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EVALUATION OF *IN-VIVO*ANALGESIC AND ANTIPYRETIC ACTIVITIES OF *MELIA. AZEDARACH* LINN. AND *PSIDIUM. GUAJAVA* LINN. LEAF EXTRACTS IN MICE

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ABSTRACT

Algesia and pyrexia are central to numerous pathological conditions. Synthetic drugs used for the treatment of these conditions cause various undesired effects. Several studies are in progress worldwide to discover therapeutic agents from natural sources with improved safety profile. The present study is aimed at evaluating *in-vivo* analgesic and antipyretic potential of methanol and ethanol extracts of leaves of *Melia. azedarach* Linn.and *Psidium. guajava* Linn. Analgesic activity was assessed by a tail immersion and acetic acid-induced writhing methods while Brewer's yeast induced pyrexia method was employed to ascertain antipyretic profile. In analgesic method methanolic extract of *P. guajava* L. showed higher pain threshold (11.86± 0.21 sec) and maximum pain inhibition at a dose of 400 mg/kg at 120 min after administration which is almost equivalent to that of standard (12.14± 0.21 sec).While, in the second analgesic model, methanolic extracts of *P. guajava* L. (400mg/kg) showed maximum protection of 60.51% with least no. of writhes (7.4±1.28) and *M. azedarach* L. 400mg/kg ethanolic extract showed 42.90% protection with 10.7±1.16 score. In pyrexia method, *P. guajava* L. methanolic and ethanolic

400mg/kg dose groups showed significant changes in pyrexia i.e., 37.99± 0.26 and 38.43±0.12 while *M. azedarach* L. extracts showed (38.19± 0.16, 38.53±0.16) in a dose-dependent manner with increase till 2hrs later followed by steep decrease. Overall, it is concluded that *P. guajava* L. extracts especially methanolic groups showed marked analgesic and antipyretic activities in three models studied, this strengthens the ethnopharmacological uses of both plants as analgesic and antipyretic plant.

Keywords: Analgesic, Antipyretic, *Melia. azedarach* L., *Psidium. guajava* L., Acetic acid-induced writhing, Tail immersion method and Brewer's yeast induced pyrexia

1. INTRODUCTION

Medicinal plants comprise of major constituents of most indigenous medicines and many of them include one or more components of plant origin. Pharmaceutical drugs that are used at present are not certainly the same as those that were used in ancient times or even in the recent past. *Melia. azedarach* L. (*M. azedarach L.*) is an indigenous deciduous tree, belonging to family Meliaceae and contains flavonoids, carbohydrates, terpenoids, and limonoids, rutin and palmitic acid derivatives **[1]**. While *Psidium. guajava* L. (*P. guajava* L.) is well known tropical and subtropical perennial tree belonging to Myrtaceae family majorly containing polyphenolics, flavonoids, limonene, and terpenes **[2]**. These medicinal plants could be of great help for the treatment of symptoms of tropically neglected diseases such as dengue fever. Pain relieving medications like acetaminophen are given during treatment to relieve from pain and fever without altering consciousness **[3]**. Painful responses can be generated in experimental animals by employing noxious thermal stimuli or chemical irritants such as acetic acid as a source of pain which are associated with tissue damage **[4]**.

Two methods were used in the study which includes writhing approach for the assessment of peripheral analgesic activity, while tail immersion method for estimation of central analgesic activity. Intraperitoneal injection of acetic acid produces pain reaction which is depicted as writhing response. Constriction of abdomen, twisting/ turning of trunk and hind limb extensions are undertaken as reaction parameters to estimate chemically induced pain. Fever or pyrexia is generally produced as a consequent effect of infection, inflammation, tissue damage, and tumor or other diseased states.Usually, diseased, or injured tissue initiates the higher formation of pro-inflammatory cytokines like interleukins and TNF-α derivatives, which enhance the production of prostaglandin E_2 (PGE_2) adjacent to preoptic hypothalamus region and thus triggers the hypothalamus to increase body temperature **[5]**.

Most of the analgesic and antipyretic drugs used in the treatment of pain, fever, inflammation usually inhibit COX-2 expression to lower the increased body temperature by reducing PGE_2 biosynthesis in central nervous system and causes toxicity and organ damage sometimes. Nevertheless, their use is associated with adverse effects, so to overcome the toxicity of these drugs, the development of new drugs is still need of hour and medicinal plants could have an advantage in discovering new natural COX-2 inhibitors with fewer side effects **[6]**. After careful literature review, we did not find any detailed *in vivo* studies with the above-mentioned methods with respect to the plants chosen. So, we explored the analgesic and antipyretic activities of both these plants that could be used for dengue treatment.

