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ORIGINAL ARTICLE

Comparative Anti-Inflammatory and Analgesic Activities of Qurs-Saffron and Quknar Polyherbal Tablets in Mouse Model

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ABSTRACT

***Objective*:** We aimed to study the anti-inflammatory and analgesic effects for two polyherbal tablets in animal model.

***Methods*:** Suspensions of Qurs-Saffron and Quknar polyherbal formulations (p.o.) were evaluated for their anti-inflammatory (in carrageenan-induced paw edema) and analgesic activities (in formalin-induced paw licking test) in albino mice using Ibuprofen (50 mg/kg, orally) as standard drug. Acute toxicity testing of suspensions from Qurs-Saffron and Quknar tablets was also performed at doses 1, 2 and 3 gm/kg, orally. Parametric data were evaluated by one way ANOVA followed by Tukey's test while graphics were made using GraphPad Prism.

***Results*:** Administration of Qurs-Saffron (1000, 1500, 2000 mg/kg) and Quknar (500, 750, 1000 mg/kg) suspensions showed significant ($P < 0.05$) inhibition of paw edema and at later time point (after 3 hours), both suspensions showed highly significant ($P < 0.001$) inhibition of paw edema. In another experiment (formalin-induced paw licking test), Qurs-Saffron (2000 mg/kg) and Quknar (1000 mg/kg) showed the highly significant ($P < 0.001$) analgesic activity.

***Conclusion*:** Qurs-Saffron and Quknar tablets possessed anti-inflammatory and analgesic effects that were most likely due to flavonoids and phenolic compounds with free radical scavenging properties which significantly reduced inflammation and pain in treated mice and were also found safe in toxicity testing.

Keywords: Anti-inflammatory, analgesic, carrageenan, Qurs-Saffron, Quknar, Ibuprofen.

INTRODUCTION

Inflammation is more prevalent in multiple disorders with beneficial effects on host defence, while pain is physical distress caused by injury or illness and is one of the important signs of inflammation [1]. Inflammation is linked with release of mediators including prostaglandins and leukotrienes which may result in free radicals mediated tissue damage [2]. Oxidants are involved in production of reactive oxygen species (ROS) responsible for inflammation and pain while anti-oxidants possessing free radical scavenging activities can prevent free radical related tissue damage [3]. Conventional drugs such as NSAIDs & corticosteroids are commonly used as anti-inflammatory and analgesic agents, but these have high cost and multiple adverse effects like renal, hepatic and gastrointestinal disorders [4]. So, the alternative sources including herbal formulations could be used for their anti-inflammatory, anti-oxidant and analgesic potentials with low cost and less adverse effects [5]. Moreover, polyherbal formulations

such as peedantak, vati, aujaie and surangeen [6, 7] have the advantage of multiple active ingredients with synergistic effects that might not be achieved with individual plants *i.e*. *Moringa oleifera* [8]. In addition, multinational companies, *i.e*. Glaxo, Merck and Boehouringer have established research and development departments dedicated to herbal remedies that are in the process of scientific approval and validation [9].

