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Study the anti - inflammatory effect of *Stevia rebaudiana* sweetness *in vitro*

<p>Authors Names a. Meison Abdulbary b. Suaad Lateef Ibrahim c. Albasheer Ammar d. Muhanad Mohammed e. Wafaa Yousif</p> <p>Article History Received on: 26/6/2021 Revised on: 20/7/2021 Accepted on: 25/7/2021</p> <p>Keywords: <i>Stevia rebaudiana</i>, sweetness, anti - inflammatory</p> <p>DOI: https://doi.org/10.29350/jops.2021.26.4.1404</p>	<p>ABSTRACT</p> <p>Because of the high rates of diabetes, overweight disorders and fatness, the need and interest in finding alternatives to sugar has increased, especially in the industrial sector in parallel with reducing the daily intake of sugar by human. This research was developed to identify the active ingredients which include in the plant aqueous extract of <i>Stevia rebaudiana</i>. Chemical examination showed the glycosides and phenolic compounds presence of in the <i>Stevia rebaudiana</i> plant. The effect of <i>Stevia rebaudiana</i> leaves products aqueous extract as anti-inflammation measured in vitro with two procedures, The repression of protein denaturation and hemolysis stimulated by heating and results showed <i>Stevia rebaudiana</i> efficiency with compared to typical medicine (Aspirin) and negative control groups.</p>
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1. Introduction

Because of the high rates of diabetes, overweight disorders and fatness, the need and interest in finding alternatives to sugar has increased, especially in the industrial sector in parallel with reducing the daily intake of sugar by human [20]. Thus, attention was drawn to the natural sweeteners produced from plants and herbs as *Stevia rebaudiana* Bert. Plant. that, stevia leaves have an important non calories secondary metabolites in glycosides form which are used in abundance in food industry as one kgm of stevia products is about 200 times sweeter than sucrose products without calories [27,17].

Stevia rebaudiana Bert. is a branched bushy shrub, the plants first grew in South America, especially in Paraguay, and is now grown in North American countries such as Canada, as well as parts of Asia

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and Europe [13]. Its leaves contain many phytochemicals such as flavonoids, hydrodynamic acid and sugar-free amino acids [15].

In addition to sweetening properties, leaves of *S. rebaudiana* has therapeutic properties like anti carcinogenic, antimicrobial, antiviral , anti-hypertension , antihyperglyceamia , antitumor, antioxidant , antidiarrheal, diuretic,, hepatoprotective, immunomodulatory [7,28,10,4]. Also it plays an important role as a harmless and safe alternative source to regular sugar in human daily use and does not pose any danger to health [20].

The defense response of living cells to damage such as burns or wounds and contamination by foreign bodies for restricting and localizing irritation, infection and causative agents called inflammation [24]. The signs of these response involve redness , heat, pain, swelling as well as lack of function [13]. In the living body, Inflammation begin during few minutes to several hours from harm start with acute manner and inflamed tissues may healed near few days and when it persist for more period will turn into chronic which may continue to along time near several months [4].

Many drugs used to treat inflammation as diclofenac and mefenamic acid and many other treatments [8]. Because of the wickedness harm and toxicity of some synthetic treatments, the use of non-synthetic treatments which obtained from medicinal plants are becoming popular [23] that the plants such yarrow, bastard myrobalan , turmeric and myrrh are used to deal the inflammation in numerous cases [2]. *Stevia rebaudiana* plant product anti-inflammatory activity had been studied *in vitro* in this study.

Materials and methods:

2.1 Stevia rebaudiana Bert extract:

The sweeteners natural plant products were collected from Al – Najaf city markets, add 25 gm of plant sugar powder to 250 ml of distilled water after mixed with small amounts of DMSO, mixed , filtered and reduction of size and dried, finally stored in refrigerator till it used, also the investigation of active constituents presence tests was done [7].

