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The assessment of Anti-diabetic and Anti- Inflammatory Activities Using crude extract of *Acacia nilotica*

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Abstract

Diabetes mellitus, which can be inherited or acquired, is a chronic disease caused by insufficient or ineffective insulin production by the pancreatic beta cells. High blood glucose levels lead to insulin insufficiency, which damages several bodily systems, most notably the neurological and cardiovascular systems. Inflammation is a critical response initiated by immune system of the body to fight infections. But prolonged and abandoned inflammation causes problems such as diabetes, cancer and cardiovascular complications. Now its treatment plays an important role for current era researchers. As the imitated drugs like diclofenac sodium and diclofenac potassium have high inflammatory potential but they can result in intestinal distension, flatulence, nausea and diarrhea. Therefore, plants extract with slight or no side effects are used as inflammatory medications. *Acacia nilotica* is an essential medical plant of the world used in traditional treatment of many diseases such as inflammation, skin outbreaks, blisters, swellings and spots. In this study *Acacia nilotica* extract was investigated for its anti-diabetic and anti-inflammatory properties. *Acacia nilotica* extract was checked by performing anti-diabetic assay Glucose uptake by yeast cells assay (57 % at 80µg/ml) and its anti-inflammatory properties by membrane stabilizing assay of red blood cells (70.07 % at 100µg/ml). Significant activities were shown by each one which gave substantial results, providing the importance *Acacia nilotica* extract therapeutically. Much more studies and research are needed in this aspect, to look for medicinal properties in our local substances and find out the hidden treasures of substances used traditionally over the years.

KEYWORDS

Anti-diabetic and Anti- Inflammatory, plant extract, *Acacia nilotica*

1.0 INTRODUCTION

Around the world, medicinal plants are used to treat a wide range of ailments. Due to the existence of many kinds of chemicals, medicinal plants have biological use. Medicinal plants are a significant source of natural goods that are made in a selective and cost-effective manner [1]. They can be found primarily in tropical

regions of Arabia, Africa, Zanzibar, Mascarene Islands, Madagascar, and southwest in India [2]. Plants have been utilized for thousands of years as beneficial
ingredients in medications, cosmetics, flavours, drinks, and colours in order to keep people well and improve the quality of their lives. The foundation of herbal medicine is the notion that plants naturally possess the capacity to improve health and treat disease. The emphasis being placed on plant research at the moment has produced a plethora of information demonstrating the enormous potential of medicinal plants employed in many traditional systems. Currently, the usage of herbal therapies is receiving a lot of public interest [3]. The World Health Organization (WHO) estimates that over 21,000 plants are utilised worldwide, many of which are employed for therapeutic purposes [4]. They found that, in around 74% of cases, 119 medicines derived from plants are used in contemporary medicine. It also estimates that 80% of the world's population, or 4 billion people, currently use herbal remedies for medicinal purposes [5]. The major source of healthcare for between 75 and 80 percent of the world's population continues to be herbal treatments created from medicinal plants, minerals, and organic materials, which have been promoted and grown in popularity for hundreds of years in both developed and developing countries [6]. Because they contain a wide range of active compounds, including alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes, and phenols, herbs have therapeutic benefits [7]. Herbal medicines' effectiveness in treating age-related conditions like memory loss, osteoporosis, immunological issues, etc. for which there is no modern treatment has increased exponentially in recent years due to their natural makeup, wide availability, and rising popularity [8,9]. *Acacia nilotica*, sometimes called Acacia, is a member of the Mimosaceae family. The Hausa-speaking people who live there refer to it as "Bagaruwa." The organism is a tree with long, gray pods that are squeezed between seeds and yellow, mimosa-like blooms. Black and cracked on the limbs and bark. Spikes that are 2 centimetres long cover the branches. The leaves are five-lobed and very hairy, with three to six pairs of pinnae, every of which has ten to twenty pairs of thin, parallel-margin leaflets with rounded tips and a densely packed center midrib. The inflorescence has half-up stalks with bright yellow flowers in auxiliary heads. The flowering period of the shrub is from November to March [10]. To cure severe diarrhea, mix the plant's powdered bark with a little salt. [11]. its name is derived from the Greek word for thorn, akis, which describes its distinctive thorns. The word "akakia," which was given to the curative tree A by the ancient Greek physician and botanist Pedanius Dioscorides (40-90), is the source of the species' name. In Materia Medica, a book he wrote, Nilotica is referenced. Linnaeus named the most well-known of the trees in this area—along the Nile River—Nilotica. Around 960 of the 1300 species in the genus Acacia are native to Australia [12].