2. MATERIALS AND METHODS

2.0 Plant extractpreparation

Leaves of *M. azedarach* L. and leaves of *P. guajava* L. were collected from local trees of Chirala and from herbal garden of Acharya Nagarjuna University, Andhra Pradesh. Voucher specimens were deposited for species identification and authenticated by taxonomist P. Satyanarayana Raju at the Department of botany & microbiology, Acharya Nagarjuna University. 100 gm of each of both dried and powdered leaf materials were soxhelated successively multiple times using soxhlet extraction technique at 55 ^{o}C with 500 ml of 50% (v/v) of different solvents such as ethanol and methanol (18 hour each) for extracting the active principles. The extracts obtained were filtered and further evaporated using rotary evaporator at 90-220 rpm and at temperature of 60-80 ^{o}C depending on the solvent. The gummy concentrated solutions of green color obtained were freeze-dried and then stored in sterile glass desiccators at room temperature for further analysis and experimental purposes **[7]**.

Chemicals and reagents: Brewer's yeast was procured from Hi media, India. diclofenac sodium, Paracetamol, Acetic acid were purchased from Ankachem, Telangana, India), Normal saline was used as control and while preparing extracts.

Equipment used: Rotary evaporator (Buchi R100),Mice restrainers, Hot water bath.

2.1 Test animals

For screening of *In-vivo* analgesic, and antipyretic potentials of methanol (ME) and ethanol extracts (EE) of leaves of *Melia.*

azedarach L. (MA) and *Psidium guajava* L. (PG), healthy Swiss albino mice of either sex were used. They were procured from Mahaveera enterprises, Hyderabad, India. The animals were acclimatized for a week before the experimentation and maintained under laboratory conditions **[8]**. The animals were fed with standard feed and were provided ad libitum. Ethical clearance was obtained from the Institutional Animal Ethical Committee (IAEC) and the study was conducted according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Regd. No: 1725/GO/Re/S/13/CPCSEA and with approval number: ANUCPS/IAEC/AH/P/10/2017.

3. *In-vivo* analgesic and antipyretic activities of *M. azedarach* L. and *P. guajava* L. leaves

3.1. Animal grouping and treatment protocol

For the two analgesic methods sixty mice of either sex (n=6) weighing (20-25g) were taken and divided into ten groups. The animals were administered test extracts, control and standard as follows. Group I was pretreated with negative control (10ml/kg of 0.1 N normal saline-NS), group II with positive control as standard drug diclofenac sodium (DS) -I. P of 10 mg/kg body weight), and groups (III–X with the plant extracts at dose of 200 and 400 mg/kg body weight for each group alternatively) group III, IV serves for 200 and 400mg ethanolic *M. azedarach* L. extracts. Group V and VI with 200 and 400 mg methanolic *M. azedarach* L. extracts, respectively. Group VII, VIII is intended for 200 and 400mg ethanolic *P. guajava* L. extracts. Group IX and X with 200 and 400 mg methanolic *P. guajava* L. *extracts,* respectively. Animals were subjected to respective stimuli 30 min after intragastric administration of respective drugs/extracts.

3.1.1 Analgesic activity using tail immersion method.

The analgesic activities of methanol and ethanol extracts of leaves of *M. azedarach* L.and*P. guajava* L. were assessed by a thermal test called tail immersion method/assay **[9]**. On the day of experimentation mice were intragastrically treated as explained in **Table 1**. The animals were held in posture in a suitable restrainer with the tail stretching out. 3-4 cm distal section of the tail was marked and immersed in the water bath thermostatically retained at 55±0.5°C. The tail flick latency period or reaction time which is the time-lag between the production of heat from water bath and the withdrawal of the tail from hot water (in seconds). Before giving the extracts or drugs, basal reaction time of animal to react to radiant heat was observed by keeping the tip of the tail in the water bath maintained at 55±0.5°C **[10]**. The tail withdrawal from the heat source which is considered as tail flick response was noted as the end point.

Analgesics generally increase the reaction time. A cut off period of 10 secs was kept for withdrawing tail to prevent damage. Those animals which are not responding within 10 secs were withdrawn from trail by giving maximum score of 100% to avoid further tissue damage. Three basal reaction times for each rat at a difference of 5 min were taken for the validation of the result **(Figure 1)**. After observing the basal reaction time, the test extracts of both plants at a dose of 200 and 400 mg/kg body weight and standard drug Diclofenac sodium (10 mg/kg) were given orally **[11]**. The reaction time was noted at 0 (immediately after drug administration), 30, 60, 120 and 180 minutes after giving the extract or drug. Difference in tail flick latency or mean increase in pain reaction time following drug administration was used to imply the analgesia produced by test extracts and standard drugs. Analgesic activity or tail flick latency difference was calculated as follows. Analgesia = Tail flick latency observed after drug administration (B) - Tail flick latency observed before drug administration (A).

