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Original Research Article

Anti-inflammatory Activity of *Impatiens balsamina* Roots and Stem

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Abstract: Medicinal plants and their products play a vital role in human health for the treatment of inflammatory conditions. The validity of claims of many of these preparations has been substantiated by modern scientific methods and techniques. This present study was aimed to evaluate the anti-inflammatory activity of *Impatiens balsamina* roots and stems. Ethanol and water extracts of *I. balsamina* roots and stems were separately prepared by cold maceration method. The anti-inflammatory activity was evaluated using carrageenan induced paw edema method in wistar albino rats. Extracts at a dose of 50mg/kg was orally administered to the experimental rats 30mint prior to administration of carrageenan (0.1ml of 0.1%w/v). The paw volume was measured using digital plethysmometer at specific intervals. All three extracts exhibited significant anti-inflammatory activity. Ethanol extract of *I. balsamina* roots and ethanol extract of *I. balsamina* stems showed high significant ($p<0.001$) anti-inflammatory activity followed by water extract of *I. balsamina* stems at the 2nd hour. Ethanol extract of *I. balsamina* roots exhibited high spectrum of anti-inflammatory activity than ethanol extract of *I. balsamina* stems. The anti-inflammatory action of *I. balsamina* may be due to the presence of alkaloids, glycosides, terpenoids, flavonoids, phenolic compounds and tannins in the extracts. The findings suggest that the extract of *I. balsamina* roots and stems explore anti-inflammatory activity and leads the further development of novel natural anti-inflammatory agent.
Keywords: *Impatiens balsamina*, roots, stem, anti-inflammatory, carrageenan induced paw edema.

INTRODUCTION

Inflammation is a significant element of the body's immune response. After an injury happened, it is the body's effort to repair, guard itself against foreign particles, such as bacteria and viruses as well as repair the damaged tissues. Wounds or cuts would also irritate and can cause infections without inflammation. Usually inflammation is categorised by warmth, swelling, redness, and at times pain and small immobility. Biochemical processes discharge proteins called cytokines as "emergency signals" during injury that bring in body's immune cells, hormones and nutrients to repair the problem [1].

In today's phenomena due to the imbalance in eating and living habits of human being, health practitioners face many challenging problem in saving human life from various illness. Many herbal preparations have been found in the pharmacopeia for the treatment of various human ailments. The validity of claims of many of these preparations has been substantiated by modern scientific methods and techniques. Now herbal preparations are actively use in the modern clinical practice [2].

Malaysia is gifted with huge natural products as the resource for the medicinal plants. Also, Malaysia is rich in traditional practice modalities and the herbal medicines are used based on practical experiences, observations and rituals derived from socio religious beliefs passed down from one generation to another [3]. *Impatiens balsamina* is belonging to the family, Balsaminaceae and commonly known as Balsam plant in English, Pokok Keembung in Malay, Kasi Thumbai in Tamil and Tou Gu Cao in Chinese [4]. It has cathartic, diuretic, emetic, anti-haemorrhoidal and antibacterial properties [2]. Moreover, the leaves are crushed and used as a poultice on wounds and skin inflammations. They are combined with salt and castor oil to make a poultice that is packed around a finger affected by whitlow; the whitlow disappears in a short time [5]. The previous study revealed that the significant analgesic and anti-inflammatory effects of water extract of *Impatiens balsamina* leaves [6]. There is no scientific report for the anti-inflammatory effect of

Impatiens balsamina roots and stems. Hence, the present study was undertaken to explore and prove scientifically the anti-inflammatory property of *I. balsamina* roots and stems in experimental animals.

MATERIALS AND METHODS

Plant material

The fresh roots and stems of *Impatiens balsamina* was obtained from the plant nursery, Pasir Gudang, Johor, Malaysia in the month of May 2016. The plant materials were identified and authenticated by Pharmacognosist, KPJ Healthcare University College, Nilai, Malaysia. (KPJUC/CRI/PA/2016(06))

Preparation of extracts

The roots and stems of *I. balsamina* was washed thoroughly, shade dried, pulverized into coarse powder. Then it was equally divided into four portions and extracted with ethanol and water separately by cold maceration technique using for 6 days. The extracts was collected separately and concentrated at 40 ^0C – 50 ^0C using rotary vacuum evaporator under reduced pressure [7]. The colour, consistency and the percentage yield was noted and tabulated. All the extract of *I. balsamina* roots was stored in dessicator until future use.

