ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
July 2016 Vol.:6, Issue:4

Anti-Inflammatory Properties of Hesperidin in Guinea Pigs

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submission: 30 June 2016
Accepted: 5 July 2016
Published: 25 July 2016

Keywords: Anti-inflammatory activity, carrageenan, cotton pellet granuloma, Hesperidin

ABSTRACT

Objective: To evaluate the anti-inflammatory activity of flavonoid (Hesperidin 40 mg/kg) on acute inflammation (carrageenan-induced paw edema) and chronic inflammation (cotton pellet granuloma) in guinea pigs. Methodology: Studying samples of guinea pigs were divided into three groups. Group I was used as control received 1ml of 5% carboxymethyl cellulose suspension, Hesperidin at a dose of 40 mg/kg was given orally to group II and group III was treated with indomethacin (10 mg/kg). 1.0 ml of carrageenan was injected s.c. to plantar region of right hind paw of each guinea pig. The change in paw volume was measured at 0, 1, 2, 3 and 4 hours intervals. For chronic model of inflammation, sterilized cotton pellets weighing 50±1 mg each, were implanted into both sides of the groin region of each guinea pig, under light anesthesia. Drug treatment was given for 7 days. On the eight day, cotton pellets along with granuloma were removed surgically and wet pellets were weighed, after that dried at 60^0C overnight and then the weight of dry pellets was taken. Results: The results indicates that Hesperidin at a dose of 40 mg/kg body weight exhibited significant inhibition ($P<0.05$). The Percent Inhibition with the control, standard (Indomethacin) and the test compound (Hesperidin) were 0%, 74% and 77% in the carrageenan induced paw edema model and were 0%, 37%, and 27% respectively in cotton pellet induced granuloma method. Conclusion: This study concludes that, Hesperidin have significant anti-inflammatory effects in both acute and chronic inflammatory conditions.

1. INTRODUCTION

The inflammation term is originated from the Latin word “inflammare” which means to burn. It is one of the most important processes involved in the defense systems against local injury and infections; however, it progresses to painful or chronically harmful disease requiring pharmacological treatment [1]. Typical inflammatory disease such as rheumatoid arthritis, asthma, colitis and hepatitis are the leading cause of death and disability in the world [2]. Chronic inflammation also contributes to the development of variety of diseases including cancer, cardiovascular and neurodegenerative disorders [3].

Inflammatory response is a chain of different effective mechanism comprising of specific vascular, humoral and cellular events that are described by the movements of fluids, plasma and inflammatory leukocytes (neutrophils, eosinophil and macrophages) to the site of inflammation[4].A different chemical mediators or alert molecules such as histamine, serotonin, leukotrienes, prostaglandins and oxygen obtained as free radicals are produced by inflammatory and phagocytic cells predominantly in the sequence which participates in onset of inflammation[5].

Inflammatory response occurs in two phases as (a) acute (b) chronic, and each is apparently mediated by a different mechanism.

Acute inflammation lasts from few minutes to hours or one to two days. The fundamental signs of acute inflammation are those described by Celsus in the 1st century like calor (heat), dolar (pain), rubor (redness) and tumor (swelling)[6].

Chronic inflammation is distinguished by perfusion of mononuclear cells (macrophages and lymphocytes), proliferation of fibroblasts, collagen fibers and synthesis of connective tissues. Degeneration is mainly mediated by reactive oxygen species and protease produced from infiltrated inflammatory cell[7]. These reactive oxygen species are mutagenic and during process of repeated tissue damage and regeneration, they interact with DNA in proliferating epithelium resulting in perpetual genomic modification such as spot mutations, deletion or re-organization[8].