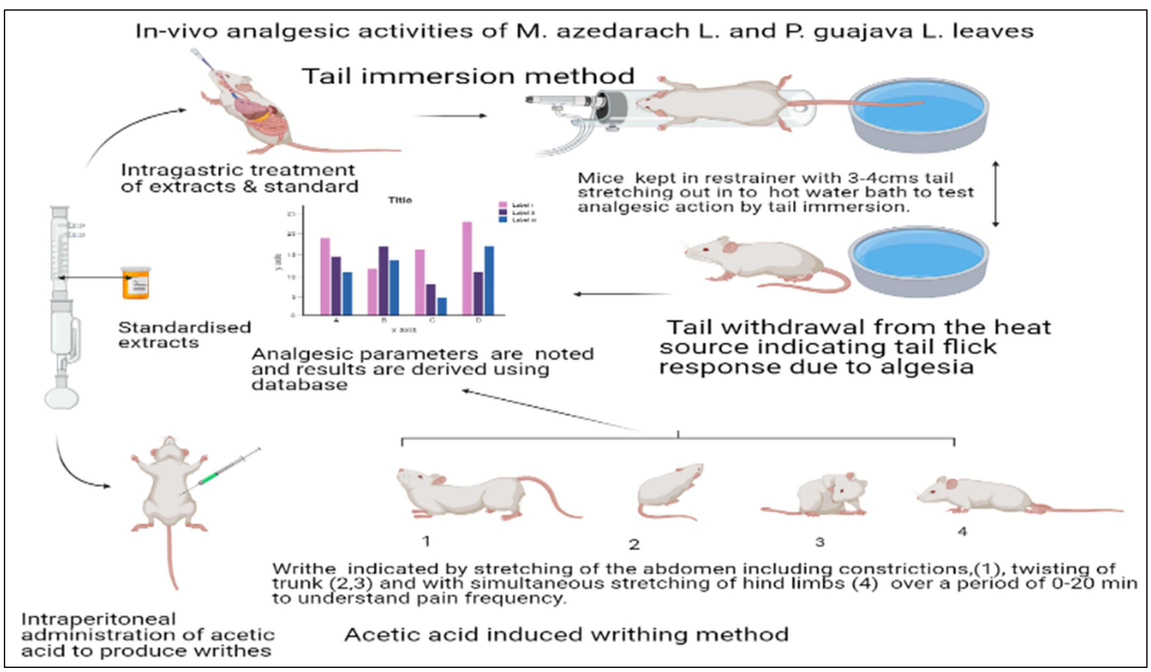

Figure 1: Schematic representation of analgesic activities using tail immersion and acetic acid induced writhing methods

3.1.2 Analgesic activity usingAcetic acid induced writhing in mice.

The analgesic activity of both extracts was studied using acetic acid induced writhing model in mice **[12]**. On the day of experimentation mice were divided and treated as explained in **Table 1**. All the test extracts, standard drugs were given 30 minutes before injecting acetic acid. A dose of 1 mL/100 g body weight of 0.6 % v/v glacial acetic acid in distilled water was injected intraperitoneally which is intended to produce writhing/stretching syndrome. After 15 min of time, the mice of each group were placed separately for further observation. The mice were then observed for signs of writhing over a period of twenty minutes and the number of writhes were recorded for each animal **[13] (Figure 1)**. For scoring purposes, a writhe is indicated by stretching of the abdomen including constrictions, twisting of trunk and with simultaneous stretching of hind limb over a period of 0-20 min with 10 min interval in-between each trail. The results were tabulated, percentage inhibition of writhes or percentage protection against acetic acid induced writhes were calculated [14]. Percentage protection against acetic acid induced writhes = {(Amount of writhing's in control animals (N_c) - Number of writhing's (N_t) in test animals)/ Amount of writhing's in control animals (N_c)×100} i. e, %Protection = (N_c- N_t)/N_c×100.

3.2 Antipyretic activity usingBrewer's yeast-induced pyrexia method.

This experiment was conducted out as explained by Gujaral and Khanna with slight variation as per our experimental need [15, 16]. On the day of experimentation mice were divided and treated as explained in Table 2. Animals were kept on fast overnight but were given drinking water ad libitum. The initial rectal temperature of all mice was recorded in next day morning manually using thermometer. Then, to induce fever 12.5% of dried Brewer's yeast suspension in normal saline was prepared and injected subcutaneously at a dose of 1 ml/100 g body weight per each animal. Animals must be handled carefully to minimize the possible stress during experimentation.

After 18 h of yeast injection, mice which demonstrated an increase in temperature of at least 0.3 -0.6°C were held for the study. Normal saline was administered to the control group I. The standard group (II) received Paracetamol at a dose 100mg/kg body weight of animal. The test doses of 200 and 400 mg/kg each of *M. azedarach* L.and*P. guajava* L. extracts were administered to treated groups in accordance with earlier groups respectively (Group III-X). The post rectal temperature of each animal in group was recorded after 1 h, 2 h, and 3 hours of drugs administration. For each time interval difference between the actual rectal temperature and initial rectal temperature was recorded. The maximum reduction in rectal temperature in comparison to control group was recorded **[17]**. Every result was calculated as the mean of three readings.

