IJP (2016), Vol. 3, Issue 1 **(Research Article)**

Received on 15 October 2015; received in revised form, 26 November 2015; accepted, 29 November 2015; published 31 January 2016

THE STUDY EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF JAPANESE PERSIMMON FRUIT ON FORMALIN-INDUCED INFLAMMATION IN THE RAT

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Keywords:

Japanese Persimmon, Inflammation, Formalin

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ABSTRACT: Acute and chronic inflammation is the most important issue in the field of health. Also, due to the side effects of synthetic drugs, herbal products are widely used in the control of inflammation. In this study was investigated the effect of a hydro-alcoholic extract of Japanese persimmon fruit on the formalin-induced inflammation in the rat paw. 30 male Wistar rats were divided into 5 groups: negative control, group treatment with 300 mg/kg of aspirin (positive control group) and the group's treated with 200, 400 and 800 mg/kg hydro-alcoholic extract of persimmon, respectively. The inflammation was established to sub-planter injection of 2.5% formalin in rats. Level of inflammation was measured to plethysmometer in six stages in the rat paw. These results were shown that in the rat's treated with different concentrations of extract; inflammation was lower than the positive control group ($p \leq 0.05$). Also, the compression of between groups' treatment with the extract was determined that reduction of inflammation was as dose depend manner ($p \leq 0.05$). We conclude that Japanese persimmon may be useful for the management of inflammation but need to perform further studies.

INTRODUCTION: The inflammation response occurs by lymphocytes, macrophages, and monocytes that resulted in the formation of inflammatory agents, such as TNF-α, IL-1, IL- 6, MCP-1 IL- 8 prostaglandins, leukotrienes, thromboxane, Lipoxin [1-3]. It is a process for clearing microorganisms and damaged cells, but it can have side effect [1]. Tissue damage ultimately leads to an infection that causes cells locality activation, an increase of vascular permeability, edema and pain [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.3(1).26-29

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(1).26-29

Because adverse complications of the inflammatory drug include gastrointestinal problem, nausea, and liver toxicity, thus always is require to strategies for the preparation of new drugs with lessening side effects [5].

Japanese persimmon (*Diospyros kaki*) belongs to Ebenacea family that has simple leaves with flowers spherical or slightly stretched and grown in North America [6, 7]. It fruit contains large amounts tannin, sucrose, triox nizol, malic acid, carotene and Vitamins A, B, C and has antioxidant property [8, 9]. In traditional medicine, persimmon fruit is used to the soothing astringent chest and calm hiccups [9]. It has been reported that extract obtained from persimmon fruit is an appetizer and recommended for hypertension [10-12]. Also, it dried fruit used to the treatment of leukemia [13]. The compounds such as isoquercitrin and hyper in with antioxidant activity property from the peel of persimmon were isolated

[14]. In previous studies has been showed that the amount of tannin in persimmon fruit is very high and probably this herb has anti-inflammatory and antioxidant properties [15, 16]. Here, we examined the effect of the hydroalcoholic extract obtained from Japanese persimmon fruit on formalin-induced inflammation in the rat.

MATERIAL AND METHOD:

Animal: In this study, 30 male Wistar rats weighing 150-180 gr were prepared from the research center and experimental animal house Jundishapur University of Medical Sciences, Ahvaz. The animals were housed in stainless cages with constant temperature 23-25 ºC and photo-cycle 12/12 h light/dark. In during period of study, they were fed to rodent standard food along with access freely to water.

Plant Preparation: Persimmon fruit was prepared of province Khuzestan (west of Iran) and confirmed the Department of Pharmacology, School of Pharmacy Ahvaz. Extraction was performed based on the maceration method. At first, the plant was located in the shade and fully dried in the room temperature and followed by crushed by the grinder. Then, 500 gm of dried fruit was soaked to ethanol 70%-water 30% solution for 72 h. After 72 h, the extract was concentrated by vacuum distillation and for finally dry was placed in a temperature of 30-35 ºC. At the end was obtained the clear and yellowish orange extract.

The desired dose was prepared by desired extract concentration per kg of body weight resolved in 5ml of normal saline, and then the extract was injected as intra-peritoneal into rats.

Experimental Groups: Thirty male Wistar rats were divided into 5 groups (n= 6):

1. The group was received 5ml/kg normal saline as negative control.
2. The group was received 300 mg/kg aspirin as a positive control.
3. The animals were treated with 200 mg/kg of the hydro-alcoholic extract prepared from persimmon.
4. The group was treated by 400 mg /kg of the hydro-alcoholic extract prepared from persimmon.
5. The rats were treated with 800 mg/kg of the hydro-alcoholic extract prepared from persimmon.

Treatment with normal saline and extract by oral administration and for aspirin by intraperitoneal injection was performed. In all groups was induced inflammation by formalin.

Formalin-induced inflammation procedure: The model of formalin-induced inflammation is widely used to study the inflammatory process and the screening of anti-inflammatory agents [17]. 1 week before the study, all animal was adapted to study environment. For inflammation induction, at first, were measured the size of the paw in rats (before injection of formalin) by the plethysmometer (Ugo basile 7140 Italy) and considered as zero time. Subsequently, treatment with normal saline, aspirin, and the extract was performed and after 30 min 0.1 ml of 2.5%, formalin was injected to paw rat as sub planter**.** Then was measured paw volume changed through plethysmometer at 15, 30, 45, 60, 120, 180, 240 and 300 min after inflammation induction and calculated to the following formula.

$$\text{Relative Paw Edema} = v_2 - v_1 \times 100 / v_1$$

V_1 = Paw volume before injection of formalin (zero time).
V_2 = Paw volume in 15 to 300 min after injection of formalin.

