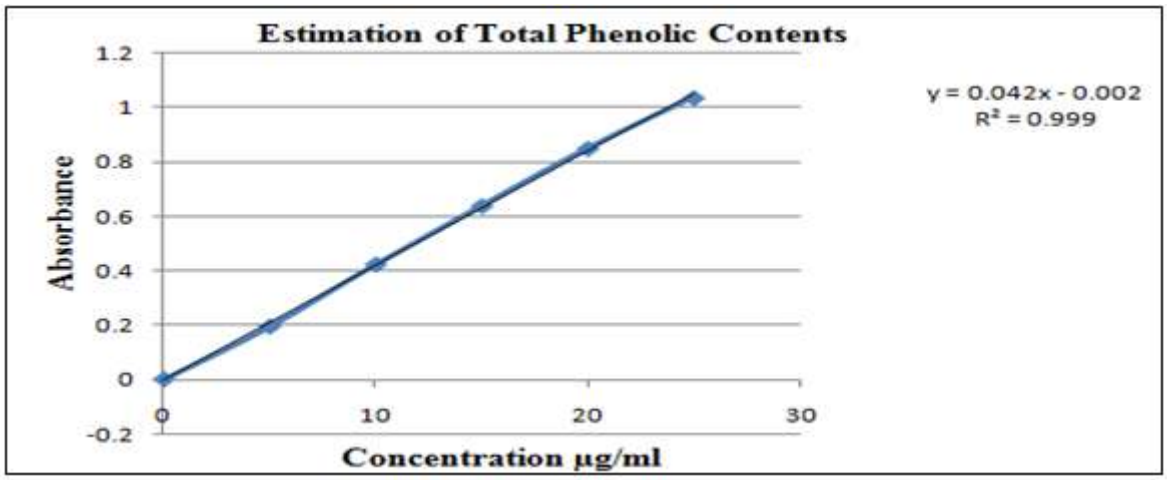

Fig.2 Graph of estimation of total phenolic content

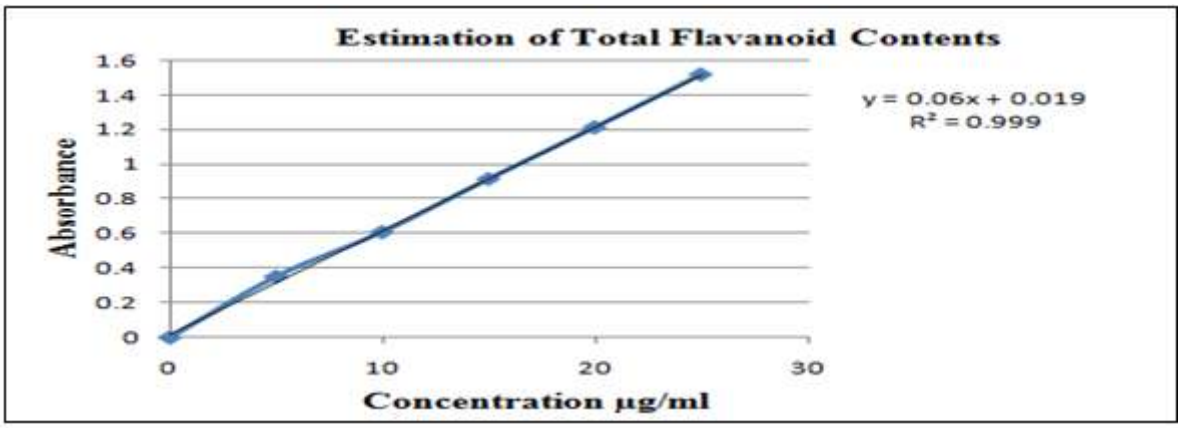

Fig. 3 Graph of estimation of total flavonoid content

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self-mutilation, walking backward etc. were observed. Gait and posture reactivity to handling or sensory stimuli, grip strength was all normal. There was no significant difference in body weights between control and treatment groups. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the hydroalcoholic extract of Acacia catechu leaves was safe to a single dose of 2000 mg/kg body weight. Hence, 100 and 200 mg/kg of body weight, of the maximum safe dose were selected for studying in vivo antipyretic activity. It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, inflammation and fever. Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the hydroalcoholic extract of Acacia catechu leaves at the doses of 100 and 200 mg/kg significantly decreased the rectal temperature of the rats. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard paracetamol (45 mg/kg, p o) and hydroalcoholic extract of Acacia catechu leaves (100 and 200 mg/kg) treated rats were compared with that of control and a significant reduction (*P<0.05; **P<0.01; ***P<0.001) against yeast induced pyrexia was observed. Hydroalcoholic extract at a dose of 200mg/kg, after 4 h showed more effect as compared to standard drug Table 4.

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Table 4 Effect of hydroalcoholic extract of Acacia catechu leaves on yeast induced pyrexia in rats

Group	Normal temperature before yeast administration	Pre-drug control, 1 h before drug admin.	Rectal temperature after drug administration (% decrease)			
			1h	2h	3h	4h
Group I Normal control	96.51±0.66	102.17±0.78	102.29±0.42 (0.30%)	102.21±0.36 (0.39%)	102.12±0.48 (0.50%)	101.09±0.56 (0.57%)
Group II Standard Control	95.58±0.84	99.98±0.68	98.60±0.59* (1.67%)	97.87±0.56*** (2.40%)	96.08±0.40*** (4.18%)	95.77±0.42*** (4.97%)
Group III Treatment Group	95.78±0.69	99.87±0.49	99.87±0.29* (0.99%)	98.76±0.29** (2.09%)	97.80±0.27*** (3.04%)	96.97±0.24*** (3.86%)
Group IV Treatment Group	95.81±0.42	100.64±0.51	99.46±0.31** (1.17%)	98.17±0.67*** (2.45%)	96.34±0.46*** (4.27%)	95.17±0.67*** (5.73%)

Each values represents the mean ± SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with toxicant control group (one-way ANOVA followed by Dunnett's test). Values in parentheses indicate percent decrease, calculated as 100 x (value of control – value of treatment) / value of control.

III. CONCLUSION

The hydroalcoholic extract from Acacia catechu leaves possesses antipyretic activities. Therefore, clinical studies are urgently needed in order to confirm traditional wisdom in the light of a rational phytotherapy. Even today, plants are the almost exclusive source of drugs for a majority of the world's population. Therefore, it remains a challenge for scientists to provide efficient, safe and cheap medications, especially for rural areas. Altogether, the present study results confirmed that A. catechu possess significant antipyretic activity, which may be devoted to major secondary active metabolite present in it. In conclusion we suggest that the future studies on Acacia catechu could be useful for the management of pyrexia. Quantification of individual phytoconstituents as well as pharmacological profile based on in vitro, invivo studies and on clinical trials should be further investigated and also accounts the scientific validation of reported use of the said plant in folklore uses to better understand the mechanism of such action scientifically.

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