3.3 Statistical analysis

The results are expressed as Mean ± SEM (Standard Error of Mean) using analysis of variance (ANOVA) method followed by Dunnett's post-hoc multiple comparisons test and the results obtained were compared to control group. These results were plotted using GraphPad prism 9.0. A value of $*p<0.001$ was considered statistically significant. The degree of significance was noted and inferred accordingly.

4. RESULTS AND DISCUSSION

In dengue fever characteristic pain symptoms as described by center for disease control and prevention (CDC) typically includes muscle, joint, bone and abdominal pain and inflammation.Among various treatment options available for dengue fever, non-steroidal anti-inflammatory drugs (NSAIDS) are commonly used. However, prolong use of this drugs leads to many adverse effects like ulcers, bleeding, kidney, and liver failure **[18]**. Thus, there is requirement for the discovery of new analgesic and anti-inflammatory drugs without these adverse effects. Therefore, compounds from natural plants are taken into consideration for further study.

4.1 Acute toxicity study

In acute toxicity studies, all the animals were observed to be surviving after 24 h. This implies that the extracts were observed to be safe up to the dose levels investigated. Both the extracts of *M. azedarach* L. and *P. guajava* L. leaves were found to be devoid of toxic symptoms or mortality at 1000 mg/kg dose. The extracts treated groups did not display any changes in behavior indicating that LD_{50} is greater than 1000 mg/kg as shown in **Table 3**. Hence, both the extracts seem to be safe at 1000 mg/kg dose level and was considered as LD_{50} cutoff value. However, there are slight signs of lethargy at dose 600-1000mg/kg. Therefore, 200 and 400 mg/kg were selected for the screening of anti-pyretic and analgesic activity. The dose of the animals is calculated by inducing the human dose to animals.

4.2 Analgesic activity

4.2.1 Tail immersion method

The results pertaining to this investigation using tail immersion method reveal an upsurge in sensitivity to thermal stimuli by mice that received control, which is represented by shorter latency to pain response as shown in the **Table 1** and **Figure 2**. All the extracts of both plants and standard tested demonstrated dose dependent analgesic activity with decrease in sensitivity to thermal stimuli and they have exhibited maximum effect at 120 min after administration beyond which there is no longer increase in the latency. Of both extracts, methanol extract of *P. guajava* L. showed maximum activity and higher pain threshold (11.86±0.21 sec) with maximum pain inhibition at a dose of 400 mg/kg at 120 min after administration which is almost comparable to that of standard (12.14± 0.21 sec) and observed statistically significant with P <0.001 and P <0.01 when compared to both control and standard group. And *M. azedarach* L.methanol extract of 400 mg/kg at 120 min showed better nociceptive action of 9.69± 0.23 sec. The study results showed better latency period for*P. guajava* L. than *M. azedarach* L.

4.2.2 Acetic acid induced writhing method.

In acetic acid induced writhing paradigm, methanolic extracts of *P. guajava* L. (400mg/kg) showed maximum protection of 60.51% with least no. of writhes (7.4±1.28) while and *M. azedarach* L. 400mg/kg ethanolic extract showed 42.90% protection with 10.7±1.16 score **(Table 2** and **Figure 3)**. Among both plant extracts, the methanolic extracts of both plants indicated significant analgesic action which is p<0.001, in comparison with negative control (18.74±0.97) and P<0.001, P<0.01in comparison with standard (6.3±0.56 score, 66.38% protection) respectively.

This indicates that *P. guajava* L. extract has better analgesic action which is due to inhibition of prostaglandins mediated by high levels of PGE_2 or by acting on peripheral pain mechanism by release of cyclooxygenase derivatives, so more future studies are required to screen bioactive compounds and their mechanisms involved **[19]**. The acetic acid induced abdominal writhing was also described to act indirectly by distributing endogenous mediators and further stimulating neurons that are sensitive to other central acting drugs. Preliminary qualitative phytochemical screening discloses the existence of more phenolics and flavonoids in *M. azedarach* L. and *P. guajava* L. plants. Flavonoids were described to have a role in analgesic and anti-pyretic activities primarily by targeting prostaglandins in hypothalamus. They also act by inhibiting cyclooxygenase or lipoxygenase **[20]**.

4.3 Antipyretic activity

Fever is a substitute indicator for disease activity in numerous infectious and inflammatory conditions and is mainly regulated by immune response. Analogous to the conventional point of view, the beginning of fever is stimulated by inflammatory mediators (i.e., cytokines, like interleukins, tumor necrosis factor, etc.,) that are principally liberated by triggered peripheral mononuclear phagocytes and other immune cells **[21]**. Fever is strictly controlled by the immune response. Inflammatory stimuli activating the production of pro-pyretic messages that trigger the release of endogenous antipyretic substances. PGE_2 is produced from arachidonic acid, which is discharged from cell membrane lipid by phospholipase. Arachidonic acid is processed by two isoforms of the COX-1 and COX-2. COX-1 generally is expressed constitutively and produces prostanoids responsible for homeostasis **[22]**.