The fruits are effective against dysentery, diabetes, and diarrhea [13]. A seed extract was found to boost physical vitality generally [14]. The use of root powder to treat leucorrhoea [15], beneficial for mending wounds and easing searing pain [16]. Different plant extracts used to cure cholera, syphilis, leprosy, and earaches [17]. When consumed in the morning and evening, a paste formed from the delicate growth tips, sugar, and water acts as a demulcent and eases coughing [18]. The Italian Africa uses a bark combination to treat smallpox. Because of its high tannic acid content, the bark of Acacia nilotica is recognized as a potent astringent in Australia, helping to reduce bleeding, discharge, and excessive mucus. The extremely astringent herb's extract may stop the body from creating compounds that cause discomfort [19]. Stearic acid is found in flowers and fruits, whereas 32% tannin is found in leaves and fruits. Leucocyanidin, isoquercetin, kaempferol-3-glucoside, and acid [20, 21]. The bark contains 20% tannin. Polyphenolic compounds have been reported; those that have been identified include (+) dicatechin, quercetin, and gallic acid [22, 23], and ß-sitosterol and ß-amyrin [17, 24].

2.0. MATERIALS AND METHODS

2.1. Collection of Plant

Acacia nilotica plant were collected from the area of district Swabi Pakistan's Khyber Pukhtun Khawa province in the month of June 2023. The plant sample was identified by Dr. Muhammad Ilyas Department of Botany in University of Swabi.

2.2 Extraction

The plant components were dried at room temperature for

14 days. The dried plant material was powdered down to a fine consistency. All plant ingredients were extracted after the components were ground up and steeped in methanol for 15 days. Using a rotating evaporator and reduced pressure at a temperature below 50 °C, the extracts were then concentrated.

2.3 In vitro Antidiabetic Activity

2.3.1 ***Glucose uptake by Yeast cells Assay***

Using glucose absorption assays, the plant extract's in vitro antidiabetic efficacy was evaluated. Yeast cells are utilized as a model for diabetes because of their affinity for absorbing glucose. The glucose returns to the bloodstream when the insulin is unable to connect with the cells. Consequently, the same methodology was applied in this test. The experiment was carried out using a slightly modified version of the procedure that [25] outlined.

Yeast cells show a preference for glucose. Baker's yeast was obtained from the market and repeatedly centrifuged using distilled water to cleanse it. Until a transparent type of supernatant fluids emerged, the centrifugation process was maintained. After that, a 10% (v/v) colloidal suspension of the pellet was made in distilled water. In addition to the various compound concentrations, 1 mL of a glucose (5 mM) solution was added. They were all incubated together for ten minutes at 37°C. The reaction was triggered by the addition of yeast suspension. After being vortexed, they were incubated at 37°C for an additional hour. After five minutes of centrifuging at 3800 rpm, the amount of remaining glucose was measured in precipitate. The spectrophotometer (UV5100B) was used to measure the amount of glucose absorption. Using the following formula, the percentage increase in glucose absorption in yeast cells was calculated:

$$\% \text{ activity} = \frac{(\text{control absorbance} - \text{extract absorbance})}{\text{control absorbance}} \times 100$$

Each test was performed in triplicate, and the average of the three was determined.

2.4 In Vitro Anti-Inflammatory Activity

2.4.1 Human Red Blood Cell (HRBC) membrane stabilization assay

The anti-inflammatory activities of the selected extract were checked by Human RBC membrane stabilization assay. To check out the anti- inflammatory property of extract, HRBC activity was performed. For this activity, we employed sodium chloride (NaCl), Diclofenac sodium, PBS (pH 7.4), fresh human blood, and distilled water, a range of inflammatory diseases brought on by the lysosomal enzymes released during inflammation. The non-steroidal medication acts by stabilizing or inhibiting the lysosomal membrane. Given that the lysosome and HRBC's composition membranes were similar [26]. Thus, it was anticipated that the study will look at the HRBC membrane's stability to forecast the anti-inflammatory activity in vitro. The healthy human subjects had fresh blood collected from them. The blood was mixed with EDTA, an anticoagulant agent, and centrifuged for 15 minutes at 3000 rpm in the falcon tube. After the supernatant was decanted, the leftover material was divided. We cleaned the rest with a w/v isosaline solution. To obtain a clean supernatant, three centrifugation and washing cycles were necessary. An isotonic saline solution was used to create a 10% suspension using the pellet containing HRBCs.

The reaction procedure used in this experiment is as follow

Reaction Mixture: The control group, standard, and test samples were prepared using the reaction process that is described below.

Control group (4.5 mL): The reaction mixture was supplemented with one milliliter of PBS, half a milliliter of packed cells in a 10% HRBC suspension, one milliliter of isotonic solution, and two milliliters of hypotonic solution while being closely watched.

Standard group (4.5 mL): Several quantities (1:1) of the 10% HRBC suspension, 1 ml of the isotonic solution, 2 ml of the hypotonic solution, 1 ml of diclofenac sodium, and 1 ml of PBS and 0.5 ml packed cells were added to the standard reaction mixture.