2.2: Anti-inflammatory action study:

A. The inhibition of protein denaturation:

In triplicate work, 1 ml of prepared extract with concentrations (1000 and 2000 µg /ml) was added to 1 ml albumin protein solution which is 1% aqueous solution of bovine serum. the mixtures located in incubation (20 min) first at 37°C, and second at 51°C (20 min), then cool and measured the absorbance by spectrophotometer (660 nm) [1]. Inhibition was valued as:

Inhibition rate = (Cabsorbance – Sabsorbance) x 100 / Cabsorbance.

(C:control and S:sample)

B. (RBCs) suspension:

The blood samples had been took from a robust and healthy animated donors which about 2 weeks before investigational work have not taken any inflammation treatments. About 10 ml from above fully unblemished blood samples are for 10 minutes centrifuged at 3000 round/min, then washed(diluted) with similar volume of normal saline for 3 times [21].

C. Stimulation of hemolysis by heating:

The plant extract which concentrations (1000 and 2000 µg /ml) were centrifuged by adding (1ml) of extract in tubes and (1 ml) of RBCs suspension (10% solution). Aspirin (100µg/ml) represent a standard +ve control and saline's only solution represent the -ve control also centrifuged with 10% RBCs suspension as in plant extract, all tubes (to keep them warmly) were putting in water bath at 56 °C about (30 min), and by tap water then were cooled. Lastly , the solutions with RBCs were centrifuged at 2500 round / minute for 5 min then absorbance of upper parts was measured at 560 nm [10]. Inhibition was valued as below:

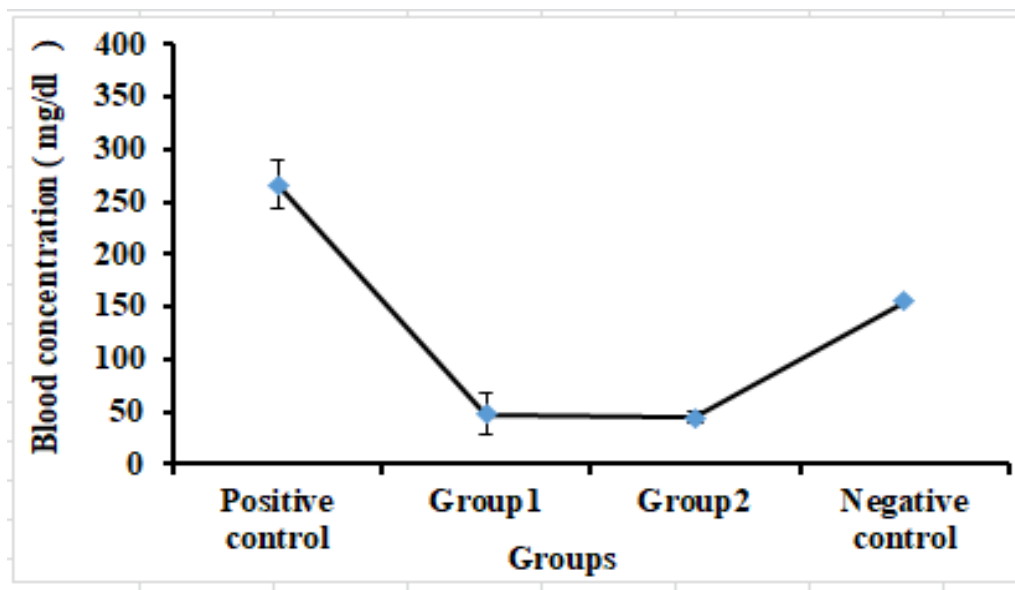
Inhibition rate = (C absorbance – S absorbance) X 100 / C absorbance.

