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ANTIPYRETIC ACTIVITY OF *CHRYSANTHEMUM INDICUM* FLOWERS EXTRACTS

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ABSTRACT

Chyrsanthemum indicum is also known as 'bunga kekwa' in Malay and 'ye ju hua' in Chinese. In Chinese traditional medicine, *C. indicum* has been used as anti-inflammatory, analgesic, antipyretic, and for the treatment of eye diseases. The present study was aimed to scientifically evaluate the antipyretic activity of *C. indicum* flowers. Water and ethanol extracts of *C. indicum* flowers were prepared using cold maceration method. The extracts were subjected to qualitative preliminary phytochemical analysis to identify the presence of secondary metabolites. The results of ethanol extracts showed presence of alkaloids, carbohydrates, glycosides, oils, fats, terpenoids, flavonoids, and volatile oils whereas constituents in the water extract also found to be the same as ethanol extract except the oils and fats. The antipyretic activity was evaluated by yeast-induced pyrexia model in wistar albino rats. Water and ethanol extracts of *C. indicum* flower were administered orally to the pyrexia-induced rats at a dose of 200 mg/kg body weight. The antipyretic activity was assessed by measuring the rectal temperature of rats using digital thermometer. Temperatures were recorded before and after the induction of yeast and after oral administration of extracts and standard drug (paracetamol 150 mg/kg) at 1h, 2h, 3h, 4h, 5h and 6h. The results showed that both water and ethanol flower extracts of *C. indicum* exhibits highly significant ($p<0.01$) antipyretic effect by decreasing the elevated rectal temperature in yeast-induced pyrexia at a dose of 200 mg/kg. These results proclaimed support scientifically the antipyretic effect of *C. indicum* flowers.

KEYWORDS

Chrysanthemum indicum flowers, Antipyretic, Extracts and Yeast-induced pyrexia.

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INTRODUCTION

Herbal products are recognized as safe and play a crucial role as an alternative medicine and also it is common element in Ayurvedic, Homeopathic, Naturopathic, siddha, Traditional Chinese medicine, as well as Native American medicine[1]. As medicinal plant consists of various chemical compounds, it plays a major role as therapeutic agents in the treatment of human diseases. For

thousands of years, medicinal plants have been used by traditional practitioners especially in the rural areas in developing countries. In several parts of the world they still used it as a primary source of medicine[2].

The secondary impact of infection, malignancy or other disease state increases the body temperature above normal in humans and this condition is called pyrexia. This occurs because of the body's defense mechanism which is the ability to create an environment free either from any infectious mediator or damaged tissue[3]. The symptoms of fever include headache, feeling very cold compared to normal person, anorexia, dehydration, depression, shivering, hyperalgesia, sleepiness, and sweating. The symptoms when fever is high are extreme irritability, confusion, delirium and convulsion[4]. Antipyretic drugs play a role in reducing the elevated body temperature by inhibiting COX-2 expression. It works by inhibiting production of prostaglandin E2 (PGE2). Moreover, it acts as an irreversibly high selective COX-2 inhibitor. However, it is toxic to the glomeruli, cortex of the brain and heart muscles as well as hepatic cells. Even though it has lesser side effects, the natural COX-2 inhibitors have lesser selectivity. This strongly suggest that finding a natural antipyretic agent with fewer or zero toxicity is necessary. Furthermore, the cost for health care is continuously rising and it is the main reason that low cost remedies such as herbal medicine are more preferred[3].

Chrysanthemum is large and diverse genus and one of the asteraceae family members. It can be found worldwide in variety of species, the original species are from East Asia. Chrysanthemum are thought to be 'cool' in the yin and yang of Chinese cuisine. It is believed that by eating Chrysanthemum longevity can be increased. It is also believed that the white hair will turn into black hair, teeth will grow back and also make body feel energetic by eating the roots, stems and flowers. As the flowers are understood to relieve sore throats and reduce fever and also the dried chrysanthemum flower petal are often made into tea[5].