Flavonoid is derived from the Latin flavus (yellow) or polyphenolic secondary metabolites were discovered in 1938 by the Hungarian scientist Albert Szent-Gyorgi was disrobing an interaction between fresh vitamin C and as yet unknown co-factors from the peels of lemons,

which scientist Alber first called "citrin," and, finally, "vitamin P"[9]. Flavonoids belong to a set of natural material and present in great number of higher plant species, principally placed in fruit, bark, seeds or flowers. These natural products were recognized for their useful effects on health long since before flavonoids were separated as the efficient substance. Flavonoids have significant function in maintaining human health and protecting it from any diseases. Furthermore, it plays significant role in preventing and fighting allergy, viruses in addition to preventing of the properties of inflammation. This last one constitute an aspect employed for a long time in the Chinese traditional medicine and the cosmetic industry represented by the compound of plant extracts, but recently it has begun to be explored in depth, in order to identify the mechanisms responsible and the possibility for use of flavonoids as anti-inflammatory agents [10].Flavonoids are accountable for the attractive color of flowers, leaves and fruit, as enzyme inhibitors, foreboding of toxic materials, prevent against ultraviolet radiation exposure and infections[11]. Flavonoids are involved in energy transfer, morphogenesis, and attracting pollinators; contribute to attract the prey, respiration, photosynthesis process, activity of plant growth hormones and organizer, gene expression and behavior[12]. The best described property of nearly all group of flavonoids is their ability to act as anti-inflammatory. Flavonoids regulate the inflammatory response by inhibition of produce and release of pro-inflammatory mediators, alteration of eicosanoid formation, inhibition of activated immune cells[13]. Flavonoids are reported to possess anti-inflammatory activity *in vitro* and *in vivo*[14].

Flavonoids have been specified more than 8000 in plants. It occurs as phenyl benzopyrones compounds which have a basic structure characterized by two benzene rings attached with a heterocyclic pyrane or pyrone with low molecular weight[10]. Depending on molecular structure flavonoids are divided into four basic classes: flavones, flavanones, flavanoles and anthocyanin's but, they all share the same common flavan nucleus base structure substitution on the C ring defines each class of flavonoids and substitution on A or B ring distinguish individual compound within a class[15].

Hesperidin, one of the most important flavonoids, belongs to the flavanone class of flavonoid, with the formula $C_{28}H_34O_{15}$. Hesperidin consists of aglycone (the chemical structure lacking sugar moieties), IUPAC name: (2S)-7-[[6-O-(6-Deoxy-alpha-L-mannopyranosyl)-beta-D-glucopyranosyl] oxy]-2, 3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one. Hesperidin is the predominant flavonoid in citrus fruits, primarily in sweet

orange (in young immature oranges it accounts for above 14% of the fresh weight of the fruit and lemon, and consequently in juices made of these citruses[16].

Hesperidin has significant anti-inflammatory activity by blocks the produce pro-inflammatory mediators, especially the arachidonic acid, thromboxane A2 and prostaglandins F2, E2[17].

Treatment of inflammation is a long discussion as the conventional NSAIDS are well known to cause adverse drug reactions. Moreover, there is undertaking study to promote safer and more effective drugs for the therapy of inflammation .In view of this and on account of assumed worthlessness of this compound in the traditional treatment, the current investigation was done to assess the anti-inflammatory property of Hesperidin in experimental animal sample.

MATERIALS AND METHODS

Chemicals

Flavonoids (Hesperidin), Carrageenan, Indomethacin were purchased from Sigma Aldrich Co. (St Louis, MO, USA). Polysorbates 80 USP (Tween 80) as a suspending agent for Indomethacin, Carboxy-methyl-cellulose 5% as a suspending agent for flavonoids were purchased from Himedia Chemical Co. India.

Experimental Animals (Care and Maintenance)

Healthy adult male guinea pigs weighing between (350-650 g) were obtained from the animal house of Biology Department, Ibb University-Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions (25±5C°, 55±5% humidity and 12 h light–dark cycle) to acclimatize with free access to diet and water *ad libitum.* Guinea pigs were given grass and carrots. The study protocol was approved by Institutional Animal Ethics Committee of Ibb University-Yemen.

METHODOLOGY

Animals were randomly divided into 3 groups of 6 pigs each; group I: Control (1ml of 5% carboxymethyl cellulose suspension); group II: Test drug (Hesperidin 40mg/kg); group III: Standard drug (Indomethacin 10mg/kg). All the doses were administered orally. The *in vivo* anti-inflammatory activity was studied using carrageenan induced paw edema.

Carrageenan Induced Rat Paw Edema Model [18]

It is devoted to investigate the effect of single oral administration of Hesperidin in guinea pigs induced by injecting of 0.1ml of carrageenan (suspension in saline) into the sub-plantar superficies of the right hind paw. Eighteen healthy male guinea pigs were used in this experiment. The animals were divided into 3 groups, 6 animals in each group.