Paracetamol is an analgesic but is also an efficient febrifuge and a poor inhibitor of cyclooxygenase in the incidence of peroxides that are uncovered in inflammatory lesions. In contrast, its antipyretic effect may be described by its capacity to hinder cyclooxygenase. In the current study, impact of *M. azedarach* L. and *P. guajava* L. ethanol and methanol extracts on rectal temperature in rats is shown in **Table 3 and Figure 4**.

The subcutaneous injection of yeast suspension significantly elevated the rectal temperature after 18 hours of administration. In the yeast control group, there was steady rise of +0.4°C after 1 hr. (36.4±0.22°c) and 2hrs (36.8±0.22°c) of testing and substantial slight decrease after 3hrs **(**36.4±0.21°c**)** in contrast to the early values. In the standard group also the rise in temperature was significant (36.2±0.20°c, 37.1±0.19°c and 36.5±0.18°c); though, the enormity was slightly less in analogy to that in the yeast control group. *P. guajava* L.methanol and ethanol 400mg/kg extract groups showed temperature changes after 3 hrs. i.e., 36.7± 0.09 and 37.2±0.14 while *M. azedarach* L. extracts showed (37.3± 0.13, 37.7±0.14) in a dose-related manner with increase till 2hrs later followed by steep decrease after 3hrs. And the observed results were relative to paracetamol-treated group. Overall, *P. guajava* L. extracts especially methanolic groups lowered the body temperature of rats showing better antipyretic effect with $P < 0.001$, $P < 0.05$ significance when compared to control which is followed by ethanolic groups indicating the efficiency of *P.*

guajava L. methanolic extracts in decreasing pyrexia.

It is understood from previous literature that most of the analgesic drugs also possess antipyretic action also which are attributed due to presence of flavonoids, glycosides, alkaloids and saponins **[23]**. Here in the present study both the plants also revealed presence of the above listed phytochemical constituents. Therefore, it is believed that these compounds or their sub compounds may be liable for the noted analgesic, anti-pyretic activities.

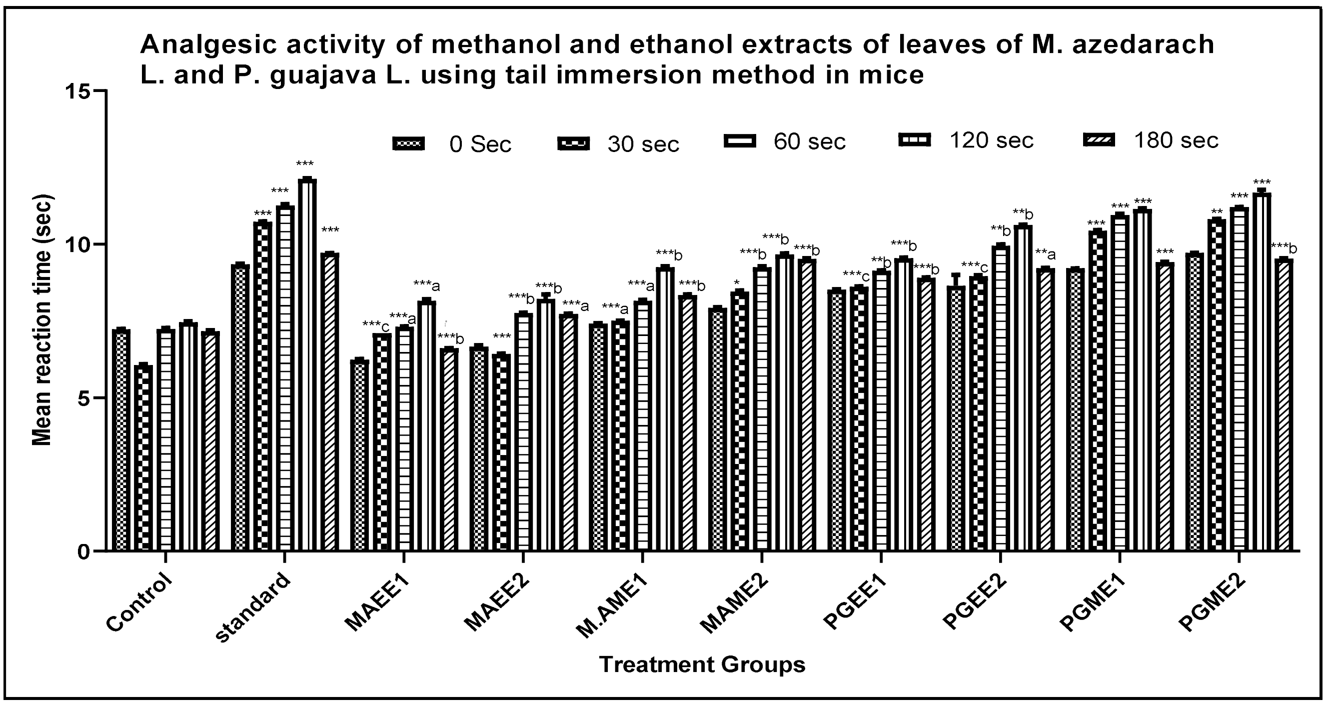

Figure 2: Analgesic activity of methanol and ethanol extracts of leaves of *M. azedarach* L. and*P. guajava* L. using tail immersion method in mice