Chrysanthemum indicum flower is also known as 'bunga kekwa' in Malaysia and 'ye ju hua' in Chinese. In Chinese traditional medicine, *C. indicum* has been used to reduce pain, fever, inflammation and to treat eye diseases. *C. indicum* flowers in Japanese pharmacopeia are listed as plant used for treatment of vertigo and eye inflammation. In Indochina and China, the leaves possess purifying and detoxifying effects besides the anti-migraine activity. In Vietnam. *C. indicum* flowers are also used externally for skin diseases and itchiness of the skin. For infection of cervix, the flowers are made into emulsion and applied on the affected parts. It is also being used by women to treat hysteria and pre-menstrual problems in Guam. In Malaysia, *C. indicum* are commonly used to treat fever and headaches and the whole plant or flower are used to treat whooping cough. Malaysians use the flower to treat sore eyes while Indians use it as a stomachic and laxative[6]. The literature review revealed that the various parts of *C. indicum* possess anticancer, antioxidant, cardio protective, antihypertensive, antidiabetic, otoprotective, antiosteoporotic, antiplasmodial, analgesic, anti-inflammatory, anti-allergy, antimicrobial, antiviral and larvicidal activities[7,8]. Yet there was no scientific study about the antipyretic activity of *C. indicum* flowers extracts. Therefore the present investigation was undertaken to determine the therapeutic efficacy of extracts of *C. indicum* flowers in reducing the elevated body temperature.

MATERIAL AND METHODS

Plant Material

The whole plant of *C. indicum* was purchased from Paling Horticulture, Green Lane, Sungai Buloh, Selangor in the month of July, 2017 and authenticated by Dr. Mohd Firdaus Ismail, Biodiversity Unit, Institute of Bioscience, University Putra Malaysia (UPM). The voucher specimen was deposited at the herbarium of Institute of Bioscience, UPM. (Reference No: UPM/IBS/UB/H69/17).

Preparation of extracts

The flowers of *C. indicum* were washed thoroughly with tap water and dried under shade. The dried *C. indicum* flowers pulverized into coarse powder and divided into two portion. Then it was extracted with ethanol and water separately by cold maceration technique for 6 days. The extracts were collected separately by filtration and concentrated at 40ºC -

50ºC using rotary vacuum evaporator under reduced pressure[9]. The colour, consistency, and the percentage yield of both ethanol and water extracts were recorded. All the extracts were stored in desiccator until further use.

Qualitative Phytochemical Analysis

C. indicum flowers extracts were subjected to qualitative phytochemical screening using suitable chemical tests to identify the presence of secondary metabolites such as alkaloids, carbohydrates, proteins, fixed oils and fats, seponins, terpenoids, flavonoids, steroids and volatile oils[10,11]. The results are presented in Table No.1.

Evaluation of Antipyretic Activity

Animals

Wistar albino rats (150–200 g) of either sex were acquired from KPJUC vivarium, KPJ Healthcare University College, Nilai, Negeri Sembilan. All rats were housed in plastic cage with commercial pellet food and drinking water. The animals were maintained under room temperature (25 ± 2ºC), 12 h light/12 h dark cycle and 35-60% relatively humidity at the animal house. This study was approved by Institutional Animal Ethics Committee (IAEC), KPJ Healthcare University College, Nilai, Negeri Sembilan (Reference No: KPJUC/RIC/BPS/EC/2017/63). All the experiments were performed in accordance with the Code of Practice for the Care and Use of Animal for Scientific Purpose.

Screening of antipyretic activity

The antipyretic activity was assessed on experimental rats. The animals were divided into four groups (n=6). Animals in Group I were served as control and received only distilled water. While group II was treated with standard drug, paracetamol (150 mg/kg*/p.o*). Group III and IV received water and ethanol extracts (200 mg/kg/*p.o*) respectively. The rectal temperature was noted before and after administration of the extracts and standard drug. 20% w/v Brewer's yeast suspension (10 ml/kg) was injected subcutaneously in the back below the nape of the neck of the animals except the normal control group. After 19 hours of yeast administration, the rectal temperatures were noted. The animals which showed an increase of 0.3 to 0.5ºC in rectal temperature were only used for this study. *C. indicum* extracts (200 mg/kg) and standard drug (150 mg/kg) was administered orally to the respective groups of animals and rectal temperature was measured using digital thermometer at 1h, 2h, 3h, 4h, 5h, and 6h[12,13] and recorded in Table No.2.