Animals

Wistar albino rats (150-200g) were procured from KPJUC Vivarium, Nilai, Malaysia and kept in plastic cage. The animal were maintained under room temperature (25±4 ^{0}C), 12 h light/12 h dark cycle and 35-60% relatively humidity at the animal house. The animal had free access to the food which is a commercial pellet and drinking water. The study was approved by Institutional Animal Ethics Committee (IAEC), KPJUC, Nilai, Malaysia (KPJUC/CRI/BPS/EC/2016/24). All the experiments were performed in accordance with the Code of Practice for the Care and Use of Animal for Scientific Purpose.

Screening of Anti-inflammatory activity

Wistar albino rats (150-200g) were used for this study. The animal were divided into six groups (n=6) and fasted for 24h prior to the experiment. The anti-inflammatory activity of various extracts of *I.balsamina* roots and stems was assessed by carrageenan-induced hind paw edema method [8] with slight modification using digital plethysmometer. Prior to any treatment, each rat was weighed properly and the initial paw volume was noted for each rat. Carrageenan was administered in subplantar region of right hind paw of each mice in every group except normal control. Animals in group 1 received 0.5% Sodium CMC (10ml/kg) and served as normal control group. Animals in Group 2 received standard drug, diclofenac sodium (10mg/kg b.wt.) and animals in group 3 – 6 received the respective extract (50mg/kg b.wt.) orally 30 min prior to administration of carrageenan (0.1 ml of 1% w/v). The paw volume was measured at 30, 60, 120, 180 and 240 min after administration of extract and standard drug. The anti-inflammatory activity was determined by observing the paw volume after two hours of carrageenan administration.

Statistical Analysis

The results will be expressed by mean ± SEM. Statistical significance were analyzed by ANOVA followed by Dunnet 'T' - test, the statistical analysis were conducted with SPSS software (v.20, SPSS, USA) at significant levels of 0.001, 0.01 and 0.05 [9].

RESULTS

All the extracts were clearly distinguished by their physical data (Table 1). Among all the extracts, ethanol extract of *I. balsamina* root (EEIBR) had the highest percentage yield (9.74%) followed by water extract of *I. balsamina* stem (WEIBS) 7.28% and ethanol extract of *I. balsamina* stem (EEIBS) (5.86%). But the yield of water extract of *I. balsamina* root (WEIBR) was very low (0.64%).

Table 1: Colour, percentage yield and consistency of various extracts of *I. balsamina*

Extracts	Colour	Percentage yield	Consistency
EEIBR	Dark brown	9.74%	Sticky
WEIBR	Dark brown	0.64%	Semisolid
EEIBS	Dark brown	5.86%	Mucilageous
WEIBS	Dark brown	7.28%	Solid

The different qualitative chemicals tests were performed for establishing profile of the extracts of *I. balsamina* roots and stem to identify the phytoconstituents presence. Alkaloids, carbohydrates and glycosides, terpenoids, flavonoids and phenolic compounds and tannins are present in all the extracts tested. The results were tabulated in Table 2.

Table 2: Preliminary phytochemical screening of various extracts of *I. balsamina*

Phytoconstituents	EEIBR	WEIBR	EEIBS	WEIBS
Alkaloids	+	+	+	+
Carbohydrates & Glycosides	+	+	+	+
Proteins & Amino acids	-	-	-	-
Oils & Fats	-	-	-	-
Saponins	-	-	-	-
Terpenoids	+	+	+	+
Flavanoids	+	+	+	+
Sterols	-	-	-	-
Phenolic compounds & Tannins	+	+	+	+
Gums & Mucilage	-	-	-	-

In the present study, EEIBR, WEIBS and EEIBS exhibited the reduction in paw volume induced by carrageenan induced paw edema (Table 3). After the injection of carrageenan in the right hind paw of each animal, a gradual increase in paw volume was observed in the right hind paw of each animal up to 1 hour. There was no change in paw volume in control group. The experimental animals treated with test extracts except WEIBR exhibited anti-inflammatory activity at 2nd h onwards. At 2nd, 3rd and 4th h, EEIBS showed high significant ($p<0.001$) anti-inflammatory activity followed by EEIBR ($p<0.01$) and WEIBS showed less significant ($p<0.05$) anti-inflammatory activity. There was no anti-inflammatory activity was observed in the experimental animals treated with WEIBR. All the results were well comparable with standard drug, diclofenac sodium.