In Pakistan, two commonly used polyherbal tablets for the management of pain, inflammation and some other problems are Qurs-Saffron and Quknar. To the best of our knowledge, the said polyherbal tablets have not been scientifically evaluated for their anti-inflammatory and analgesic activities so far. Qurs-Saffron is usually prepared by 13 medicinal plants including *Colchicum autumnal* (colchicine), *Colchicum luteum* (β-lumicolchicine), *Curculigo orchioides* (curculigoside), *Chlorophytum borivilianum* (alkaloids), *Anacyclus pyrethourum* (pyrethouric acid), *Smilax chinenis* (saponins), *Mentha piperita* (menthol), *Anaethum graveoles* (phellandrene) *Crocus sativus* (saffron), *Piper nigrum* (pinene), *Piper longum* (piperine), *Pimpinella anisum* (anethole) and *Moringa oleifera* (vitamin A). Quknar is usually prepared by 12 medicinal plants including *Apium graveolens* (glycosides), *Cuminum cyminum* (pinene), *Elettaria cardamomum* (flavonoids), *Vanda tessellate* (anthocyanins), *Tribulus terrestris* (saponins), *Coriandrum sativus* (terpinene), *Terminalia chebula* (gallic acid), *Terminalia bellerica* (sitosterol), *Phyllanthus emblica* (polyphenols), *Cyperus scariosus* (polyphenols), *Zingiber officinalis* (zingiberene) and *Embelia ribes* (embelin) [10]. Majority of these plants have medicinal value in traditional medicine for treating pain and inflammation such as *Curculigo orchioides*, *Colchicum luteum* and *Zingiber officinalis* have antirheumatic, anti-gout and anti-inflammatory activities [11, 12]. The present study was aimed to scientifically evaluate and compare the Qurs-Saffron and Quknar tablets for their anti-inflammatory and analgesic effects in animal model to understand the pharmacological basis for the treatment of inflammatory diseases.

MATERIALS AND METHODS

Polyherbal Formulations and Other Chemicals

Polyherbal formulations; Qurs-Saffron (manufactured by Mahfooz Dawa-khana, Taxila, Pakistan) and Quknar (manufactured by Azeem Dawa-khana, Rawalpindi, Pakistan) were procured from Chinioti Dawa-khana, Sargodha, Pakistan. Ibuprofen (Maan-Gee Distributors, Sargodha, Pakistan), formalin and gum tragacanth (Merck, Germany) were also purchased.

Experimental Animals

Male and female Swiss albino mice weighing between 20-30 g (6 animals / group) were housed in standard polypropylene cages under controlled environment and allowed free availability to water and *ad libitum* diet.

Ethical Approval

The study was approved by the Institutional Ethics Committee (20-IEC-32 UOS), University of Sargodha, Sargodha, Pakistan. All the procedures were performed in accordance with the rules of NIH guidelines [13].

Experimental Design

Administration of Suspensions to Animals

Suspensions of polyherbal tablets were prepared in vehicle (gum tragacanth 0.5% w/v in distilled water) according to doses mentioned in the traditional medicine literature [14]. Animals were sustained in an upright position and the solutions were directly administered to the stomach *via* oral gavage.

Determination of Anti-Inflammatory Activity in Carrageenan-Induced Paw Edema

Anti-inflammatory activity of herbal preparations was evaluated by carrageenan-induced paw edema in mice. The mice were kept free of food for 24 hours with water *ad libitum*. Group I and II were treated with vehicle and standard drug (Ibuprofen) respectively. Various doses of herbal preparations were administered orally to remaining 6 groups (III-VIII). After 1 hour, inflammation was induced to hind paw by injecting 0.1 mL of freshly made carrageenan suspension (1% in normal saline) into right hind paw of animals and anterio-posterior diameter (mm) of the paw was measured at intervals of 1, 2 and 3 hours using vernier calliper. As previous studies on different polyherbal formulations had been conducted at 3 to 5 hours interval [15, 16] and in the present investigation polyherbal tablets showed maximum effects at 3 hours so, experiment was not conducted further on later time points. The difference in diameter between basal and calculated values at various intervals of

time was recorded as amount of edema as well as percentage inhibition by applying following formula [16].

$$\% \text{ inhibition of paw edema} = \frac{(Vt - Vo)^{control} - (Vt - Vo)^{treated}}{(Vt - Vo)^{control}} \times 100$$

Where Vt is the mice paw volume at time 't', Vo is the initial mice paw volume (basal value), $(Vt - Vo)^{control}$ is edema produced in control group and $(Vt - Vo)^{treated}$ is oedema produced in treatment group.