Statistical Analysis

The data is expressed by mean ± SEM. Statistical significance was analyzed by one way ANOVA followed by Dunnet 'T' test using SPSS software (V. 20) at significant levels of 0.01 and 0.05[12].

RESULTS AND DISCUSSION

Characteristics and Percentage Yield of Extracts

The colour, consistency and percentage yield of water and ethanol extracts of *C. indicum* flower were noted. Water extract was brown whereas ethanol extract was dark green in colour. The consistency of water extract of *C. indicum* flower was sticky semi solid while ethanol extract were semi solid in nature. Ethanol extract showed highest percentage yield (6.56%) than water extract (4.32%).

Qualitative Phytochemical Analysis

Table No.1 shows the presence of phytochemicals in water and ethanol extracts of *C. indicum* flowers. The qualitative phytochemical analysis showed that the presence of alkaloids, carbohydrates, fats, terpenoids, flavonoids, and volatile oils while absence of oils/resins, phytosterol, polyphenol, steroid, protein and amino acids were observed in both extracts but oils and fats were only found in ethanol extract.

Antipyretic Activity

Table No.2 shows the antipyretic activity of both water and ethanol extracts of *C. indicum* against brewer's yeast induced pyrexia method in experimental rats. The antipyretic activity of *C. indicum* was evaluated by measuring the rectal temperature in yeast-induced pyrexia rats after the treatment with water and ethanol extracts (200mg/kg) at 1h, 2h, 3h, 4h, 5h and 6h. All the results were well comparable with standard drug, paracetamol (150mg/kg). In the 1st h, no antipyretic activity was observed in the experimental animals, while in the 2nd and 3rdh, a decreased in rectal temperature was observed in the group treated with water extract but not was significant. Nevertheless, the animals treated with ethanol extract exhibited significant ($p < 0.05$) antipyretic effect at 2nd and 3rd

h. In the 4th, 5th and 6th high significant ($p<0.01$) antipyretic effect was noted. Though antipyretic effect was observed on all the experimental rats that were treated with water and ethanol extracts, the ethanol extract of *C. indicum* flower presented effective antipyretic activity than water extract. The antipyretic effect of ethanol extract started from 2nd h, whereas water extract showed antipyretic effect at the 4th h only.

DISCUSSION

The body usually responds to any invasion which may be caused by microorganism, is called pyrexia. Pyrexia occurs when defense mechanism in the body is combating an infection. The defense mechanism is mainly due to white blood cells (WBC), which are active in responding to such invasion and infections. WBCs release the substances known as cytokines and interleukins that are responsible in increasing body temperature. The elevated body temperature causes increase in enzymatic reaction and cellular processes associated with the non-specific immune response of the body[14].

Stimulation of pro-inflammatory mediators such as interleukin-1β, interleukin-α, interleukin-β, and TNF- α, usually happen when there is an infection or damage. Consequently, the stimulation increases the synthesis of PGE2 near hypothalamic area and thus triggering the hypothalamus to increase the body temperature. Whereas both TNF-α and prostaglandin synthesis are induced by brewer's yeast hence the test drug reduces the elevated body temperature by inhibiting the synthesis of high level of prostaglandin E2 at the same time maintaining the constant body temperature level[15].

The activation of arachidonic acid pathway occurs when cytokine migrates into circumventricular organs of the brain and bind with either endothelial receptors on vessel walls or local microglial cells. Arachidonic acids pathway stimulates the synthesis of prostaglandin E2 (PGE2). Phospholipase A2, cyclo-oxygenase-2 (COX-2) and PGE2 synthase are enzymes found in this pathway which are in charge of producing PGE2. The final mediator for fibrile response is the PGE2, it also stimulates the hypothalamus to produce more formation of heat by minimizing the heat loss through cyclic adenosine mono-phosphate (cAMP) pathway[16].

In the present study, both water and ethanol extracts of *C. indicum* flowers exhibited effective antipyretic effect by decreasing the elevated temperature in yeast-induced pyrexia at a dose of 200 mg/kg. The antipyretic activity was well comparable with standard drug, paracetamol (150 mg/kg). It was demonstrated that the mechanism of action of aspirin and other NSAIDs are inhibiting the formation of prostaglandins, as well as local factors that are associated with pain, fever and inflammation. Nonetheless, paracetamol does not inhibit prostaglandin synthesis although its action is similar to those of the NSAIDs. Only now, the mechanism for the basic pharmacological effects of paracetamol has become clear and it is now recognized to be an inhibitory of prostaglandin synthesis in cellular systems and a selective cyclooxygenase-2 (COX-2)[17].

