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Original Article

POTENTIAL HEALING EFFECTS OF HIBISCUS SABDARIFFA L. FLOWERS ON ARTHRITIS

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ABSTRACT

Objective: The present study has been focused to assess the anti-inflammatory activity and healing effects of the aqueous extract of *Hibiscus sabdariffa* L**.** Flowers on induced arthritis in mice and compare it with meloxicam (Mobic®), one of the most conventional drugs used to treat arthritis.

Methods: The water extract of *Hibiscus sabdariffa* was administered orally at a dose of 300 mg/kg, 400 mg/kg and 500 mg/kg body weight for 14 days after induction of arthritis with incomplete fruend's adjuvant.

Results: (T1, T2 and T3) showed a significant increase in body weight when compared with T4, negative and positive control groups. A significant decrease in the levels of RBC and Hb was observed in all groups subjected to arthritic (T1,T2,T3,T4 and T5) when compared to the negative group (T6). The administration of the aqueous extract of *Hibiscus sabdariffa* L. flowers to arthritic mice in (T1, T2 and T3) improved the levels of Hb and RBC to near normal. A significant reduction (P≤0.01) in spleen weight ,WBC, ESR, CPR and serum copper level was found at all treatment groups with the water extract of *Hibiscus sabdariffa* in comparison with the groups treated with meloxicam, the positive and the negative.(T1, T2 and T3) reveled a significant (P≤0.01) reduction of the inflammation in comparison with the other treatment groups (T4, T5). A better activity was observed at 500mg/kg body weight in mice.

Conclusion: The water extracts of *Hibiscus sabdariffa* L**.** flowers reveled a significant anti-inflammatory activity in albino mice at a dose of 300, 400 and 500 mg/kg body weight. Whereas 500 mg/kg in mice proved to possess better healing effects

Keywords: Hibiscus sabdariffa L, meloxicam (Mobic®), Erythrocyte sedimentation rate (ESR), C-reactive protein level (CPR) andthe serumm copper level.

INTRODUCTION

The field of natural product biotechnology and ethnopharmacology has received renewed attention in the recent years. The concept of ethnopharmacology specifically aims to develop plant-based drugs from the more widespread local use either as pure phytochemical compounds or plant extracts (phytotherapy)[1].

Hibiscus sabdariffa L. Is a genus of the Malvaceae family, a plant known for its large, beautiful and colorful flowers. Different types of hibiscus have been used around the world as a famous beverage and herbal remedies. The water extract of hibiscus is named in the middle east as "Karkade". In Iraq it is called ''Kojorat'' and in Iran it is called sour tea. While in most English speaking countries it is called red sorrel. About 15–30% of the plant is made of plant acids, such as citric, malic, and tartaric acids, as well as allo-hydroxycitric acid lactone (hibiscus acid). *Hibiscus* also contains alkaloids and flavanoids, L-ascorbic acid, anthocyanin, beta carotene, beta sitosterol, citric acid, polysaccharides (arabins and arabinogalactans), quercetin, and gossypetin, In addition *Hibiscus* contains small amounts of galactose, arabinose, glucose, xylose, mannose, and rhamnose [2].

In recent years, many researchers have been exploring the health effects of this delicious drink and the chemicals in this plant. *Hibiscus sabdariffa* extract has almost no side effect as its LD (50) was found to be above 5000 mg kg-1. (3). In the other hand, experimental and clinical studies have shown that *Hibiscus* has an antihypertensive activity and an ability to reverse cardiac hypertrophy. [3-7]. In another study hibiscus extract blocked adipogenesis, through suppression on the expression of adipogenic transcription factors, including C/EBPalpha and PPARgamma [8]. Also, some clinical studies used *Hibiscus* to study its effect on reducing lipid profile [9,10]. Moreover, *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis [11]. In addition, *Hibiscus* exhibited a protective effect for erythrocytes from protein degradation, lipid peroxidation. [12]. Also, a study proved the inhibitory effect of a phenolic acid isolated from *Hibiscus sabdariffa* L. named protocatechuic acid (PCA), on tumor promotion in mouse skin cancer (13). Furthermore, *Hibiscus sabdariffa* L. Induced apoptosis in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expressions [14]. Additionally, recent scientific findings combined with traditional knowledge suggest that hibiscus water extract can be used as an alternative medicinal beverage in the management of obesity [15].

