

# Analgesic and Antipyretic Properties of Ethanol Extract of *Plumeria Acuminata* Leaves in Male Wistar Rat Pain Models: Perspectives in Clinical Pain Management

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Submitted: 10 November 2022. Published: 12 March 2023

## ABSTRACT

**Background:** Pain, whether acute or chronic, is debilitating and gradually reduces one's quality of life. *Plumeria acuminata* (*P. acuminata*), commonly known as Frangipani in English, is a plant that has traditionally been used in traditional medicine to relieve pain and inflammation, among other uses. **Aims and objectives:** To evaluate the antipyretic and analgesic properties of ethanol leaf extract of *P. acuminata* in wistar rat models of pain. **Materials and Methods:** The experimental rats weighing 100-150g were randomly assigned into six groups (n = 6), control group (0.9% normal saline), paracetamol (150 mg/kg), *P. acuminata* ethanol extract (250, 500, 1000 mg/kg). Analgesic activity was assessed using acetic acid induced writhing reflex and tail immersion protocols. Pyrexia was induced by subcutaneous injection of 20% w/v brewer's yeast. Rectal temperature was recorded hourly for four hours using digital thermometer. **Results:** Antipyretic activity was observed at 1000 mg/kg of the extract, indicated by decrease in rectal temperature, which was not statistically significant (p>0.05). The extract exhibited analgesic activity at 1000 mg/kg in the acetic acid tests. The analgesic activity observed was similar to that of paracetamol, evident in the reduction of IL-1 concentration in the groups that received 500 mg/kg and 1000 mg/kg of the extract. There was no significance recorded for the tail immersion test. **Conclusion:** The ethanol extract of *P. acuminata* leaves possesses antipyretic and analgesic effects that may have acted on peripheral but not central nociceptive pathways. The effects are equivalent to paracetamol and may have clinical significance for the treatment of painful conditions.

**Key words:** Pain, IL-1, *Plumeria acuminata*, Antipyrexia, Analgesia, Antinociception.

## INTRODUCTION

Inflammation is an abnormal physiological response characterized by pain in which the affected area of the body becomes swollen, reddish, and hot, often in response to injury. It can be either local or systemic. When inflammation

occurs, the body's white blood cells release inflammatory mediators such as interleukins into the blood or damaged tissues to protect against foreign substances. The release of mediators increases blood flow to the site of injury or

### Access to the article



Website: [www.jmbsr.com.ng](http://www.jmbsr.com.ng)

eISSN: 2971-7833

pISSN: 2971-7108

10.5281/zenodo.7744106

### How to cite this article

Ogbole N E, Ahmed-S I, Dingwoke E J, Umar A H. Analgesic and Antipyretic Properties of Ethanol Extract of *Plumeria Acuminata* Leaves in Male Wistar Rat Pain Models: Perspectives in Clinical Pain Management. *J Med & Bas Sci Res.* 2023;4(1-2):95-102. DOI: 10.5281/zenodo.7744106

infection, resulting in the apparent redness and warmth. Globally, pain is a major health problem.<sup>[1]</sup> It is estimated that one in every five adults suffers from pain, and that one in every ten adults is diagnosed with chronic pain each year.<sup>[2]</sup> Pain not only reduces quality of life, but it also has significant health and economic implications. Unfortunately, the available analgesic drugs have a variety of side effects, and the majority of them are either excessively or under-potent.<sup>[3]</sup> It has been noted that multimodal analgesia has a wide range of intraoperative and postoperative applications.<sup>[4]</sup><sup>[5]</sup> Multimodal analgesia is the gold standard for pain management since no one analgesic can treat all forms of pain.<sup>[6]</sup> Among the many factors that contribute to the emergence of pain is the inflammatory response.<sup>[8]</sup> Effective postoperative pain management is critical for encouraging recovery and avoiding complications. Although opioids have long been the cornerstone of surgical pain therapy, the adverse effects reduce patient satisfaction and lengthen hospital stays, which raises treatment costs.<sup>[8],[9]</sup>

Medicinal plants have long been recognized as a rich source of new chemical compounds with analgesic and antipyretic activities.<sup>[10]</sup> As a result, research into plants with analgesic and anti-inflammatory qualities is being conducted in quest of novel and effective analgesic and anti-inflammatory agents.<sup>[11]</sup> More importantly, the search for new analgesic compounds to use as therapeutic alternatives is critical.<sup>[11]</sup> *P. Acuminata*, also known as Frangipani, is a flowering plant in the Apocynaceae family (Dogbane family). The plant is native to Nigeria and is distinguished by its different colored pink (*Plumeria rubra*) and yellow-white flowers (*Plumeria acuminata*). The *P. acuminata* species of interest is found throughout tropical countries, including Nigeria. Its leaves are oval-shaped, tapered at both ends, and somewhat hard, with prominent leaf veins. Frangipani flowers are fragrant and appear in clusters at the ends of the branches. *P. acuminata* is related to *oleander* (*Nerium oleander*), but unlike