Results and discussion:

The chemical investigation for phytochemicals may present in aqueous extracts of *Stevia rebaudiana* by reagents was showing the occurrence of phenolic composition and glycosides which as showed in prior researches [17]. Elements of lysosomes membranes are similar to that which found in human red blood corpuscle (HRBC) membrane [22], therefore, the human red blood corpuscle (HRBC) were thoughtfully preferred to studying the actions of many substances especially the toxics because of their availability and simplicity of getting them [18].The membrane stability is highly importance in lysosomes membranes since during inflammation, the lysosomal enzymes will be free activate that lead to cell damage breakdown by rupture of the cells membranes resulting lack or absence of cations from the membranes [5] in addition protein configuration will be crashed or broken by denaturation [11]. Aspirin which is consider as non-steroidal anti-inflammation drugs plays a role in the lysosomal membrane stability beside the lysosomal enzymes action inhibition [25]. The probable effect of plant extract is resulting in membrane stability and avoiding protein denaturation which was reflected in vitro with stimulation of hemolysis by heating and the inhibition of protein denaturation that explained by figures (1) & (2).

Stevia rebaudiana plant indicated significant action when compared to Aspirin when studied RBCs hemolysis induced by heat at different effective concentrations. Also *Stevia rebaudiana* played a role in avoiding denaturation of protein with studied concentrations. The effects indicated that plants include active constituents aid in preserving lysis and maintains the RBCs membranes efficiently and may be inhibit the lysosome enzymes liberation and develop the membrane stability since of glycosides presence will enhance the reaction of attachments to bivalent cations as Ca^{+2} and Mg^{+2} [15]

beside that, anti-inflammatory action which avoid denaturation of protein perhaps because alkaloid, polyphenolic compounds and phenolic acid may present in extract [3].



Figure(1) : Stimulation of hemolysis by heating on RBCs with *Stevia rebaudiana*. (group1: 1000 µg/ ml, group2: 2000 µg/ ml plant extract).

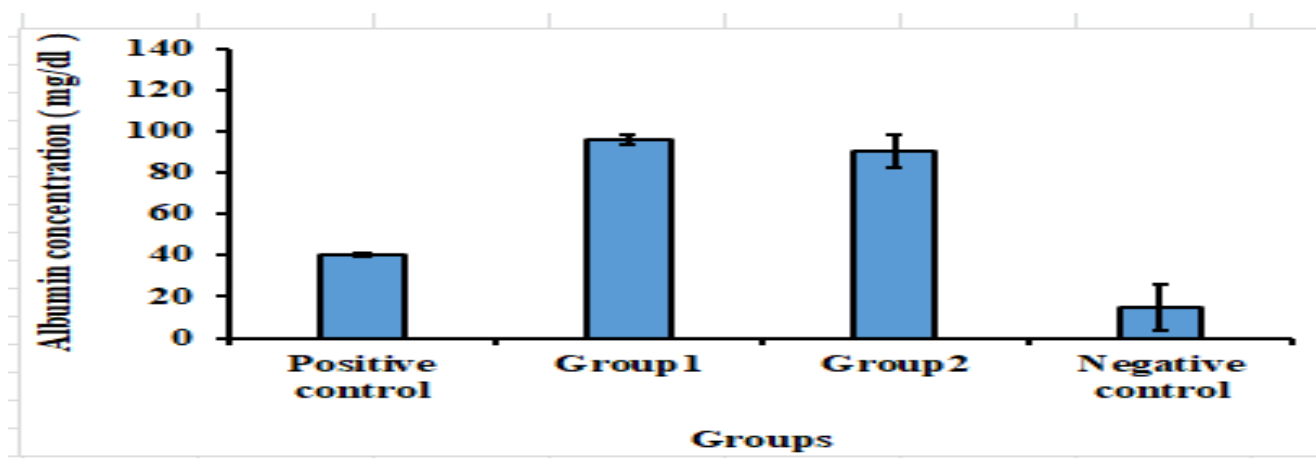


Figure (2) : The inhibition of protein denaturation activity of *Stevia rebaudiana* extract results. ((group1: 1000 µg/ ml, group2: 2000 µg/ ml plant extract).).

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