Arthritis is a sum of conditions effects the health of the bone joints in the body. It is considered one of the most widespread chronic health problems worldwide. Around 43 million adults in the united states are reported to be diagnosed with types of arthritis including rheumatoid arthritis, ankylosing spondylitis or fibromyalgia [16]. The most frequent signs of arthritis are swelling in the joints, stiffness around the joint, constant or recurring pain with tenderness in the joint, difficulty in moving the affected joint and finally the warmth and redness in the joint.

Arthritis can appear for a number of reasons including infection injury, abnormality of the immune system and aging.[17] The aim of arthritis therapy is to reduce or prevent functional impairment and structural damage that can occur over a patient's lifetime. Long-term control is often best obtained through the adaptation of treatment based on disease activity. [18].

The conventional treatments for arthritis are mostly non steroidal anti-inflammatory drugs such as aspirin®, ibuprofen® and voltaren®. This group may cause many side effects and risks such as potential heart attack, stroke, and stomach bleeding. Since *Hibiscus sabdariffa L*. Has exhibited a variety of therapeutic effects with no associated toxicity. Also, Hibiscus illustrated an anti-inflammatory effect [19]. Thus, this herb was considered as a potential therapy for arthritis.

Therefore, this study was designed to study the healing effects of the water extract of *Hibiscus sabdariffa* L**.** flowers on induced arthritis in mice and compare it with meloxicam (Mobic®), one of the most conventional treatments used to treat arthritis.

MATERIALS AND METHODS

Plant Material

Dried flowers of *Hibiscus sabdariffa L.* Were collected from the local market in Baghdad city. They were identified at the department of Pharmacognosy and medicinal plants of the college of pharmacy-University of Baghdad. Flowers were grinded into a fine powder.100 g of *Hibiscus sabdariffa L.* Was extracted for 24 h with distilled water. Then, the solution then was sieved using a sterile gauze to get rid of coarse particulars and filtered using Whitmann® filter. Later the filtrate was concentrated by a rotavap and dried over a water bath at 45°C with intermittent vigorous shaking. The Aqueous Extract of *Hibiscus sabdariffa L.* (AEHS) obtained were stored in a refrigerator for the *in vivo* study [20].

Determining concentrations of (AEHS) used in this study

According to OECD Guideline no. 423 (Organization for Economic Cooperation and Development). The aqueous extract of *Hibiscus* has been stated as nontoxic even at a dose of 2000 mg/kg body weight (19). Moreover, *Hibiscus sabdariffa* extract was found to have an LD (50) above 5000 mg kg-1 (2). Therefore, three different doses of (AEHS) 300,400 and 500 mg/kg doses were selected for the *in vivo* study.

Experimental Animals

Thirty eight albino mice weighting (30-40g) obtained from the Laboratory Animal Facility at the college of Pharmacy/ University of Baghdad was used for this experiment. Animals were placed in cages subjected to constant environmental conditions. Standard rodent diet (commercial feed pellets) and Tap water was freely available.

Induction of rheumatoid arthritis

0.1 ml of incomplete fruend's adjuvant was injected in the right tarsal joint of each animal and repeated after 7 days. Arthritis signs appeared after 14 days [21].

The main *in vivo* experiment

Thirty six mice were divided equally into six groups. Treatment was administrated daily by G-tube for 14 days:

First Group (T1): Was treated with a dose of 500 mg/kg B. W of (AEHS).

Second Group (T2): Was treated with a dose of 400 mg/kg B. W of (AEHS).

Third Group (T3): Was treated with a dose of 300mg/kg B. W of (AEHS).

Fourth Group (T4): was treated with 0.2 mg/kg meloxicam (Mobic®),

Fifth Group (T5): Was considered as positive control and treated with distilled water only.

Sixth Group (T6): Was considered as the negative control group.