Details are as follows:

Group I was served as a non-treated control. They received 5% carboxymethyl cellulose. Group II were given oral single dose of Hesperidin (40mg/kg). Group III received oral single Indomethacin in a dose of (10mg/kg). Indomethacin (as a standard anti-inflammatory agent) was dissolved in 5% Polysorbate 80 (Tween 80) with the concentration of 10 mg/kg. It was prepared just before use[19].

The edema (acute inflammation) was induced in each of the guinea pigs by injecting of 0.1ml of carrageenan suspension in saline into the sub-planter superficies of the right hind paw 1h after flavonoids administration. The volume of paw edema was determined directly after carrageenan injection and during 1, 2, 3 and 4h by using a plethysmograph apparatus(VGO Basile, Italy) and compared with the volume of the right hind paw before edema induced. The percentage inhibition of inflammatory effect of the flavonoids was calculated using the formula:

$$\text{Anti-inflammation activity (\%)} = [(Vc-Vt)/Vc] \times 100$$

where:

Vc = The average paw edema volume in the control group.
Vt = The average paw edema volume in the flavonoids-treated (group II) or (group III) Indomethacin-treated pigs at the same time.

Cotton Pellet-Induced Granuloma [18]

It is devoted to investigate the effect of Hesperidin on guinea pig, which induced (chronic inflammation) by implant of sterile 100% pure cotton pellets (50±1 mg). Eighteen male guinea pigs were used in this experiment. The animals were divided into 3 groups, 6 animals on each. First group served as anon treated control. The animals received 5% carboxymethyl cellulose single orally. Second group of animals was administered with Hesperidin

(40mg/kg). The animals of third group were given oral dose of Indomethacin (10mg/kg) once daily as aqueous suspensions using 5% Polysorbate 80[19] for 7 successive days from the 1st day of cotton pellet implantation of each.

After 30 minutes, all experimental animals were anesthetized with light ether. A sterile cotton pellet weighing (50 mg±1) was implanted in each pig on both sides in the scapular region under the skin. On the 8th day all animals were anaesthetized with chloroform. The cotton pellets were isolated, dried at 60°C, and the dry weight was determined. Chronic inflammation was indicated as an increase of weight of cotton pellet as inflammatory response [20].

Statistical Analysis

The effects of different drugs under study was presented by calculating the mean and SD of the outcome parameters. One way Analysis of Variance (ANOVA) and independent samples T-test was applied to see the difference between any two groups at a time (5% significance level). SPSS for windows (version 15) was applied in the statistical analysis.

RESULTS AND DISCUSSION

Anti-Inflammatory Studies

Carrageenan Induced Rat Paw Edema

The anti-inflammatory effect of the Hesperidin using carrageenan induced edema tests is expressed in (Table1). In this test, the positive control (Indomethacin 10mg/kg) significantly ($P<0.05$) decreased the paw edema by 65%, 71%, 72% and 74% after 1, 2, 3 and 4 hours compared with non-treated control group value. Guinea Pigs with the Hesperidin at 40 mg/kg/ body weight significantly decreased ($P<0.05$) the carrageenan induced edema paw volume edema by 50%, 51%, 63% and 77 % after 1, 2, 3 and 4 hours respectively compared with non-treated control group values.

Cotton Pellet Induced Granuloma in Rats

The result of anti-inflammatory activity of Hesperidin in cotton pellet induced granuloma is shown in Table 2. Hesperidin (40mg/kg) showed significantly decreased ($P<0.05$) in granuloma formation as compared to control group. The percent of inhibition were

significantly 0%, 37%, and 27% for non-treated control, (10 mg/kg) Indomethacin and (40 mg/kg) Hesperidin, respectively (Figure 3).