Determination of Analgesic Activity in Formalin-Induced Paw Licking

Animal groups I and II were administered the vehicle and the standard drug, Ibuprofen, respectively. Various doses of herbal products were administered orally to 6 groups (III-VIII). After 1 hour, 20 µL of 1% v/v formalin solution was injected subcutaneously under the skin of left hind paw of animals and analgesic responses were monitored for 30 minutes [17, 18].

Determination of Acute Toxicity Testing in Animals

Acute toxicity of herbal preparations was carried out at doses 1, 2 and 3 g/kg according to organization for economic cooperation and development (OECD) guidelines [19]. Mice were kept under keen observation, morbidity and mortality rate was measured for 48 hours and changes in weight, behavioural patterns and respiration were monitored on daily basis for 14 days [20].

Statistical Analysis

As the data was normally distributed so, the sum results were presented as means ± SEM (standard errors of means). Parametric data were evaluated by one way ANOVA (analysis of variance) followed by Tukey's test. Graphics and statistical hypothesis testing were done using GraphPad Prism (version 5.0) and IBM SPSS version 19.

RESULTS

Effect on Anti-Inflammatory Activity in Carrageenan-Induced Paw Edema

There was successful induction of edema paw by administration of carrageenan suspension. The suspensions of polyherbal formulations showed significant inhibition in paw edema while, Ibuprofen (50 mg/kg) also produced very significant inhibition ($P < 0.01$) of paw edema in albino mice throughout the time course as depicted in Figures **1**, **2** and **3**. A dose dependent highly significant inhibition (paw edema) was shown by polyherbal formulation at 1 hour after edema induction (Figure **1**). At early follow-up (2 hours), there was a significant inhibition of edema ($P < 0.01$) in paw as shown in Figure **2** at low doses of polyherbal formulations while at high doses the results were highly significant. Both preparations showed highly significant and dose dependent inhibition in paw edema at 3 hours; Qurs-Saffron (1000, 1500 & 2000 mg/kg) and Quknar (1000 mg/kg), respectively as depicted in Figure **3**. Of note, higher degree of edema inhibition was observed with Qurs-Saffron as compared to Quknar while the same was observed with Quknar only at maximum administered dose.

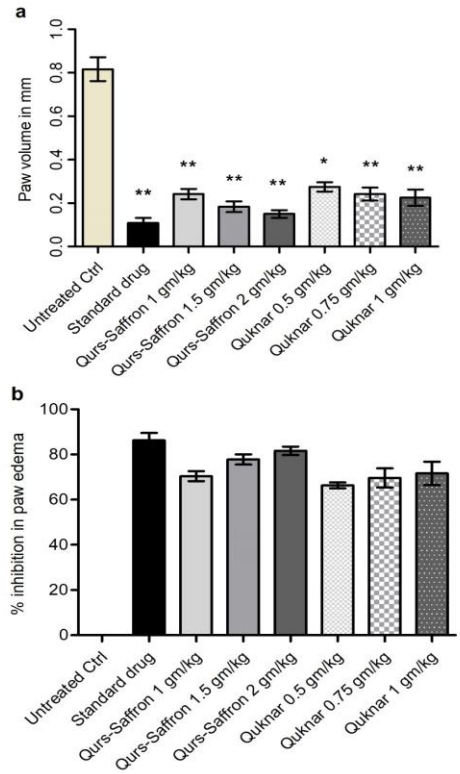

Figure 1. Comparative anti-inflammatory activities of Qurs-Saffron and Quknar (p.o) at 1 hour after carrageenan-induced edema: Different groups of albino mice were treated with Qurs-Saffron, Quknar and Ibuprofen as Standard drug followed by edema induction. **a:** Treatment groups (II-VIII) *vs*. control group; one way ANOVA followed by Tukey's test (* = $P < 0.05$ and ** = $P < 0.01$). **b:** Percentage inhibition in paw edema was calculated by comparing paw volume of treatment and control groups.