Table 3: Anti–inflammatory effect of different extracts at *I. balsamina* against Carrageenan induced paw edema

Treatment	Paw volume before inject Carrageenan	Paw volume after inject Carrageenan	Paw volume after treatment with extracts (ml) (Mean±SEM)				
			30min	1h	2h	3h	4h
Control	0.41 ± 0.04	0.41 ± 0.24	0.41 ±0.39	0.42 ± 0.48	0.43 ± 0.15	0.41 ± 0.11	0.43 ± 0.14
Standard drug	0.37	1.03 ± 0.09	1.12 ± 0.03	1.28 ± 0.16	0.97 ±0.11*	0.99±0.14*	0.73 ± 0.04*
Ethanol extract (Root)	0.36	0.96 ± 0.38	1.02 ± 0.15	1.04 ± 0.27	0.99 ±0.38*	0.91±0.52*	0.71±0.11***
Water extract (Stem)	0.39	0.89 ± 0.14	0.94 ± 0.19	0.98 ± 0.03	0.91 ±0.08*	0.91±0.11*	0.64 ±0.11**
Ethanol extract (Stem)	0.42	1.02 ± 0.12	1.25 ±0.6	0.98 ± 0.04	0.89±0.07**	0.73±0.06**	0.68 ±0.13**

Values are mean ± S.E.M.; **P*<0.05, ***P*<0.01, ****P*<0.001 statistically significant between control group, n=6

DISCUSSION

Anti-inflammatory activity of natural products in animal models was assessed by inducing the inflammatory mediators using external stimuli such as egg white, dextran, histamine, carrageenan and formalin. Inflammation induced by carrageenan is one of the suitable experimental models to establish the oral anti-inflammatory agents [8]. The first phase of inflammation which is observed during the first and second hour is attributed to a release of histamine and serotonin due to administration of carrageenan and the second phase is due to a release of prostaglandin like substances such as cyclooxygenase products and lipoxygenase products [10]. Cyclooxygenase and lipoxygenase pathways form metabolites of arachidonic acids. Thus, two important types of inflammatory mediators which is prostaglandins particularly prostaglandin E2 (products of the cyclooxygenase pathway) and leukotriene B4 (product of lipoxygenase pathway) are formed. The standard drug diclofenac sodium is a cyclooxygenase inhibitor and inhibits the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against Carrageenan induced paw edema [8]. In the present study, the inhibition of Carrageenan induced paw edema by the ethanol and water extracts of *I. balsamina* roots and stem might be due to its inhibitory activity on the lipoxygenase enzyme.

The various spectrum of observed anti-inflammatory activity of the extracts of *I. balsamina* roots and stem depends on the concentration of these phytoconstituents. The amount of active phytochemicals extracted from plants was greatly relied on the type of solvents used for extraction procedure because every phytochemical constituent has its own solubility competency in different solvent [11]. In the present study, the preliminary phytochemical screening

on the extracts of *I. balsamina* roots and stem revealed that the presence of alkaloids, carbohydrates and glycosides, terpenoids, flavonoids and phenolic compounds and tannins. The phytoconstituents present in *I. balsamina* roots and stem might be responsible for the anti-inflammatory activity observed. An earlier study supports that the secondary metabolites present in the plant inhibit the inflammatory pathways in a similar manner as NSAIDS [12]. An another study stated that the herbal constituents such as phenolic compounds, condensed tannins, gallotannins, flavonoids, alkaloids, saponins, sterols, terpenoids and essential oils are being prescribed widely for the treatment of inflammatory conditions [13].

Secondary metabolite, diterpenoids present in the medicinal plants exhibited anti-inflammatory action due to their ability to inhibit NF-kappaB [14]. In addition, Vijay et al., 2010 reported that the presence of alkaloids in root bark of *Plumeria acutifolia* is responsible for the significant anti-inflammatory activity [15]. It was hypothesized that the presence of flavonoids in the plant, the chance of having anti-inflammatory action is high. Another study on leaves of *I. balsamina* reveals that the flavonoids inhibit the enzyme prostaglandin synthetase more specifically the endoperoxidase [6]. This finding supports the presence of flavonoids in the roots and stem of *I. balsamina* and it might be more responsible for the anti-inflammatory potential of *I. balsamina* roots and stem.

CONCLUSION

The present study can be suggested that the extracts of *I. balsamina* roots and stem posses various spectrum of anti-inflammatory activity. The presence of bioactive constituents might be responsible for the anti-inflammatory activity of *I. balsamina* roots and stems. Furthermore, extensive studies are required to elucidate the exact mechanism of action and active principle responsible for anti-inflammatory activity of *I. balsamina* roots and stems so that new potent and safe anti-inflammatory agents can be developed from the natural resources.

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