DISCUSSION

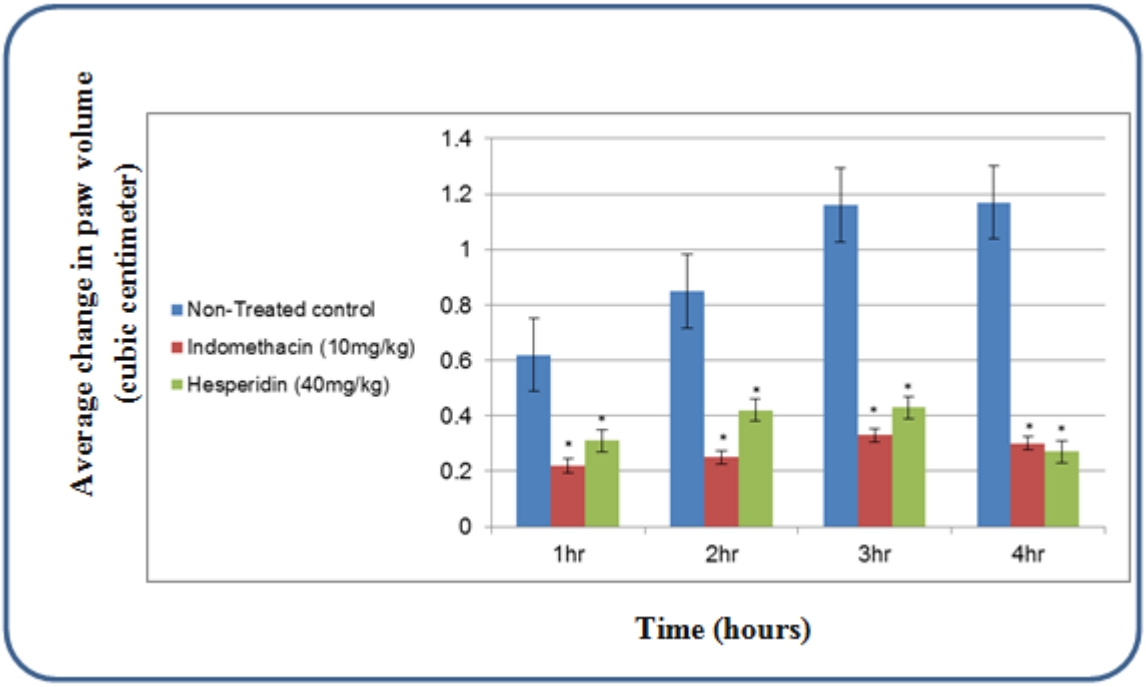
Carrageenan-induced inflammation is widely applied model to estimate the anti-inflammatory potency of compounds or natural products [21]. The potential mechanism of action of carrageenan-induced inflammation is biphasic; the first phase is characterized by the release of histamine, serotonin and kinins; while the 2nd phase is referred to the release of lysosome enzymes and prostaglandins at 2 to 4 hours[22]. This phase is sensitive to different anti-inflammatory medications[23]. The results of present study revealed that flavonones (Hesperidin) were proved to most potent anti-inflammatory agent (77%, $P<0.05$) compared to a dose of Indomethacin 10 mg/kg (74%, $P<0.05$). A possible mechanism of the anti-inflammatory activity of these Flavonoids is the inhibition of the enzymes in the arachidonic acid pathway; prevent the synthesis of prostaglandins [24]. Flavonoids have been reported to possess high anti-inflammatory activity[25]. Indomethacin block the action of cyclooxygenase enzymes I and II which are necessary for the synthesis of inflammation mediating factor like prostaglandin E2 (PGE2) from arachidonic acid[26].

Cotton pellet is widely used to induce granuloma for the estimate of the transudate, exudative and proliferative components of chronic inflammation. The absorption of the surrounding fluid by the cotton pellet influences the wet weight of the granuloma[27]. Flavonoids were capable of inhibiting the development of cotton pellet induced granuloma, used as a model of proliferative phases of inflammation, In the present experiment, there was significant improvement evidenced by reduction of weight of implant compared with control non-treated cotton pellet implanted animals. The percent of inhibition were significantly 37% and 27% for Indomethacin (10mg/kg), and Hesperidin (40mg/kg) respectively. The results refer that the flavonoids (Hesperidin) prevent the synthesis of collagen and mucopolysaccharides, elevated number of fibroblasts through granuloma tissue synthesis in the chronic inflammation stage. The anti-inflammatory activity of Flavonoids may due to COX-2 enzyme inhibition. This effect may be due to the cellular immigration to lesion sites and accumulation of collagen. Cell migration occurs due to many various processes involved like adhesion and cell mobility[28].