*oleander*, it has no associated toxicity. Aside from its use as an ornamental plant, *P. acuminata* has been used in the treatment of a variety of diseases in traditional medicine<sup>[1]</sup>. The plant material is widely used as a purgative, diarrhoea remedy, and itch treatment. The milky juice has been used to treat inflammation and rheumatism. The bark has been used to treat hard tumors, diarrhoea, and gonorrhoea. The flower is used as a form of contraception.<sup>[12]</sup> This study looked at the analgesic and antipyretic activities of an ethanol extract of *P. acuminata* leaves in male Wistar rat pain models.

## MATERIALS AND METHODS

### Chemicals, Reagents and Drug

Enzyme-linked immunosorbent assay (ELISA) kits for IL-1 and analytical grade acetaminophen (PARACETAMOL) were purchased from GenAsia Biotech Co. Ltd, Shanghai, China. A 10% low-odor formalin solution containing 4% wt/vol formaldehyde, 0.075 M sodium phosphate, 1.5% wt/vol methanol, and phosphate buffer were used for all experiments (Formalde-Fresh; Fisher Scientific, Waltham, MA). All other chemicals used were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO, USA).

### Plant material and preparation of extract

*P. acuminata* fresh leaves were collected from its natural habitat in Sabon-gari Local Government Area, Zaria, Kaduna State. Taxonomists from the department of Botany at Ahmadu Bello University Zaria identified and authenticated it at the Herbarium, and voucher specimen number 131 was deposited for reference. The leaves were thoroughly washed with clean tap water and air-dried at room temperature for 21 days before being mechanically ground to a fine powder in the mortar, sieved, and stored in an airtight container in the laboratory. Fifty-five grams (55 g) of powdered plant leaf were extracted using a soxhlet extractor after 48 hours of cool maceration in triplicate with 750 ml of ethanol. The extract was filtered using Whatman's filter paper No.1 and allowed to evaporate completely in open air at room

temperature. The obtained semisolid mass (18.4%) was reconstituted in normal saline and served as the crude extract for the current study. The extract's percentage yield was calculated by dividing the mass of the extract by the mass of the plant material and multiplying by 100.<sup>[13]</sup>

#### Oral Acute toxicity test

The lethal dose (LD<sub>50</sub>) of the plant fraction was determined by the method of Lorke (1983)<sup>[14]</sup> using 12 rats and the median lethal dose (LD<sub>50</sub>) was calculated. This method consists of two phases which are phases 1 and 2 respectively. Phase 1 required nine animals. The nine animals were divided into three groups of three animals each. Each group of animals were administered orally different doses (10, 100 and 1000 mg/kg) of *P. acuminata* leaf extract. The animals were observed for 24 hours to assess their behavior as well as morbidity and mortality. Phase 2 involved the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of *P. acuminata* leaf extract and then observed for 24 hours to assess morbidity as well as mortality. Then the LD50 was calculated by the formula:

D<sub>0</sub> = Highest dose that gave no mortality,  
D<sub>100</sub> = Lowest dose that produced mortality.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

#### Experimental Animals and grouping

Apparently healthy male Wistar rats weighing 100 to 150 g purchased from the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University Zaria, and housed in the Department of Human Physiology, Ahmadu Bello University, Zaria for 14 days of acclimatization prior to the commencement of the experiments and allowed access to animal feed and water *ad libitum*. The animals were randomly grouped into polyacrylic cages of five animals (n=5) per group

as follows: Group I (control) animals were orally administered normal saline 0.9 mg/kg body weight. Group II (standard drug) were treated with 150 mg/kg paracetamol. Group III were treated with 250 mg/kg of *p. acuminata* extract. Group IV were treated with 500 mg/kg *p. acuminata* extract. Group V were treated with 1000 mg/kg *p. acuminata* extract. The Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) approved these experiments, with approval number ABUCAUC/2022/047, which followed the guidelines for the use of experimental animals. The study was designed in such a way that the number of animals used and pain experienced by the animals was kept to a minimum. Efforts were made to ensure that the animals did not suffer excessively.