Table 1: Analgesic activity of methanol and ethanol extracts of *M. azedarach* L. and *P. guajava* L. leaves using tail immersion method

S. No	Group No	Treatment	Dose (P.O) (mg/kg)	Mean reaction time in (seconds)				
				0 sec	30 sec	60 sec	120 sec	180 sec
I	Control (I)	Normal saline	10 ml/kg	7.23 ± 0.25	6.08 ± 0.22	7.26 ± 0.23	7.48 ± 0.18	7.18 ± 0.20
II	Standard (II)	Diclofenac	10 mg/kg	9.36 ± 0.22	10.74 ± 0.25 ***	11.29 ± 0.27***	12.14 ± 0.21 a***	9.71 ± 0.23 a***
III	*M. azedarach* L. (III-VI)	Ethanolic extract + Diclofenac	200 mg/kg (MAEE1)	6.25 ± 0.18	7.10 ± 0.11***c	7.32 ± 0.16 ***a	8.19 ± 0.22*** a	6.61 ± 0.26*** b
IV			400 mg/kg (MAE E2)	6.69 ±0.21	6.43 ± 0.38***	7.76 ±0.26***b	8.48 ± 0.29*** b	7.73 ± 0.14***a
V		Methanolic extract + Diclofenac	200 mg/kg (MAM E1)	7.42 ± 0.18	7.51 ± 0.22***a	8.18 ± 0.27***a	9.27 ± 0.14 ***b	8.36 ± 0.13***b
VI			400 mg/kg (MA ME2)	7.94 ± 0.18	8.48 ± 0.24*	9.27 ± 0.26***b	9.69 ± 0.23***b	9.53 ± 0.14a***b
VII	*P. guajava* L. (VII-X)	Ethanolic extract + Diclofenac	200 mg/kg (PGEE E1)	8.53 ± 0.30	8.62 ± 0.19***c	9.14 ± 0.26 ***b	9.56 ± 0.12 **b	8.91 ± 0.16a***b
VIII			400 mg/kg (PGEE2)	8.80 ±0.21	8.98 ± 0.38***c	9.98 ±0.26** b	10.63 ± 0.29**b	9.25 ± 0.14**a
IX		Methanolic extract + Diclofenac	200 mg/kg (PGME1)	9.21 ± 0.19	10.45 ± 0.3***	10.98 ± 0.27 ***	11.16 ± 0.24***	9.42 ± 0.17***
X			400 mg/kg (PGM E2)	9.72 ± 0.22	10.82 ± 0.17**	11.21± 0.19 ***	11.86 ± 0.21***	9.54 ± 0.09***b

Values are expressed as mean ± SEM (Standard error mean) (n = 6); Significance was measured using two-way ANOVA followed by Dunnett's multiple comparison test; Comparison to control: * indicates P<0.05, ** indicates P<0.01, ***indicates P<0.001, and nsP= non-significant; Comparison to control: a indicates P <0.001, b indicates P <0.01 and c indicates P <0.05

Table 2: Analgesic activity of methanol and ethanol extracts of leaves of *M. azedarach* L.and*P. guajava* L. using acetic acid induced writhing in mice

Group	Extract/Drug	Treatment Dose (P.O) (mg/kg)	No. of writhes (Mean ± SEM)	% Protection
I	Normal saline (10 ml/kg) + glacial acetic acid	Toxic control	18.74±0.97	-
II	Standard (10ml/kg) + glacial acetic acid	Positive control	6.3± 0.56***	66.38
III	*M. azedarach* L.(200, 400mg/kg)+ glacial acetic acid	MAEE1	11.6±0.79***a	38.10
IV		MAEE2	10.8±1.32***a	42.36
V		MA ME1	11.2±1.17***b	40.97
VI		MAME2	10.7±1.16***a	42.90
VII	*P. guajava* L. (200, 400mg/kg) + glacial acetic acid	PGEE1	9.5±1.25***ns	49.30
VIII		PGEE2	8.9±0.97***b	52.55
IX		PGME1	8.8± 0.83***ns	53.04
X		PGME2	7.4±1.28***ns	60.51

One way ANOVA trailed by multiple Dunnett's comparison test; Values are mean ± SEM, in each group n= 6. ***indicates P<0.001, ** indicates P<0.01 when compared with control group. aindicates P<0.001, bindicates P<0.01, cindicates P<0.05, nsindicates non-significant with respect to standard group