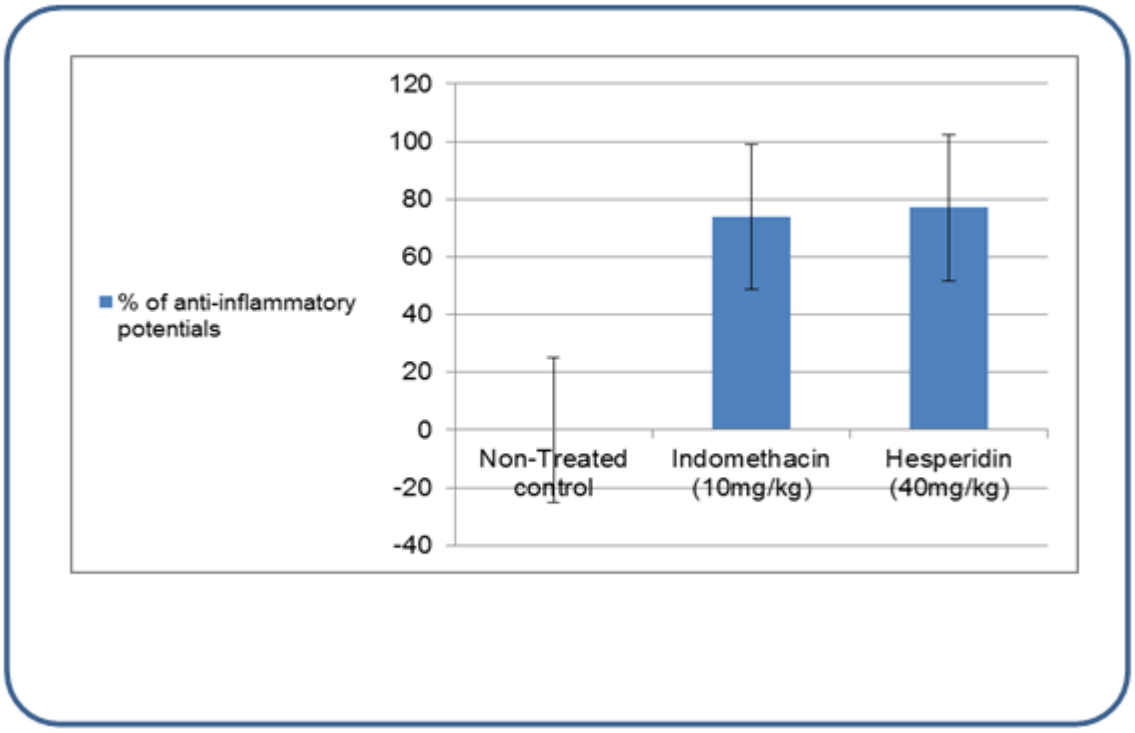
(Table 1): Effect of Hesperidin and Indomethacin on carrageenan induced edema paw volume in male guinea pig

Groups	Mean paw edema(cm)± SD				%Percent inhibition at 4 hours
	1hr.	2hr.	3hr.	4hr.	
Non-treated control	0.62 ±0.008	0.85 ±0.005	1.16±0.009	1.17 ±0.006	0.0%
Indomethacin (10mg /kg)	0.22 ±0.008*	0.25 ±0.005*	0.33 ± 0.009*	0.30 ±0.006*	74%
Hesperidin(40mg/ kg)	0.31 ±0.012*	0.42 ±0.005*	0.43 ±0.014*	0.27 ± 0.006*	77%

Values are expressed as mean ± SD, n=6 animals per group, *P<0.05ascompared to control (one-way ANOVA).



(Graph 1): Effect of Hesperidin and Indomethacin on carrageenan-induced edema paw volume in male guinea pig.

