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Effect of Propolis hydroalcoholic extract on formalin- induced inflammation in male rat paw

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ABSTRACT

Propolis has long been used in folk medicine for the management of different diseases. In this study we evaluated anti-inflammatory effect of a propolis hydroalcoholic extract on formalin-induced edema in rat paw. Propolis hydroalcoholic extract was prepared using maceration method. Male Wistar albino rats were divided into 6 groups (n=6). Then different doses of extract (100, 200, 400 and 600mg/kg,IP) were injected in 4 groups, positive control group received indomethacin (5 mg/kg , IP) and negative control received normal saline(5 ml/kg, IP) . Thirty minutes later all groups received 100 µl formalin 2.5% was injected (SC) then the rat paw volumes were evaluated using plethysmometer apparatus once every hours between the first and fifth hours. Extract with doses of 100 and 200mg/kg had less effect on decreasing the paw edema in comparison with group received indomethacin($p<0.05$). More effect on decreasing the paw edema was seen in dose of 400 and 600mg/kg of the extract that difference between two groups and indomethacin was not statistically significant. Propolis hydroalcoholic extract with doses of 400 and 600 mg/kg and indomethacin (5mg/kg) significantly decreased the paw edema volume in the subject field rats in comparison with group received 5ml/kg of normal saline. Moreover, as the fewer side effects are to be expected for lower dose the most appropriated dose was selected400mg/kg for this extract.

Keywords: Propolis, Indomethacin, Inflammation , Rat paw , Plethysmometer

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INTRODUCTION

Inflammation is the complex biological response of tissues to harmful stimuli such as pathogens, damaged cells or irritants. The inflammation process involves production or release of mediators from neurons or damaged tissues, which are responsible for different responses including redness, heat ,swelling, pain, and loss of Function (1, 2).

Therapeutic agents that used in inflammatory diseases such as NSAIDs (non steroidal anti inflammatory drugs), glucocorticoids and immunosuppressive drugs have a high level of side effects, therefore use of plant and natural substance that has anti-inflammatory effect without side effect can be good replacement for this drugs.

Propolis, which is a resinous sticky substance that honeybees produce by mixing their own waxes with resins collected from plants, is used as a sealant and sterilant in honeybee nests (3-6). It has been used as a folk medicine since ancient times. In modern times, it has been found to have a wide range of biological activities, such as antibacterial, anti-inflammatory, antioxidative, hepatoprotective effects, and/ or tumoricidal activities(3-7).

Moreover, propolis may also prevent dental caries. Because of its biological activities, propolis have been used in the composition of toothpastes. Chemical studies conducted with propolis extracts revealed the existence of a very complex mixture of different naturally-occurring constituents. More than 300 constituents identified in propolis to date such as phenolic acid, terpenes, cinamic acid, caffeic acid, several esters, and also flavonoids (8-10). In the present study, we sought to investigate the Anti-inflammatory effect of propolis by using rat paw edema model that no such investigation has been reported.

MATERIALS AND METHODS

Preparation of the extract:

Propolis Was a gift from BEES WADI (Saudi Arabia) . Propolis was extracted through maceration in ethanol for 72 h. Then the extract was concentrated using rotary vacuum evaporator to get the solid mass.

Formalin and Indomethacin:

Formalin purchased from merck and indomethacin powder was a gift from Amin Pharmaceutical Co (Isfahan, IRAN)

Animals:

For this study, Wistar albino rats (average weight: 200 g) were used. Animals were kept in a clean holding room on a 12 h light and dark cycle with relative humidity 45-55% and

temperature 23±2°C. During the experimentation, all rats were fed with concentrated food pellets (Pars Khurakdam Shushtar, Iran) and tap water *ad libitum*. The animals were randomly divided into 6 groups (n = 6)(11,12). At first, the rat paw volumes were measured in all groups by plethysmometer (UGO Basile 7140) as initial volume (V_1) in each case.

Experimental groups:

The Propolis extract (100, 200, 400 and 600 mg/kg,IP) was administered in the first to fourth groups. The 5th group received indomethacin (5 mg/kg, IP) and 6th group received normal saline (5 ml/kg, IP). Then 30 minutes later , 100 µl of formalin 2.5% was injected (SC) in the right hind paw of animals and then the rat paw volumes were evaluated using plethysmometer apparatus once every hours between the first and fifth hours

The following equation was used for calculation of the percent of resulted edema as follows in animals:

$$\text{Relative paw edema (\%)} = \frac{V_2 - V_1}{V_1} \times 100$$

Where:

V_1 = The animal paw volume before injection of irritant

V_2 = The paw volume from the 1st to 5rd h after the injection so the irritant

Statistical analysis:

Results of the experiment were statistically analyzed using one-way ANOVA and Tukey tests. Statistical differences between control and treated groups was shown as ($p<0.05$).

RESULTS

Comparison of anti-inflammatory effect between different doses of propolis extract (100,200,400 and600mg/kg) and negative control group (normal saline) showed this effect in all of extract groups were significantly more than negative control group($p<0.05$), (Figure 1).