#### Experimental protocols

##### Acetic acid induced writhing test

The acetic acid-induced writhing test was performed as previously described.<sup>[15]</sup> Following the administration of the extract, the animals were intraperitoneally injected with 0.6% acetic acid solution at a dose of 10 ml/kg and placed individually in an observation cage for thirty minutes. For each animal, the number of writhing reflexes (characterized by a wave of abdominal contraction and hind limb extension) was recorded for 10 minutes after the Acetic acid injection.

##### Tail immersion test

The tail immersion test was performed using a modified method described by Chopra *et al*<sup>[16]</sup>. The rat's tail was immersed in a hot water bath at 52 °C ± 1 °C until tail withdrawal (flicking response). The reaction time (latency period) was measured using a stopwatch.<sup>[17]</sup>

##### Brewer's yeast-induced pyrexia

We used the previously described method to induce pyrexia.<sup>[18]</sup> Pyrexia was induced by administering 20% w/v brewer's yeast subcutaneously near the animal's nape at a dose of 10 mg/kg body weight. A digital thermometer inserted 3 - 4 cm into the

rectum was used to measure the temperature. A rise in temperature of more than 1°C confirmed the induction of pyrexia, and animals with a rise in temperature of less than 0.6°C were excluded from the study.

#### Assessment of Interleukin 1 (IL-1)

The IL-1 concentration was determined using the Liu *et al* method.<sup>[1]</sup> Blood was drawn immediately after the last temperature readings were taken, and the serum was separated by centrifugation and stored at -80°C. IL-1 levels in serum were determined using an ELISA Kit (GenAsia Biotech Co. Ltd, Shanghai, China) according to the manufacturer's instructions. The absorbance was measured at 450 nm wavelength.

#### Data Analysis

The experimental data were pooled and expressed as mean ± standard error of mean. The results were analyzed using one-way analysis of variance (95% confidence interval) with the statistical package for social sciences (SPSS Inc., Chicago, V.17.0).  $P < 0.05$  was considered statistically significant.

## RESULTS

Yield of successive extraction of leaves of *P. acuminata* and Qualitative phytochemical screening

The extraction yielded 18.4%. The phytochemical analysis revealed the presence of alkaloids, tannin steroids, flavonoids, saponin, cardiac glycoside, and anthraquinone.

#### The acute toxicity test results

Experimental phase	Dose (mg/kg)	Number of deaths in 24 hours
<b>Phase I*</b>		
	10	0/3
	100	0/3
	1,000	0/3
<b>Phase II*</b>		
	1,600	0/1
	2,900	0/1
	5,000	1/1

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

$D_0$  = Highest dose that gave no mortality,

$D_{100}$  = Lowest dose that produced mortality.

$$LD_{50} = \sqrt{(2900 \times 5000)}$$

$$\begin{aligned} LD_{50} &= \sqrt{2900 \times 5000} \\ &= 3807 \text{ mg/kg} \\ &\approx 3.8 \text{ mg/kg} \end{aligned}$$

#### Acetic acid induced writhing test

Percentage inhibition of induced pain of peripheral origin by the intraperitoneal injection of acetic acid is presented in Table 1. Overall, the extract administration delayed the onset of writhing in mice in a dose-dependent manner. The extract reduced the frequency of writhing in the groups of animals that received 500 mg/kg and 1000 mg/kg doses of the extract ( $13.50 \pm 1.55$  and  $10.50 \pm 0.50$ , respectively).

#### Tail immersion test

The central analgesic activity of *p. acuminata* assessed using the tail immersion test is presented in Table 2. When compared to paracetamol, the standard analgesic used in this study, and the control, the extract did not produce antinociception. However, when the rats were given 1000 mg/kg of *P. acuminata* extract, a higher threshold was observed.

#### Brewer's yeast-induced pyrexia

The results show that the extract has antipyretic activity, as shown in Table 3. Following the inhibition of IL-1, the rectal temperature was found to have decreased in the group of animals given the extract.

