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Detection about the Anti-Inflammatory Effect in Alcoholic Extract of Alhagi maurorum in vitro

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ABSTRACT

This study was conducted to detect the presence of some active compounds in the extract of Alhagi maurorum plant, which was extracted by soxhlet using methanol as a solvent. The chemical survey showed the presence of glycosides, tannins, alkaloids and phenolic in the studied plant.

The anti-inflammatory activity of the plant extract had been studied in vitro by two methods , albumin denaturation inhibition test and hemolysis stimulated by heating test and results showed the effectiveness of the Alhagi maurorum plant extract with compared to standard medicine (Aspirin) and negative control groups.

Keywords-- Alhagi maurorum, Anti-inflammatory, Albumin, Aspirin

I. INTRODUCTION

Inflammation is defined by pathologists as ordinary defense reaction of the living body cells to the hurt or injury that is represented as the manner to restrict and localize the irritation, infection and the causative agents[1]. The living body in inflammation state shows many signs which include rubor (redness), calor (heat), dolor (pain), tumor (swelling) and loss of function[2]. It can be divided into two kinds: acute and chronic , the acute inflammation is began within minutes to few hours of injury onset and fixed or the tissues healed with hours to days , if this is not occurred the inflammation become chronic inflammation which may persist to weeks or months[3,4].

The inflammation generally treat by many drugs include steroidal and nonsteroidal agents (NSAIDS) as diclofenac and mefenamic acid in addition to many other strategy for treatment[5,6]. The use of medicinal plants are becoming common because of the harmfulness and toxicity of some synthetic drugs[7] that some plants used to treat the inflammation in many cases of injury or infections such Achillea millefolium, Terminalia bellarica , Curcuma longa and Commiphora myrrha[8,9,10].

The plant (Alhagi maurorum) is used also for treatment and many cosmetic that the genus Hedysarum alhagi was well-known by Linnaeus in 1753 then the name Alhagi maurorum, replacing Linnaeus name by Friedrich Kasimir Medikus[11]. It's generally termed by a common name camel thorn which is inborn in the region between the Mediterranean and Russia, and its grown well in the Middle East area[12]. The plant contain carbohydrate thus manitol sugar is extracted from it to use in tablets made in drugs industry[13] also glycosides as anthraquinone , and hall plant has volatile oil with the exception of for the roots[14]. The plant used in pharmaceutical productions as it used to manufacturing laxatives, diuretics and sweeteners[15]. Nowadays, the plant consider as antioxidant, anti-inflammation and antibacterial agent[16].

II. METHODS

2.1 Preparation of the plant extract:

The plant (Alhagi maurorum) was collected from Al – Najaf desert, crushed to powder by mixer grinder. Soxhlet was used to extract plants by adding 250 ml of solvent (methanol 95%) to 25 gm of plant powder for 24 hours , filtration, concentration and dried then stored in refrigerator until used and for detecting the presence of active constituents the phytochemical tests occurred to investigate about secondary metabolites[17].

2.2 Evaluation of the anti-inflammatory effects of plants extracts:

A. Albumin Denaturation Inhibition:

The plant extract action against inflammation could be prepared as: 1 ml extract mixed with 1 ml bovine serum albumin (1% aqueous solution). In triplicate work, Alhagi maurorum mixtures placed for (20 min) in incubation at 37°C then for (20 min) at 51°C then cool and the

absorbance determined at 660 nm with spectrophotometer[18]. The percentage of Inhibition to albumin protein denaturation was valued as:

Inhibition percentage = (Control absorbance – sample absorbance) x 100 / control absorbance.

B. Preparation of red blood cells (RBCs) suspension:

A fully unblemished blood (10 ml) obtained from a robust and vigorous voluntary associates which did not take drugs for inflammation treatments (NonSteroidal Drugs) for 2 weeks before to the experimental work is transferred to the tubes to centrifuge for (10 minutes) at 3000 round / minute and were diluted three times with identical volume of normal saline[19].