Anti-inflammatory effect of propolis extract with dose of 100 mg/kg at all time points and 200 mg/kg at the points of 1 and 2 (h) were significantly less than positive control group (indomethacin , 5 mg/kg)($p<0.05$),(Figure 2).

There was not significantly difference between dose of 400 and 600 mg/kg at all of time, 200 mg/kg at the points of 3-5(h) and positive control group(indomethacin , 5mg/kg), (Figur3).

Anti-inflammatory effect of propolis extract with dose of 100 mg/kg was significantly less than dose 200 mg/kg at all time points($p<0.05$), (Figure 1). Anti-inflammatory effect of propolis extract with dose of 200 mg/kg at the points of 1 and 2 (h) was significantly less than

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dose of 400 mg/kg($p<0.05$) ,but there was not significantly difference at the points of 3-5 (h), (Figure 1). However , there was not significant difference between poropolis extract with dose 400 and 600 mg/kg at all time points, (Figure 1).

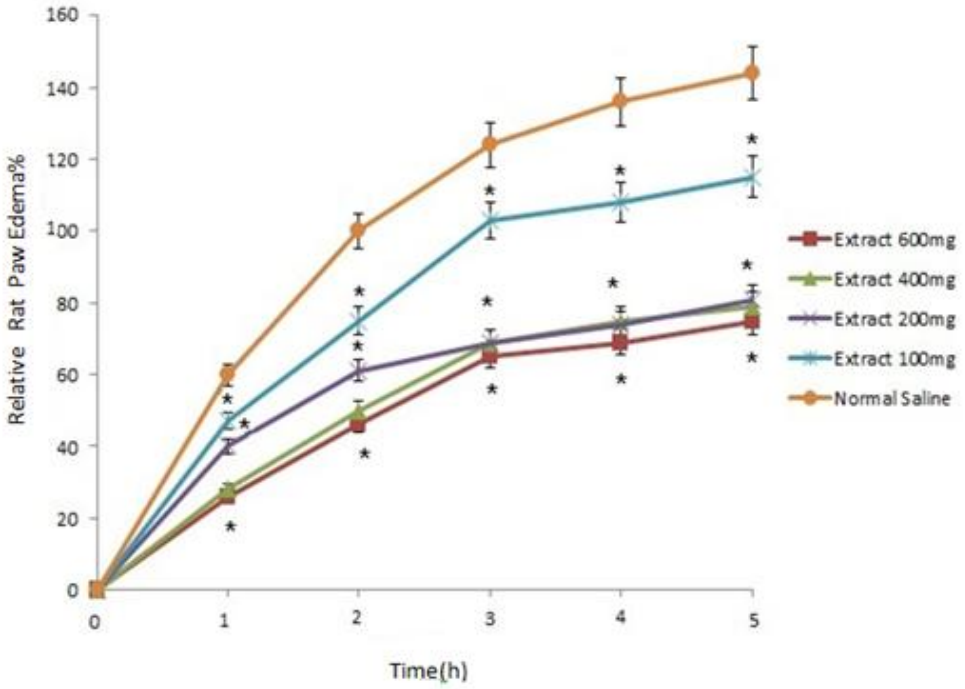

Figure 1: Effect of propolis hydroalcoholic extract (100, 200, 400 and 600mg/kg, IP) and negative control group (normal saline,5ml/kg, IP) on formalin-induced edema in rat paw . Significant difference between different doses of propolis hydroalcoholic extract (100, 200, 400 and 600mg/kg) and negative control group is shown by * ($p<0.05$, n=6).

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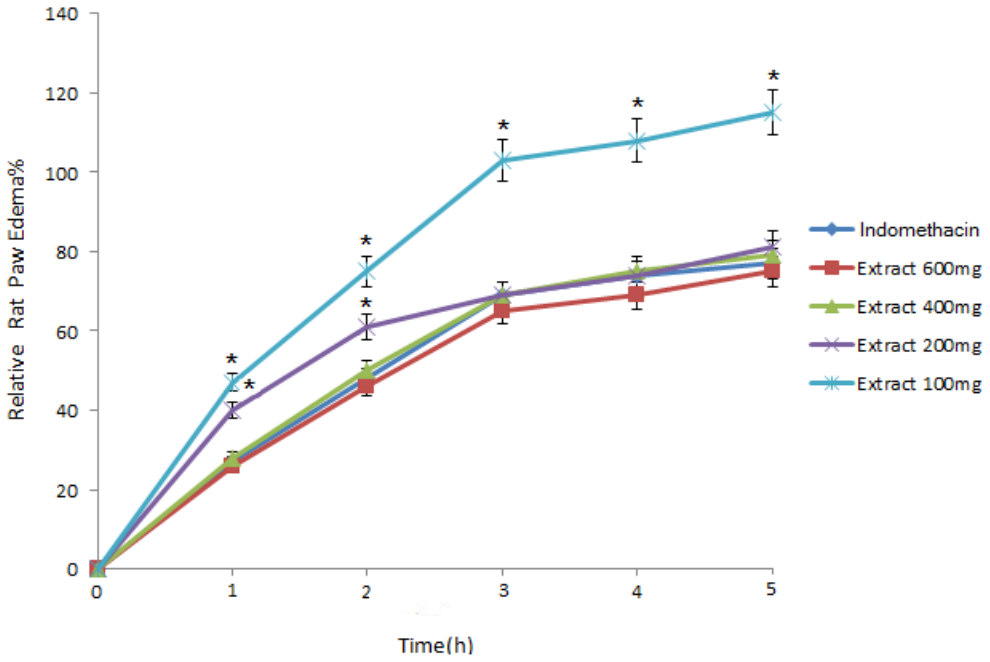