**Table 1: Effect of the ethanol extract of *pacuminata* on acetic acid-induced writhing test in rats**

Grouping	Number of writhings
Control	21.00 ± 1.00
Paracetamol	25.00 ± 3.21
250 mg/kg	25.00 ± 7.00
500 mg/kg	13.50 ± 1.55*
1000 mg/kg	10.50 ± 0.50*

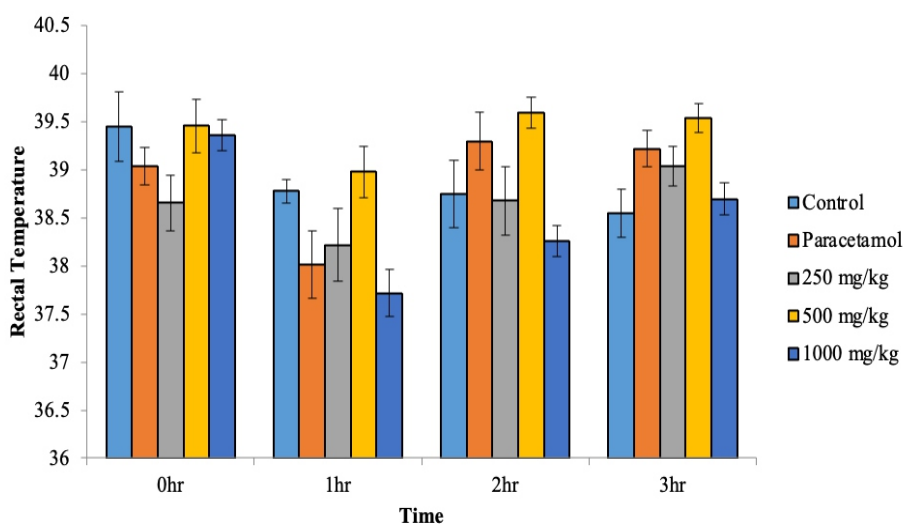
**Table 2: Tail Flick Profile**

Grouping	Latency (Seconds)
Control	2.75 ± 0.2
Paracetamol	2.90 ± 0.26
250 mg/kg	2.07 ± 0.03*
500 mg/kg	1.87 ± 0.32*
1000 mg/kg	2.53 ± 0.12

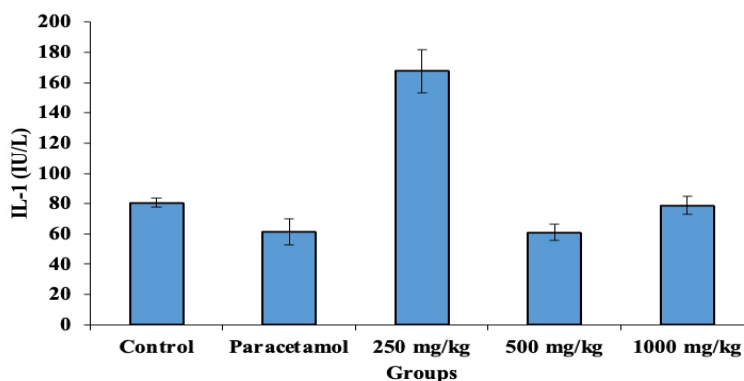
**Table 3 : IL-1 Profile**

Grouping	IU/L
Control	80.66 ± 2.99
Paracetamol	61.43 ± 8.4
250 mg/kg	167.64 ± 14.37^
500 mg/kg	61.04 ± 5.28
1000 mg/kg	78.86 ± 6.07

\*In comparison to 250 mg/kg



**Figure 1: The effect of ethanol leaves extract of *P. acuminata* on rectal temperature in mice with Brewer's yeast-induced pyrexia.**



**Figure 2: The effect of ethanol leaves extract of *P. acuminata* on IL-1 activity in mice with Brewer's yeast-induced pyrexia.**

## DISCUSSION

Plants and plant parts are used in ancient African folk medicines to treat various ailments, and they are an important part of African traditional medicine. Herbs are used in almost all ancient civilizations to treat diseases and revitalize the body system, according to research.<sup>[19]</sup> In this study, the antipyretic and antinociceptive effects of ethanol leaves extract of *P. acuminata* were evaluated using writhing test, tail immersion test, and yeast induced pyrexia Wistar rat models.

In our study, we observed that ethanol leaves extract of *P. acuminata* had antipyretic and antinociceptive effects on yeast-induced pyrexia animals by decreasing interleukin 1 (IL-1) levels in a dose-dependent manner. In a related study<sup>[1]</sup>, the acetic acid-induced writhing test was used, which is a widely used classical animal model of pain because acetic acid causes peritoneal inflammation. Similarly, a study found that the methanol extract of *P. acuminata* has potent anti-inflammatory activity in both acute and chronic models. Carrageenan, on the other hand, was used to cause paw oedema in the experimental animals.<sup>[10]</sup> The tail flick or tail immersion test is used to evaluate agents with central analgesic or antinociceptive activity. According to the findings of this study, *P. acuminata* did not exert its analgesic effects centrally.