Test group (4.5 mL): One milliliter of PBS, five milliliters of packed cells containing 10% of the HRBC suspension, one milliliter of isotonic solution, two

milliliters of hypotonic solution, and one milliliter of test sample with varying concentrations (1:1) of (10, 20, 40, 60, 80, and 100 µg) were the components of the reaction mixture group for the test samples.

Incubation and centrifugation: Every reaction mixture is incubated for 30 minutes at 37°C. After that, it is centrifuged for 15 minutes at 3000 rpm. **Spectrophotometry:** The ultra-violet 5100B spectrophotometer was used to analyze the centrifuged supernatants at 560 nm. The following formula was used to calculate the percentage of hemoglobin denaturation, and all tests were run using triplicate readings.

$$\% \text{ Inhibition} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Absorbance}} \times 100$$

3 .RESULTS AND DISCUSSION

3.1 Anti-Diabetic

3.1.1 Effect of different concentration of *Acacia nilotica* on glucose uptake by yeast cells in glucose solution

Many concentrations of Acacia nilotica extract were used, ranging from 10, 20, 30, 40, 50, 60, 70, and 80 µg. These concentrations demonstrated varying percentages of glucose uptake, ranging from 8% to 21%, 21%, 21%, 30%, 36.7 %, 46.7 %, and 57%, respectively. Following the typical grouping of 10, 20, 30, 40, 50, 60, 70, and 80µg/ml of acarbose, the percentages of glucose uptake that were disclosed were 15%, 19%, 25%, 33%, 39.2%, 45%, 56.7%, and 62%, respectively.

3.1.2 Anti-Inflammatory Effect of *Acacia nilotica*

The HRBC-membrane stabilizing test was utilized to investigate the extract's anti-inflammatory efficacy in vitro.

3.1.3 Human RBC effect of *Acacia nilotica*: The extract was employed at different concentrations: 10, 20, 40, 60, 80, and 100 µg. The results indicated that the extract inhibited growth at 12.88%, 24.45 %, 41.70 %, 52.2 %, 59.81 %, and 70.07 %, respectively. Using 10, 20, 40, 60, 80, and 100µg/ml of the diclofenac sodium medication as a reference group, the inhibition levels were found to be 32.66%, 54.23%, 67.38%, 74.57%, 80.95%, and 85.71%, respectively. At 10 µg, the lowest inhibition rate ever recorded was 12.88%. The maximal inhibition rate at 100µg was found to be 70.07%.

CONCLUSION

Acacia nilotica is an important medical plant of the world used in traditional management of many diseases such as inflammation, skin outbreaks, blisters, swellings and spots. In this study *Acacia nilotica* extract was investigated for its anti-diabetic and anti-inflammatory properties. *Acacia nilotica* extract was tested by performing anti-diabetic assay (Glucose uptake by yeast cells assay, 57 % at 80µg/ml) and its anti-inflammatory properties by membrane stabilizing assay of red blood cells (70.07 % at 100µg/ml). Significant activities were shown by each one which revealed considerable results, predicting the therapeutic importance of *Acacia nilotica* extract. Much more studies and research are needed in this aspect, to look for medicinal properties in our local substances and find out the hidden treasures of substances used conventionally over the centuries.

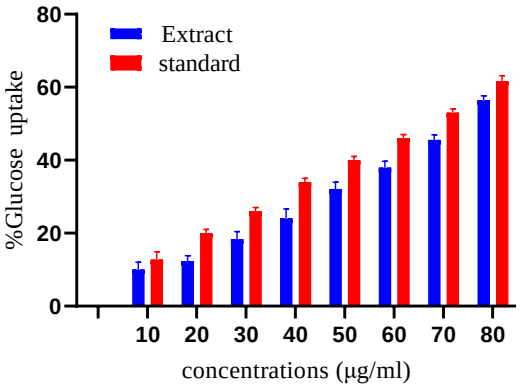

Fig.1: Shows the effect of *A. nilotica* on glucose uptake by yeast cells.

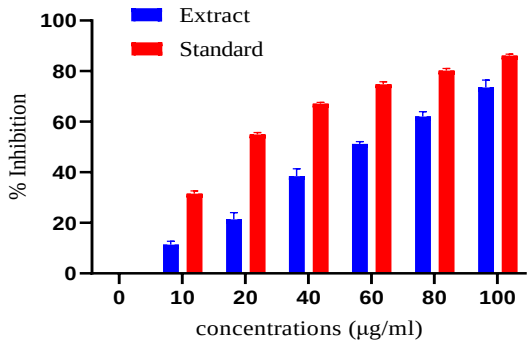

Fig.2: Shows the effect of *A. nilotica* on HRBC membrane stabilization.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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