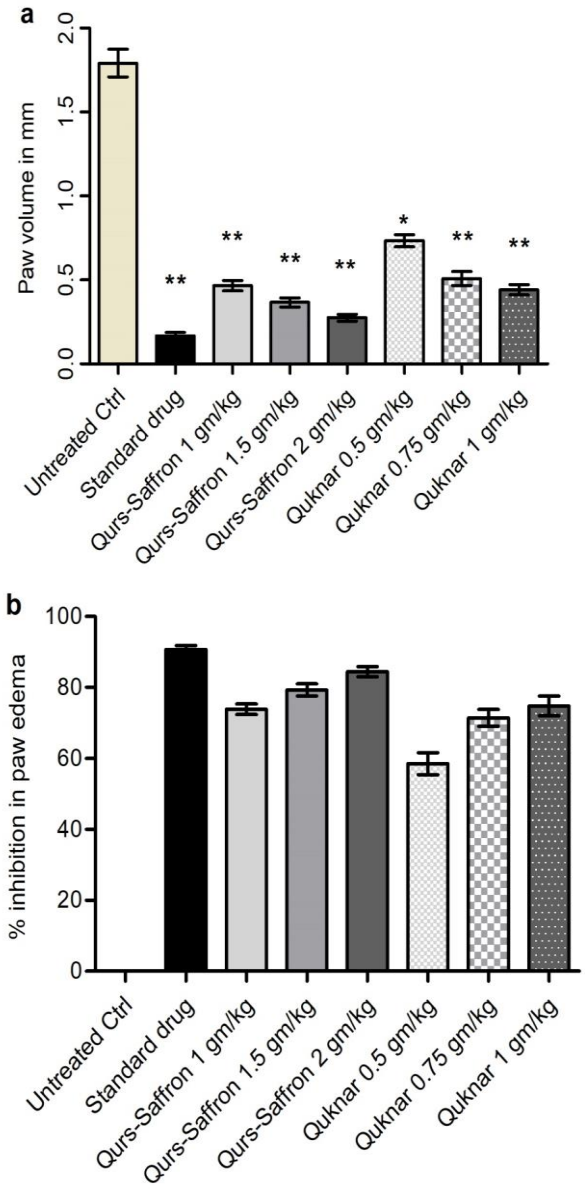

Figure 2. Comparative anti-inflammatory activities of Qurs-Saffron and Quknar (p.o) at 2 hours after carrageenan-induced edema: Different groups of albino mice were treated with Qurs-Saffron, Quknar and Ibuprofen as Standard drug followed by edema induction. **a:** Treatment groups (II-VIII) *vs*. control group; one way ANOVA followed by Tukey's test (n = 6, * = $P < 0.05$ and ** = $P < 0.01$). **b:** Percentage inhibition in paw edema was calculated by comparing paw volume of treatment and control groups.