Yeast-induced pyrexia (pathogenic fever) acts by increasing prostaglandin synthesis and is thus one of the useful models for evaluating the antipyretic effect of plant materials.<sup>[20]</sup> Because biomarkers such as IL-1 and pathogenic fever are among the characterized endogenous pyrogens, inhibition or reduction of IL-1 levels, and restoration of an abrupt increase in core rectal temperature within the normal range, indicate antinociception and antipyretic effects.<sup>[21]</sup> According to our findings, ethanol extract of *P. acuminata* reduced elevated body temperature when compared to the standard drug paracetamol. The extract's possible mechanism of antipyretic action could be

attributed to prostaglandin inhibition, similar to paracetamol's mechanism of action by inhibiting cyclo-oxygenase enzyme activity.<sup>[21]</sup> After demonstrating that antipyretics suppress fever by inhibiting prostaglandin synthetase, resulting in a blockade of prostaglandin synthesis in the brain or suppressing the rise of interleukin-1 production, it was discovered that there are several mediators for pyrexia, and inhibition of any one of the mediators can be responsible for the antipyretic effect.<sup>[10,22]</sup>

Oral administration of *P. acuminata* ethanol extract significantly restored the rectal temperature of yeast-induced pyrexia in our experimental animals to normal, and this effect is comparable to that of the standard drug, paracetamol. As a result, we hypothesize that the ethanol extract of *P. acuminata* contains pharmacologically active analgesics that interfere with prostaglandin release. This may be due to the presence or synergistic action of the various bioactive agents identified in the extract's qualitative phytochemical analysis, particularly the flavonoids and saponins. It has been demonstrated that flavonoids, such as baicalin, have an antipyretic effect that targets prostaglandins, which are responsible for pyrexia, and accomplishes this by suppressing TNF- $\alpha$ .<sup>[23], [24]</sup> Furthermore, related compounds inhibit the peroxidation of arachidonic acid, resulting in a reduction in prostaglandin levels and, as a result, a reduction in fever and pains.<sup>[25]</sup> Our findings are consistent with other similar studies<sup>[26, 27]</sup>, in which flavonoids and saponins are thought to work synergistically to exert the observed pharmacological activity against prostaglandin release.

## CONCLUSION

Our findings show that ethanol leaves extract of *P. acuminata* reduces pyrexia by inhibiting the release of the inflammatory mediator IL-1, which may be associated with the suppression of pathogenic fever. The findings shed light on the potential clinical benefits of the extract for the treatment of painful conditions and inflammatory fever, as it has potential anti-inflammatory and

analgesic properties.

### Funding

This work did not receive any external funding.