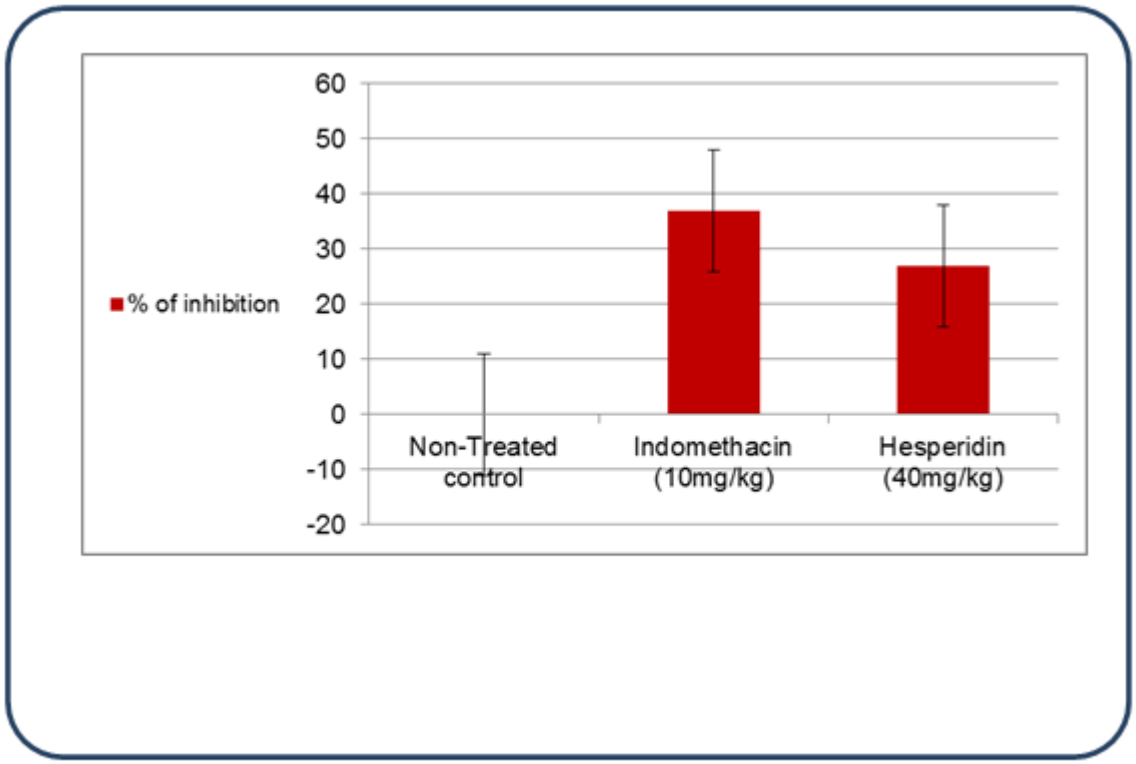


Graph 2:Percent of anti-inflammatory potentials (%) with the control, standard (Indomethacin) and the test compound (Hesperidin), in the carrageenan induced paw edema.

(Table 2):Anti-inflammatory activity of Hesperidin and reference standard drug Indomethacin (10mg kg) on cotton pellet granuloma in normal male guinea pigs.

Treatment	Weight of dry granuloma(mg)	% of inhibition
Non-treated control	86.67 ± 18.75	0%
Indomethacin (10mg/kg)	55.00 ± 6.74*	37%
Hesperidin(40 mg/kg)	63.33 ± 11.55*	27%

Results are Mean ± SD (n=6) *P<0.05 compared to control.



(Graph 3): Anti-inflammatory activity of Hesperidin and reference standard drug Indomethacin (10mg/kg) on cotton pellet granuloma in normal male guinea pigs.

CONCLUSION

Based on the aforementioned results it can be concluded that the Hesperidin (40mg/kg) showed good significantly (*P<0.05*) anti-inflammatory effects on carrageenan induced edema, which may be related to inhibition of inflammatory mediators formation.In chronic cotton pellet induced granuloma model, there were significant improvements evidenced by reduction of weight of implant compared with control non-treated animals. Percent of inhibition for indomethacin and Hesperidin were significantly 37% and 27%. This refers that the experiment samples reduce the rise in the number of fibroblasts, formation of collagen and mucopolysaccharides through granuloma tissue synthesis in the chronic inflammation stage. The results of the present study may serve as a ready reference for the standardization of flavonoids (Hesperidin) for converting them into therapeutic modalities hence the results suggest that selected flavonoids are quite safe and can be used for design and development of novel anti-inflammatory agents.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Ali Ahmed Hassan Al-baddany for his unlimited help during the laboratory work. The authors gratefully acknowledge the help by animal house of Animal Production Department, Ibb University-Yemen for conducting animal experiments.

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