The qualitative phytochemical analysis of ethanol extract of *C. indicum* flower revealed the presence of alkaloids, carbohydrates, glycosides, oils, fats, terpenoids, flavonoids, and volatile oils except, phytosterol, polyphenol, steroid, protein and amino acids. Water extract's phytochemical analysis showed the same constituents as of ethanol extract except that oils and fats. Presence of compounds such as flavonoids also suggests inhibition of arachidonic acid peroxidation, hence reducing fever by lowering prostaglandin levels[18] as flavonoids are well known to inhibit prostaglandin synthetase[13].

Moreover, it is well known that flavonoids are able to inhibit pain perception and also possess anti-inflammatory properties as they have an ability to inhibit the enzymes that stimulates the production of the chemical mediator of inflammation[19]. Flavonoids, terpenoids, steroids of many plants has been reported to possess anti-inflammatory, analgesic and antipyretic activity[20]. Steroids, tannins, triterpenoids, and coumarin glycosides are also reported to possess potential antipyretic properties[16]. Alkaloids are found to possess antipyretic activity in the roots of *Berberis* species on FCA-induced increased rectal temperature on sub-acute administration[21]. The presence of alkaloid in the water and ethanol extracts may also

contributed to the antipyretic activity of *C. indicum* flower.

The presence of flavonoids, terpenoids, and phenolic compounds in *C. indicum* flowers were identified. It was also found that *C. indicum* suppress the release of interleukin-β, and TNF- α. Therefore, these inhibitory effects of the flowers may be due to the presence of compounds that suppress the inflammatory response[22].

Since flavonoids also produce other biological effects such as anti-inflammatory, antimicrobial, hepatoprotective as well as anti-ulcer activities, hence it is possible that the antipyretic action of *C. indicum* flower extracts is due to the presence of flavonoids.

Table No.1: Phytochemicals present in the extracts of *C. indicum* flowers

S.No	Phytoconstituents	Water extract	Ethanol extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Protein and amino acids	-	-
4	Oils and fats	-	+
5	Volatile oils	+	+
6	Terpenoids	+	+
7	Flavonoids	+	+
8	Steroids	-	-
9	Phytosterols	-	-
10	Polyphenols	-	-
11	Oils/resins	-	-

+ = present; - = absent

Table No.2: Antipyretic activity of extracts of *C. indicum* flowers in Wistar albino rats

S.No	Treatment	Rectal Temperature After Treatment (ºC) (Mean ± SEM)							
		before inducing pyrexia	after inducing pyrexia	1st H	2nd H	3rd H	4th H	5th H	6th H
1	Control	36.4±0.16	36.6±0.12	36.4±0.10	36.6±0.07	36.4±0.13	36.2±0.07	36.4±0.08	36.3±0.05
2	Standard Drug (150 mg/kg)	36.6±0.20	37.8±0.23	37.8±0.21	38.0±0.21	37.4±0.18	35.8±0.20**	35.6±0.16**	35.6±0.13**
3	Water Extract (200 mg/kg)	36.1±0.24	37.4±0.28	37.5±0.35	37.5±0.30	37.0±0.24	36.0±0.06**	35.4±0.05**	35.4±0.06**
4	Ethanol Extract (200 mg/kg)	36.6±0.17	37.6±0.14	37.8±0.14	37.6±0.11*	37.2±0.08*	36.4±0.11**	36.0±0.12**	35.8±0.08**

All values are expressed as mean ± SEM; (n=6); statistical significance were calculated by one way ANOVA followed by Dunnet 'T' test, when compared to the control where *p<0.05 is significant and **p<0.01 is highly significant.

CONCLUSION

The present study reveals the antipyretic activity of *C. indicum* which might be due to the presence of flavonoids, alkaloids and terpenoids. These results scientifically supports the antipyretic effect of *C. indicum* flowers and can be used as natural antipyretic agent to reduce fever. However, further studies are necessary to isolate the bioactive phytoconstituents and to predict the exact mechanism of action to develop the efficacious natural antipyretic agent.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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