Parameters used in this experiment

Body weight was measured on day one and after a week and on the last day of the experiment. At the end of the 15th day after the approval of the animal rights committee at the college of Pharmacy/ University of Baghdad, mice were euthanized by cervical dislocation. Spleens were collected from all groups were weighted. Serum samples were collected for further biochemical assays Hb (g/dl), RBC, WBC, ESR, CRP(µg/ml) and copper (µg/ml).

RESULTS

Visual observations

Animals in (T4 and T5) were generally lethargic, inactive, redness and enlargement of the metatarsal joint, difficult in walking and lameness at day 15 in comparison with the rest of treatment groups. Also, the consumption of dietary pallets was much less in (T4 and T5) due to the loss of appetite.

Body weight

There was a significant difference in the body weight of each group after the induction of arthritis. (T1, T2 and T3) showed a significant increase in body weight when compared with (T4), the negative and positive control groups as illustrated in (figure 1).

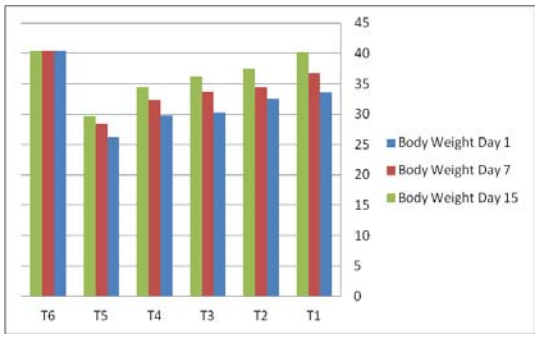

Fig. 1: The effect of 3 different doses of (AEHS) in compassion with meloxicam and Positive and negative groups on Body weight in arthritic induced rats

Spleen weight

The enlargement of spleen was found clearly in (T5). The water extract of *Hibiscus sabdariffa* L**.** flowers groups at different doses (500, 400 and 300 mg/kg) along with the group treated with meloxicam (Mobic®) produced a significant ($P \leq 0.01$) containment of the spleen weight. (T1) showed a clear suppression in spleen weight when compared with (T2,T3,T4) as showed in (figure 2).

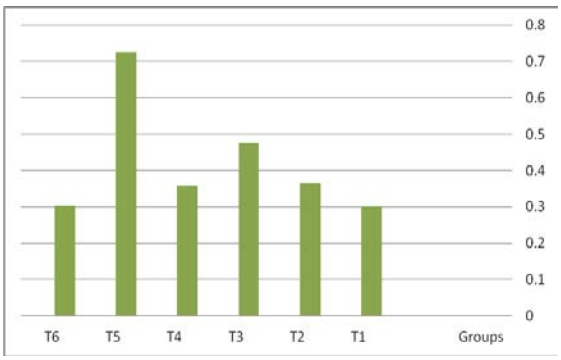

Fig. 2: The effect of 3 different doses of (AEHS) in compassion with meloxicam and positive and negative groups on Spleen weight in gm / 100 gm body weight in arthritic induced mice

Hematology

Hematological parameters taken for this experiment(Hb, RBC count, WBC count, ESR, C-reactive protein level and serum copper levels) are shown in (Figure 3).

A significant decrease in the levels of RBC and Hb was observed in all groups subjected to arthritic (T1,T2,T3,T4 and T5) when compared to the negative group (T6). The administration of *the aqueous extract of Hibiscus sabdariffa* L. flowers to arthritic mice in (T1, T2 and T3) improved the levels of Hb and RBC to near normal. The increases in WBC count, ESR, CPR and serum copper level were significantly suppressed in (T1).

DISCUSSION AND CONCLUSION

Hibiscus sabdariffa L**.** Was proven in previous studies that it possesses anti inflammatory and an analgesic effects [18]. Therefore, this plant was used in this study and was proven to possess healing effects in the treatment of rheumatoid arthritis induced in mice using incomplete fruend's adjuvant.

Loss of appetite and weight loss is one of the constitutional symptoms that come associated with rheumatoid arthritis. Results of measuring body weight for all treated groups found a significant difference between body weights of each groups immediately after induction of arthritis in animals. Moreover, there was a significant increase body weight in treated groups after day 15.