C. Hemolysis stimulated by heating:

In centrifuge tubes, in triplicate, 1 ml from plant extract of concentrations (500, 1000 and 2000 µg/ml) mixed with (1 ml) of 10% RBCs suspension from the item B, +ve control was standard drug (Aspirin, 100µg/ml) and – ve control was saline's solution only, then in water bath, tubes were kept warm at 56 ºC about (30 min), cooled with tap water. Finally, at 2500 round / minute for 5 min, the mixture of plant solutions with RBCs were centrifuged and the upper layer absorbance was measured at 560 nm [19]. The percentage of Inhibition was estimated as: Inhibition rate = (control absorbance – sample absorbance) X 100 / control absorbance.

III. RESULTS AND DISCUSSION

Phytochemical tests to alcoholic extracts of Alhagi maurorum by reagents exposed the presence of glycosides, tannins, alkaloids and phenolic compounds as indicated in previous studies[14]. The components of lysosomal membrane are analogous to the components of human red blood corpuscle (HRBC) membrane[20], thus these were carefully chosen to studying the effects of toxic materials on their membranes since of their accessibility and effortlessness[21]. The maintenance and stability of lysosomal membrane is very important because through inflammation, the enzymes of lysosome will be liberated which lead to disintegration of cell by rupture of the cells membranes which is causing loss of cations from the membranes[22] and the structure of protein will be defeated and damaged with denaturation[23]. The anti-inflammation drugs (nonsteroidal drugs as Aspirin) play a role in the stability of lysosomal membrane in addition to suppression the action of lysosomal enzymes [24]. The potential effect of plant extract to prevent albumin protein denaturation was studied in vitro and the heat induced hemolysis and the results explained by figures (1) & (2).

The results revealed that Alhagi maurorum plant extract has significant effect in constraining heat induced hemolysis of erythrocyte membrane at different effective concentrations which was comparable to the standard aspirin.

Also Alhagi maurorum plant extract was effect in preventing albumin protein denaturation at different concentrations. The results published that methanolic extracts involve contents that preserve and maintain the erythrocytes membranes from lysis effectively. The extract perhaps prevent the liberation of enzymes from lysosome and improve the stabilization of membrane because of the presence of tannins which are participated in the stabilization effects on lysosome membrane and stabilizing erythrocyte membrane with the action of attaching to bivalent cations like Ca^{+2} and Mg^{+2} [25] also the anti-inflammatory effect to prevent the protein denaturation may be due to the presence of alkaloid, polyphenolic compounds and phenolic acid[26].

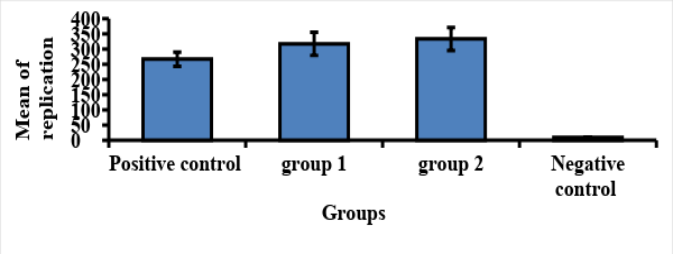

Figure (1): Albumin protein denaturation inhibition effects of Alhagi maurorum methanolic extract results. (group 1: Aspirin drug, group 2 : 1000 µg/ ml , group 3: 2000 µg/ ml and group 4 : albumin protein solution only).

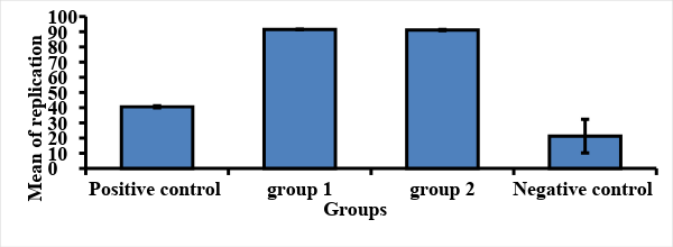

Figure (2): Heat induced hemolysis effect on erythrocytes with Alhagi maurorum methanolic extract results. (group 1: Aspirin drug , group 2 : 1000 µg/ ml , group 3: 2000 µg/ ml and group 4 : normal saline).

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