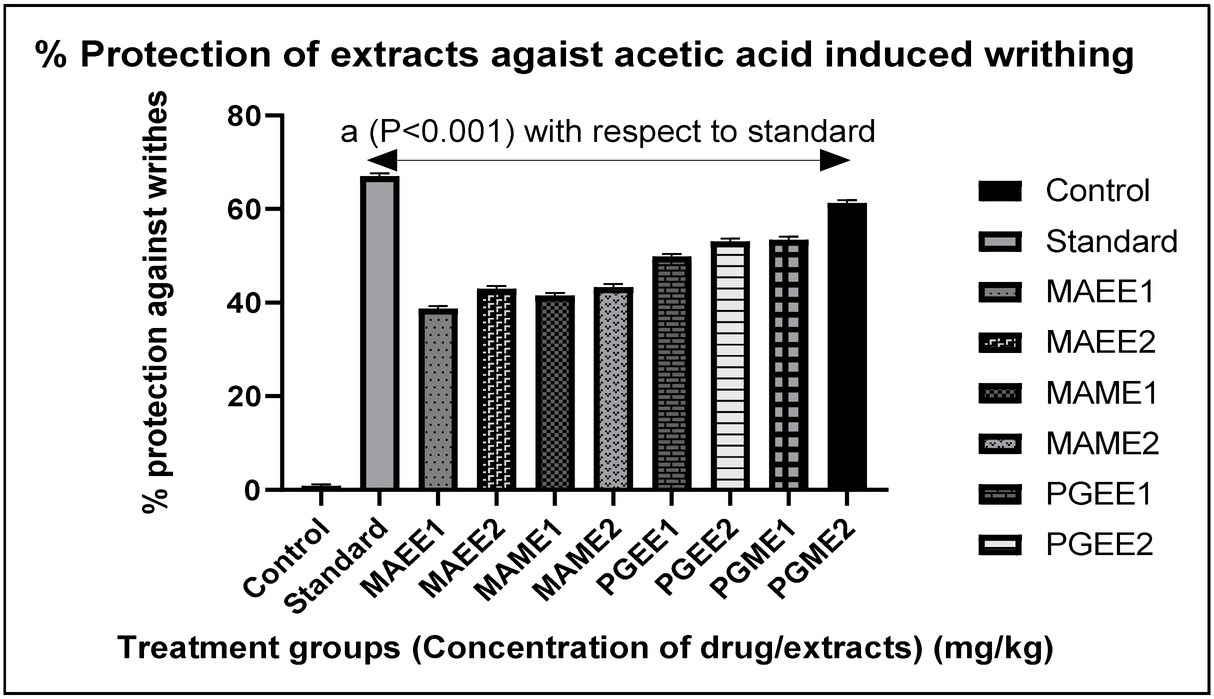

Figure 3: % Protection against writhes observed in mice after administration of two extracts in acetic acid induced writhing method