Figure 2: Effect of propolis hydroalcoholic extract (100, 200, 400 and 600mg/kg, IP) and Positive control group(indomethacin,5mg/kg, IP) on formalin-induced edema in rat paw. Significant difference between different doses of propolis hydroalcoholic extract (100, 200, 400 and 600mg/kg) and positive control group is shown by * ($p<0.05$, n=6).

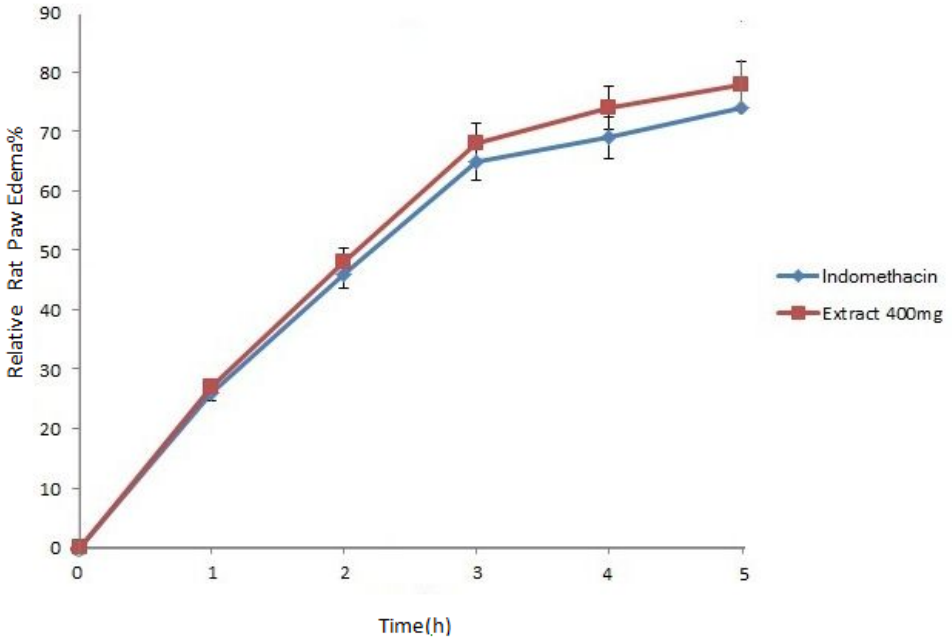

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Figure 3: Effect of propolis hydroalcoholic extract (400 mg/kg, IP) and indomethacin (5mg/kg, IP) on formalin-induce edema in rat paw. There is no significant difference between groups, (n=6).

DISCUSSION

Inflammation, the first physiological defense system in the human body can protect against injuries caused by physical wounds, poisons, etc. This defense system also called short-term inflammation can destroy infectious microorganisms, eliminate irritants and maintain normal physiological a regular physiology, i.e., asthma and rheumatoid arthritis (13).

The currency and wide of treatment inflammatory is being used of NSAIDs drug. But whereas the majority of the NSAIDs have gastrointestinal irritation therefore Natural substances with anti-inflammatory effect and minor side effect can be as a beneficial replacement for these drugs.

In some countries, propolis is used for the treatment of different diseases, such as odontological, dermatological, and gynaecological disorders, in which inflammation and pain are important components (7,14). Scavenging of free radicals, generated by neutrophils in inflammatory processes, is the principal mechanism of conventional anti-inflammatory drugs, and is also a known property of propolis (15-17).

Propolis or one of the bioactive components in propolis such as caffeic acid phenethyl ester has shown an anti-inflammatory effect through numerous mechanisms, such as the inhibition of eicosanoid production or nitric oxide production, antioxidant action, depression of neointimal formation in injured vessels and angiogenesis, modulation of calcium ion mobilization(18-27) . In addition , it is also confirmed that propolis extract inhibits PMNs migration that is also favorable for preventing exacerbation on inflammation(28) .

Study by naito et al demonstrated that topical application of propolis extract reveals a moderate, but evident anti-inflammatory effect on carrageenan induced hind paw edema of rats. The purpose of this study was to confirm the anti-inflammatory effect of injectionally applied propolis extract on formalin-induced rat hind paw edema(28).

The result of the present study showed that intraperitoneal administration of propolis hydroalcohlic extract decreased formalin-induced rat hind paw edema in the rats in comparison with the group received normal saline, it is mentionable this effect was dose dependent . There was not significant difference between dose of 400 and 600 mg/kg at all of time and indomethacin .However, as the lower side effects are to be expected for lower dose of 400 mg/kg ; the most appropriated dose was selected for this extract.

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