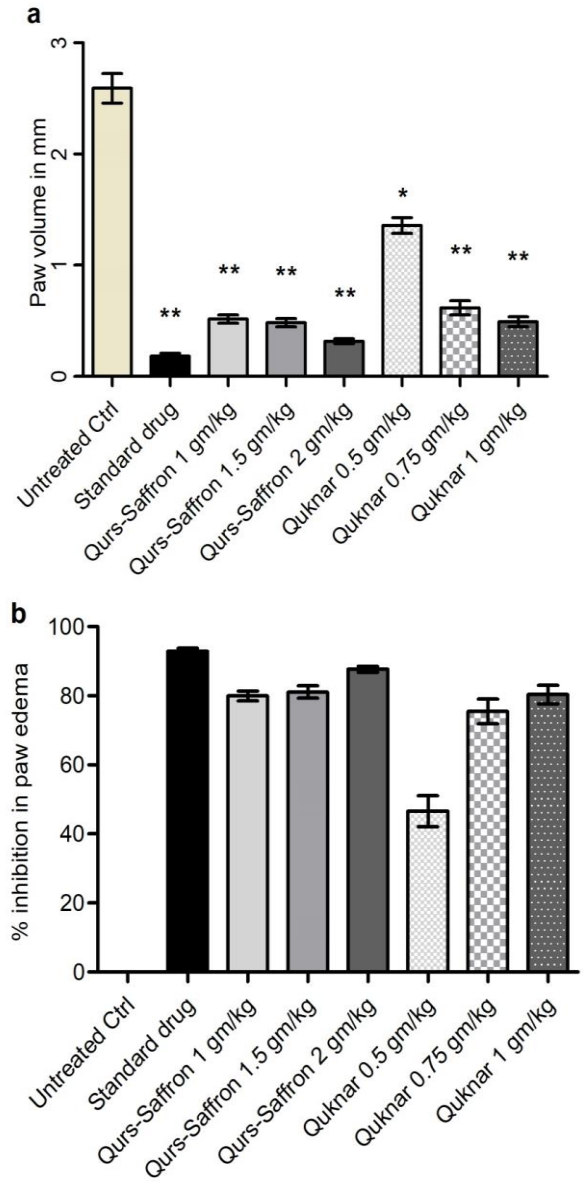

Figure 3. Comparative anti-inflammatory activities of Qurs-Saffron and Quknar (p.o) at 3 hours after carrageenan-induced edema: Different groups of albino mice were treated with Qurs-Saffron, Quknar and Ibuprofen as Standard drug followed by edema induction. **a:** Treatment groups (II-VIII) *vs*. control group; one way ANOVA followed by Tukey's test (n = 6, * = P < 0.05 and ** = P < 0.01). **b:** Percentage inhibition in paw edema was calculated by comparing paw volume of treatment and control groups.

Table 1. Comparative analgesic activity of Qurs-Saffron and Quknar in paw licking model induced from formalin.

Treatment	Dose (mg/kg)	Number of Licking(s)
Vehicle	_____	384.166±36.66
Ibuprofen	50	118.166±4.99***
Qurs-Saffron	1000	282.833±49.23**
	1500	209.166±6.76***
	2000	186.500±19.44***
Quknar	500	320.166±60.30^{ns}
	750	229.833±49.39**
	1000	195.333±12.56***

Values are expressed as mean ± SEM, where n = 6, *** = $P < 0.001$ (highly significant results); ** = $P < 0.01$ (very significant); ns = non-significant licking compared to vehicle

Effect on Analgesic Activity in Formalin-Induced Paw Licking

Polyherbal formulations; Qurs-Saffron (1500, 2000 mg/kg) and Quknar (1000 mg/kg) showed highly significant ($P < 0.001$) decrease in licking frequency as summarized in Table **1**. The standard drug, Ibuprofen (50 mg/kg orally) also showed the highly significant ($P < 0.001$) inhibition in paw licking. Of note, Quknar did not significantly reduce the licking frequency at low doses, *i.e*. 500 mg/kg however significant inhibition in paw licking was observed at higher doses of Quknar, *i.e*. 1000 mg/kg.

Acute Toxicity Testing in Animals

The results of acute toxicity testing (1, 2, 3 g/kg) in mice revealed that the animals did not show any morbidity changes like behaviour or weight and respiratory parameters or mortality which indicated that these formulations have no toxicity in higher doses and considered as safe.

DISCUSSION

Inflammation and pain are linked with pathophysiology of a variety of diseases with its high disease burden in the world and the search of new compounds or formulations has continued [21]. Currently available and commonly used remedies for inflammation and pain include NSAIDs, COX2 inhibitors, paracetamol and opiate analgesics. However, these drugs are reported to cause untoward effects like hepatic, renal and gastro-intestinal disorders [22]. The alternative options like plants or polyherbal formulation are commonly employed for the treatment of many chronic inflammatory diseases and management of pain [18]. Keeping in view the above discussion, it was expedient to search for relatively safer formulation with least side effects. The present study evaluated the comparative anti-inflammatory and analgesic efficacies of two commonly used polyherbal formulations in animal model**.** Furthermore, study on acute toxicity was also conducted to check its safety profile in animal models.