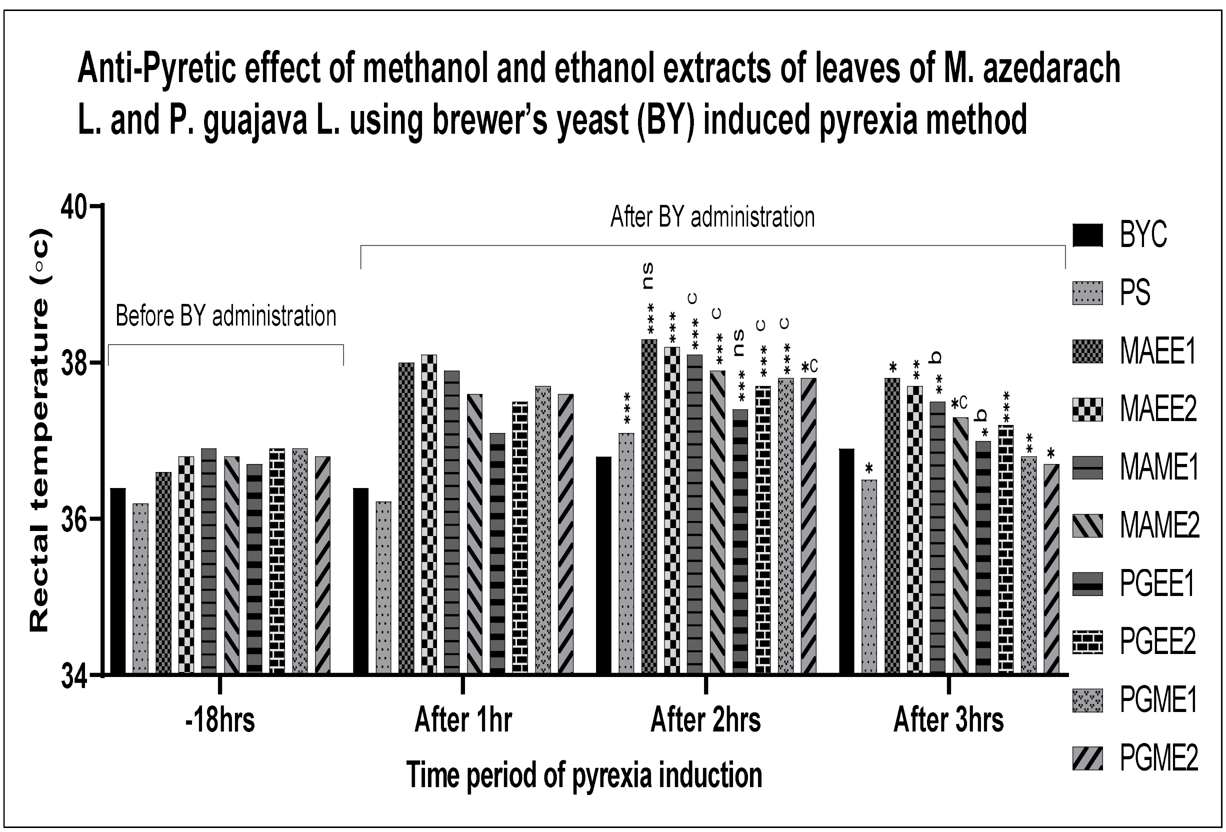

Figure 4: Change in the rectal temperatures observed in mice before and after administration of Brewer's yeast.

Table 3: Anti-Pyretic effect of methanol and ethanol extracts of leaves of *M. azedarach* L. and *P. guajava* L. using Brewer's yeast (B.Y) induced pyrexia in mice

Group	Drug or Extract	Treatment/ Dose (mg/kg)	Rectal temperature (◦c)						
			Before 18 hrs.	after 18hrs					
			-18 hrs.	After 1 hr.	Change in pyrexia	After 2 hours	Change in pyrexia	After 3 hours	Change in pyrexia
1	Yeast Control (BYC)	10mL/kg (S.C)	36.4±0.17	36.4±0.22 ↑	+0.0	36.8± 0.22↑	+0.4	36.4±0.21↓	-0.4
2	Standard (PS)	Paracetamol(100mg/kg)	36.2± 0.20	36.2±0.20 ↑	+0.0	37.1± 0.19** ↑	+0.9	36.5± 0.18*↓	-0.6
3	*M. azedarach* L. + 12.5% Brewer's yeast (S.C)	MAEE1	36.6± 0.23	38.0±0.16 ↑	+1.4	38.3± 0.19***ns↑	+0.3	37.8±0.22*↓	-0.5
4		MAEE2	36.8± 0.19	38.1±0.12 ↑	+1.3	38.2±0.16 *** ↑	+0.1	37.7± 0.14 **↓	-0.5
5		MAME1	36.9± 0.16	37.9± 0.19↑	+1.0	38.1± 0.17**c ↑	+0.3	37.5± 0.19* c ↓	-0.6
6		MAME2	36.8± 0.21	37.6± 0.18↑	+0.8	37.9± 0.16**ns ↑	+0.3	37.3± 0.13** b ↓	-0.6
7	*P. guajava* L. + 12.5% Brewer's yeast (S.C)	PGEE1	36.7± 0.16	37.1±0.16 ↑	+0.4	37.4±0.18 ***c↑	+0.3	37.0± 0.16*** ↓	-0.4
8		PGEE2	36.9± 0.09	37.5± 0.11↑	+0.6	37.7± 0.16***ns↑	+0.2	37.2± 0.14* b ↓	-0.5
9		PGME1	36.9± 0.13	37.7± 0.17↑	+0.8	37.8± 0.10*c↑	+0.1	36.8± 0.08** ↓	-1.0
10		PGME2	36.8± 0.13	37.6± 0.12↑	+0.8	37.8± 0.14***c↑	+0.2	36.7± 0.09* ↓	-1.1

Values are expressed as mean ± SEM (n = 6), Significance was measured using two-way ANOVA followed by Dunnett's multiple comparison test; Comparison: * indicates P<0.05, ** indicates P<0.01, ***indicates P<0.001, and ns P= non-significant in comparision to control; a indicates P<0.001, b indicates P <0.01 and c indicates P <0.05 in comparision to standard, ↑: Increase, ↓: Decrease

5. CONCLUSION

The results obtained for analgesic and anti-pyretic activities of both plant extracts were proved to have better activities than those of the studies reported previously. It is also evident that between both plants *P. guajava* L. methanolic extracts exhibited higher analgesic and antipyretic activities in comparision with *M. azedarach* L. extracts. There by providing evidence that administration of *P. guajava* L. and *M. azedarach* L.extracts as natural remedies during dengue fever couldsuccessfully treat pathogenic fever in a dose dependent manner. However, to know the exact mechanism of how these extracts show TNF-α and prostaglandins inhibition, further pharmacological and toxicological studies are needed.

6. Conflict of Interest

We, the authors declare that there is no conflict of interest associated with this work and publication of this paper.

7. Acknowledgments

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