### Conflicts of interest

There are no conflicts of interest to disclose

### REFERENCES

- Liu C, Hongchang SU, Hongye W, Qingxia Q, Xuan WU, Xiangying Kong, Na Lin. Forsythoside A exerts antipyretic effect on yeast-induced pyrexia mice via inhibiting transient receptor potential vanilloid 1 function. *Int. J. Biol. Sci.* 2017; 13(1): 65-75.
- Goldberg DS, McGee SJ. Pain as a global public health priority. *BMC Public Health* 2011; 11:770-780
- Crofford LJ. Adverse effects of chronic opioid therapy for chronic musculoskeletal pain. *Nat. Rev. Rheumatol.* 2010; (6) 191–197.
- Lamplot JD, Wagner ER, Manning DW. Multimodal pain management in total knee arthroplasty: a prospective randomized controlled trial. *J. Arthroplasty* 2014; 29(2): 329-334.
- Sandhu T, Paiboonworachat S, Ko-Iam W. Effects of preemptive analgesia in laparoscopic cholecystectomy: a double blind randomized controlled trial. *Surg. Endosc* 2011; 25 (1): 23–27.
- Lee BH, Park JO, Suk KS, Kim TH, Lee HM, Park MS, Lee SH, Park S, Lee JY, Ko SK, Moon SH. Pre-emptive and multi-modal perioperative pain management may improve quality of life in patients undergoing spinal surgery. *Pain Physician* 2013; 16(3): 217–226.
- Tsukada S, Wakui M, Hoshino A. Pain Control after Simultaneous Bilateral Total Knee Arthroplasty. *J. Bone Joint Surg. Am* 2015; 97(5): 367-373.
- Williams DP, O'Brein S, Doran E, Price AJ, Beard DJ, Murray DW, Beverland DE. Early postoperative predictors of satisfaction following total knee arthroplasty. *Knee* 2013; 20 (6): 442–446.
- Society KK. Guidelines for the management of postoperative pain after total Knee Arthroplasty. *Knee Surg. Relat. Res.* 2012; 24(4): 201–207.
- Gupta M, Mazumder UK, Gomathi P, Thamil SV. Anti-inflammatory evaluation of leaves of *Plumeria acuminata*. *BMC Comp. Alt. Med.* 2006; 6: 36-43.
- Elisabetsky E, Amodor TA, Albuquerque RR, Nunes DS, Carvalho ACT. Analgesic activity *Psychotria colorata* (Wild ex R. & S.) Muell Arg. *Alkaloids. J Ethnopharmacol* 1995; 48:77-83.
- Ashraf MD Farooque AMD, Shambhawe MAS and Mazumder R. Review on *Plumeria acuminata*. *Int. J Res. Pharm Chem.* 2012; 2(2): 467-469
- Adim EU, Dingwoke EJ, Adamude FA, Edenta C, Nwobodo NN, Offiah RO, Onyeka IP, Ezeaku IN. Bacteriostatic and Bactericidal effects of ethyl acetate root bark extract of *Terminalia avicennioides* on Methicillin-resistant *Staphylococcus aureus*. *Afri. J Biochem. Res.* 2018; 12(5):45-54
- Lorke D. A New Approach to Practical Acute Toxicity Testing. *Arch. Toxicol.* 1983; 54(4): 275-287.
- Tanko YA, Mohammed MA, Okasha MA, Umar AH, Magaji RA. Anti-nociceptive and anti-inflammatory activities of ethanol extract of *Syzygium aromaticum* flower bud in wistar rats and mice. *Afr. J Tradit. Complement Altern. Med.* 2008; 5(2): 209-212.
- Parle M, Yadav M. Laboratory models for screening analgesics. *Int. Res. J Pharm.* 2013; 4(1): 15-19.
- Chopra K, Tiwari V, Arora V, Kuhad A. Sesamol suppresses neuro-inflammatory cascade in experimental model of diabetic neuropathy. *J. Pain* 2010; 11(10): 950-957.
- Akapa TC, Kehinde AO, Beatrice OO, Olajide OJ. Antipyretic Activity of *Abutilon mauritianum* (Jacq.) Roots in Wistar Rats. *Int. J Pharm. Sci. Res.* 2014; 5(2): 42-46.

19. Janakiram A, Glara AF. Evaluation of antipyretic activity of “Anjanavaerathichooranam” in yeast induced pyrexia: A critical analysis. *Int. J. Med Health Res.* 2018; 4(2): 104-108.
20. Zhang T, Leng Z, Liu W, Wang X, Yan X, Yu L. Suppressed expression of mitogen-activated protein kinases in hyperthermia induced defective neural tube. *Neurosci. Lett.* 2015; 594: 6-11.
21. Chandrasekharan NV, Simmons DL. The Cyclo oxygenases, *Gen. Biol.* 2004; 5: 241-251.
22. Yin ZY, Li L, Chu SS, Sun Q, Ma ZL, Gu XP. Antinociceptive effects of dehydrocorydaline in mouse models of inflammatory pain involve the opioid receptor and inflammatory cytokines. *Sci. Rep* 2016; 6:27129
23. Rajnarayanan K, Reddy MS, Chaluvadi MR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutical potential. *Indian J Pharmacol* 2006; 68:380-384.
24. Adesokan AA, Yakubu MT, Owoyele BV, Akanji MA, Soladoye AO, Lawal O. Effect of administration of aqueous and ethanol extracts of *Enantia chlorantha* stem bark on brewer's yeast induced pyresis in rats. *Afr J Biochem* 2008; 2(7):165-169.
25. Germain ST, Elisabeth NB, Theophile D, Emmanuel T, Norbert W, Neteydji S, Amadou D, Fleur COM, Paul D, Michel W. Antipyretic and antinociceptive effects of *Nauclea latifolia* roots decoction and possible mechanisms of action. *Pharm Biol.* 2011; 49(1):15-25.
26. Zakaria Z, Loo YW, Nurul IAR, Abdul HAA, Mohd RS, Hanan KG. Antinociceptive, Anti-Inflammatory and Antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals. *Med Princ Pract* 2007; 16:443-449.
27. Gautham SA, Onkarappa R. Pharmacological activities of metabolite from *Streptomyces fradiae* strain GOS1. *Int J Chem Sci* 2013; 11(1):583-590.