Statistical Analysis: All results expressed as mean ± SD. Using SPSS software statistical analysis was performed through one-way analysis of variance (ANOVA) followed by Tukey test. The significant difference was considered to $P<0.05$.

RESULTS AND DISCUSSION: In this study, we investigated the effect of a hydro-alcoholic extract of Japanese persimmon fruit on formalin-induced inflammation in rats. In previous studies, it has been demonstrated anti-inflammatory effect persimmon, for example, has been showed that phenolic compounds of dried persimmon fruit were reduced TNF-α level in THP-1 cells and may be beneficial to inflammation Kim *et al.*, 2011 [18]. In comparison of anti-inflammatory effect among groups treated with extract to negative control group, the results was determined that paw edema volume the rats were received of 400, and 800

mg/kg hydro-alcoholic extract was significantly lower than the group received with 5 mg/kg normal saline (negative control group) at early; second, third, fourth and fifth hours ($p \leq 0.05$). In the group treated with 200 mg/kg extract paw edema volume was nearly similar to the negative control group at early, second, third, while there was a significant decrease at fourth and fifth hours ($p \leq 0.05$) **Fig. 1**. In a study was found that persimmon leaf extract or its major flavonoid (astragalin) has inhibitory activity against the histamine releasing in mice [19]. As depicts **Fig. 2**, the effect of anti-inflammatory in the groups received with 400 and 800 mg/kg was nearly similar to the group treated with 300 mg/kg aspirin (positive control group) in the all-time experiment. Nevertheless, in the group with oral administration of 200 mg/kg hydro-alcoholic extract of persimmon volume of paw edema was significantly higher than the positive control group at second, third, fourth and fifth hours ($p \leq 0.05$). Our results were also showed that concomitant to increase of dose, was promoted anti-inflammatory effect and there was most anti-inflammatory effect in the group treated with 800 mg/kg in compression with negative and positive control groups ($p \leq 0.05$).

Perhaps the anti-inflammatory effect is due to the antioxidant property of persimmon because in previous studies it has been confirmed [15]. Probably the high antioxidant activity of persimmon fruit appears due to its high tannin. Because Ahn *et al.*, 2002 has been found that persimmon has radical scavenging activity and its total tannin concentration was dramatically high. Also, it was showed that persimmon has a protective effect against oxidative stress in the liver of rats and increased catalase (CAT) and superoxide dismutase (SOD) activities [20].

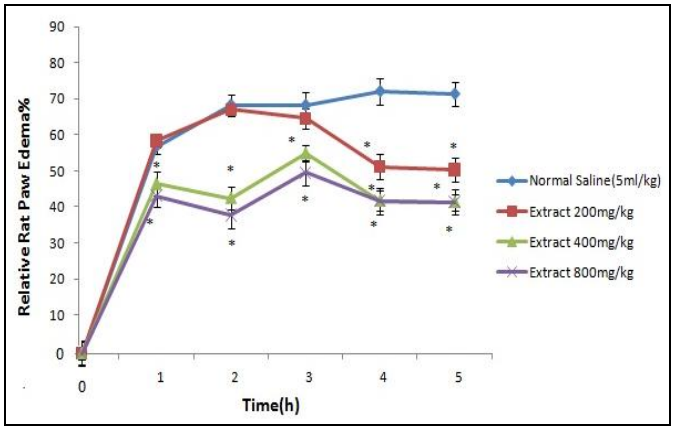

FIG. 1: THE COMPARISON OF ANTI-INFLAMMATORY EFFECT IN GROUPS TREATED WITH EXTRACT WITH NEGATIVE CONTROL GROUP

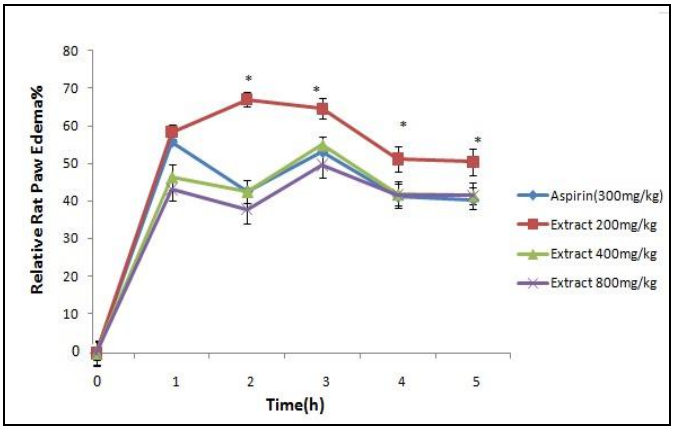

FIG. 2: THE COMPARISON OF ANTI-INFLAMMATORY EFFECT IN GROUPS TREATED WITH EXTRACT WITH POSITIVE CONTROL GROUP

CONCLUSION: In this study, we observed that hydro-alcoholic extract of Japanese persimmon could be beneficial in the treatment of inflammation. We also found that the concentration of 800 mg/kg has the most anti-inflammatory effect. However, for better understand the anti-inflammatory effect this herb is required further studies.

ACKNOWLEDGMENT: We thank our staff in the Department of Pharmacology and Toxicology, School of Pharmacy for cooperation in this research.

COMPETING INTERESTS: The authors declare that there is no conflict of interest regarding the publication of this paper.

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How to cite this article:
Hemati AA, Arzi A, Khataminia M, Karampor NS, dehghan M and Ebrahimi E: The study effect of hydro-alcoholic extract of Japanese persimmon fruit on formalin-induced inflammation in the rat. Int J Pharmacognosy 2016; 3(1): 26-29. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(1).26-29.