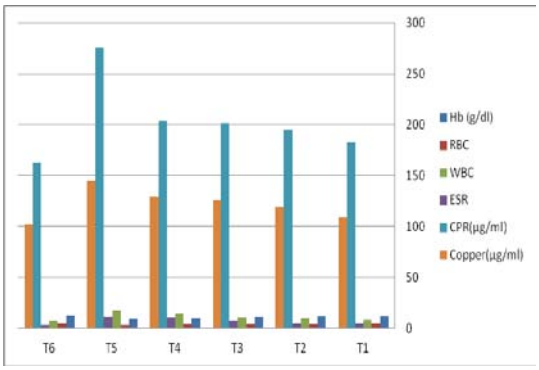

Fig. 3: The effect of 300,400 and 500mg /kg of (AEHS), mobic®, distilled water and control group on hematological parameters

In general, enlargement of the spleen occurs during inflammation, as spleen has the phagocyte nature which is marked clearly in the positive control (T5). The spleen weight also significantly decreases at all doses in the treated groups (T1,T2,T3 and T4). This result is referred to the anti inflammatory effect of the extract [19].

Anemia is noticed evidently in patients with arthritis(22). The cause of anemia is by a variety of mechanisms. The continuous inflammation caused by rheumatoid arthritis leads to elevated levels of hepcidin, and that causes the anemia because iron is poorly absorbed and also sequestered into macrophages. In addition, rheumatoid arthritis causes a warm autoimmune hemolytic anemia [23]. Moreover, gastrointestinal ulcers from arthritic medications causes blood loss and that prevents the release of iron for incorporation into red blood cells [24-25]. In this study anemia was clearly marked in (T4 and T5) as these groups showed a reduced RBC count, reduced Hb levels, and an increased erythrocyte sedimentation rate (ESR). While the negative control group and the three groups treated with different doses of (AEHS) showed no signs of anemia.

Increased white blood cell counts are a common feature of inflammatory reactions, especially those induced by rheumatoid arthritis [26-27]. WBC was highly increased in (T4 and T5) while the migration of leukocytes to the inflamed area was significantly suppressed in (T1, T2 and T3) this was indicated by the significant decrease in the WBC count. This activity may be due to presence of steroidal phytochemical compounds [28].

Erythrocyte sedimentation rate (ESR) in (T5) was high when compared to treated groups (T1,T2, T3 and T4). This may be due to the flavonoid content of *Hibiscus sabdariffa* L. flowers. As flavonoids have surface charge neutralizing effects. ESR is highly affected with the ability of red cells to aggregate into orderly stacks or rouleaux. Proteins are thought to affect the repellant surface charges on red cells and cause them to aggregate into rouleaux and thus the sedimentation rate elevates. [29]

C-reactive protein levels elevate noticeably during inflammatory processes [30-31]. The concentration of C-reactive protein in (T5) was significantly increased in comparison with (T6). While CPR levels in(T1, T2 and T3) were found to be significantly reduced in comparison with (T4 and T5).

Ceruloplasmin, an enzyme produced in the liver, it contains 8 atoms of copper in its structure. Free copper ions are powerful catalysts of free radical break. By binding copper, ceruloplasmin prevents free copper ions from catalyzing oxidative damage [32]. The increase in copper ion levels is an indication for inflammation [33]. Positive control group (T5) showed a clear increase in copper (µg/ml) in comparison with the rest of the treating groups (T1, T2, T3 T4 and T6).

Phytochemical analyses of *Hibiscus* showed the presence of the flavonoids apigenin, sitosterols, alkaloids, reducing sugars, as well as unidentified sterols [2,34-35]. Apigenin has been reported to possess anti-inflammatory activity(36). Flavonoids are anti-inflammatory agents; they may exert its anti-inflammatory activity by inhibiting the 5-lipoxygenase pathway, which collectively with COX-2 pathway, are very important in maintaining inflammation [37].

In summary, the water extract of *Hibiscus sabdariffa* L. flowers reveled significant anti-inflammatory activity in albino mice at a dose of 300, 400 and 500 mg/kg body weight. Whereas 500 mg/kg in mice proved to posses better healing effects.

CONFLICT OF INTERESTS

Declared None

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