We evaluated anti-inflammatory activity of two polyherbal tablets by using carrageenan-induced paw edema model in animals [15]. Inflammation was induced by reactive oxygen species (ROS), hydrogen peroxides, super-oxides, COX (cyclooxygenases), cytokines and leukotrienes. The COX, responsible for the conversion of arachidonic acid into prostaglandins, acts through free radical mediated reactions. Therefore, the presence of anti-oxidant phytochemicals in herbal formulation may explain their anti-inflammatory activity [23]. The results obtained in the current study revealed that the anti-inflammatory effects of Qurs-Saffron and Quknar were significant and comparable with previous studies in context of anti-inflammatory activities of other polyherbal formulations [24]. Furthermore, Ibuprofen, a standard NSAID has shown significant anti-inflammatory activity in animal model. The possible mechanisms for mild anti-inflammatory effect of Quknar, at low doses (500 and 750 mg/kg) and at later time points could be its inability to sufficiently inhibit the kinin like substance [25].

Oral treatment with Qurs-Saffron or Quknar in albino mice showed significant inhibition of formalin-induced paw edema in animal models (Table **1**). The current results showed the dose dependent augmentation in analgesic activity from both polyherbal formulations in animal models that were comparable with previously reported studies in context of other polyherbal tablets [7, 8, 26]. Moreover, Ibuprofen also showed significant analgesic activity in animal model. Mechanistically, formalin is responsible for biphasic pain reaction *via* its central and peripheral actions resulting in inflammatory and neurogenic pain [27]. Scientific data suggest that there is boost in activity of

C fiber afferent upon injection of formalin which induces a unique behaviour as determinant of pain revealed by paw licking in mice [28]. In other words, a good degree of pain should reflect the inhibitory effect of hyperalgesia; resultantly analgesic drug should be related to the behavioural indices of pain in a systematic manner. So, by applying these principles to our study the paw licking was used for the determination of the analgesic activity of polyherbal formulations.

The anti-inflammatory and analgesic effects of Qurs-Saffron and Quknar tablets were might be due to active constituents found in the formulation. Multiple constituents of these plants with antioxidant properties (which help in reduction of ROS including hydroxyl ions, hydrogen peroxide, superoxide, COX, cytokines and leukotrienes) have already been identified in many of the plants used in Qurs-Saffron and Quknar tablets [29]. These polyherbal formulations contain important phytochemical ingredients like curculigoside, saponins, alkaloids, piperine, sylvatin, sesamin, terpinene, terpenoids, polyphenols, steroids and vitamins (A, B1, B2, B3, B6) that have well documented anti-inflammatory and anti-oxidant activity and being used in traditional medicines since many years [30]. This logically explains the anti-inflammatory and analgesic effects of Qurs-Saffron and Quknar as seen in current study.

CONCLUSION

It can be concluded from the above discussion that Qurs-Saffron and Quknar possessed significant and consistent effects during inflammatory and analgesic experiments in animal models while Qurs-Saffron was more effective in relieving pain and inflammation as compared to Quknar. Mechanistically, it can be suggested that the ingredients, *i.e*. curculigoside, saponins, alkaloids, piperine, sylvatin, sesamin, terpenoids and steroids, responsible for anti-inflammatory and analgesic activity, act by inhibiting the release of inflammatory mediators. Therefore, the traditional use of Qurs-Saffron or Quknar tablets in rheumatism and other disorders has been justified by the findings of our study. However, more studies are needed for mechanistic understanding of anti-inflammatory and analgesic effects of polyherbal formulations and to document their actual efficacy and safety in clinical settings.

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