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Research Article

Investigating the Therapeutic Potential of Raw Apple Seed Extracts: Effects on Normal and Cancer Cells

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Abstract

The study was conducted in the laboratories of the College of Life Sciences at the University of Kufa, with the aim of investigating the effects of red apple seed extract (Malus pumila Mill) on the liver and kidneys, as well as its potential to inhibit cancer cells. Additionally, the study sought to identify and detect secondary compounds present in the extract using white laboratory rats (male) weighing between 100 and 150 grams.

A total of 20 rats were used in the study, divided into four groups, each consisting of five male rats. The first group received 0.5 grams of fresh apple seed extract. Observations and procedures were carried out over a period of seven days, and tissue samples were subsequently collected. The second group was administered a doubled dose of 1 gram for two weeks. Samples from this group were then collected. Based on the observed changes and to determine the dose at which tissue damage or fatal harm could occur, the third group was given 1 gram of the extract. Samples from this group were delivered to Al-Iman Laboratory in Najaf Governorate for further analysis.

Various tests were performed, including Fourier-transform infrared spectroscopy (FTIR), enzyme-linked immunosorbent assay (ELISA), and other reagent-based assays, to analyze the effects and detect the presence of secondary compounds in the apple seed extract.

1. Introduction

Apple *(Malus domestica)*, a member of the Rosaceae family—which also includes apricots, cherries, peaches, and pears—is the most widely consumed fruit in Iraq. When consumed, some individuals peel the apples and remove the seeds, while others, especially children, often eat the entire fruit, including the seeds. This practice can be concerning as apple seeds contain potentially toxic compounds that may pose health risks if consumed in large quantities. Apple seeds are particularly noted for their significant content of amygdalin (1.0–3.9 mg/g seeds), a cyanogenic glycoside known as mandelonitrile gentiobioside [3].

Historically, medicinal plants have been utilized to treat various diseases and conditions affecting both humans and animals. These plants are rich in biologically active chemical compounds. According to German physician Hartmut Haines, secondary plant compounds can be categorized based on their chemical composition and functions, including carotenoids, flavonoids, sulfides, and phytosterols, among others. Many studies have highlighted the positive effects of these compounds, such as their antioxidant properties, in reducing the risk of cancer. Haines, a member of the Cancer Control Center at University Hospital Freiburg, further noted that certain secondary plant compounds can reduce cardiovascular diseases by lowering blood pressure or cholesterol levels, such as phytosterols. Additionally, these compounds can lower blood sugar levels, inhibit inflammation, and combat blood clots. The German Nutrition Society explained that flavonoids can be obtained from consuming apples and other fruits. German Professor Bernhard Watzl from the Max Rubner Institute emphasized that the combination of various plant compounds is crucial for maximizing their health benefits, with studies showing that greater diversity of these compounds leads to greater health advantages.

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Cyanogenic glycosides are plant secondary metabolites composed of a sugar moiety and an aglycone. They are found in over 2500 plant species and are notable for their ability to produce toxic hydrogen cyanide. Vetter, J. [7]. These glycosides are stored in the vacuoles of plant cells and remain non-toxic unless the plant tissue is damaged, such as by bruising or chewing. When this occurs, endogenous enzymes (β-glucosidases and α-hydroxynitrile lyases) interact with the cyanogenic glycosides, cleaving the carbohydrate moiety and releasing cyanohydrins. These further decompose into hydrogen cyanide and aldehydes or ketones. Hydrogen cyanide can also be produced by enzymes in the gut microflora acting on ingested cyanogenic glycosides. This can lead to sub-acute cyanide poisoning, presenting symptoms like anxiety, dizziness, headache, and confusion. Acute poisoning can cause more severe effects, including decreased consciousness, hypotension, paralysis, coma, and potentially death [6].

1.1. Preparation of Plant Extracts

The dry seeds were ground using a grinder to obtain a fine powder. The powder was then soaked in distilled water to produce the extract, with a ratio of 1 gram of seed powder to 10 milliliters of extraction liquid. The mixture was placed in a shaker water bath at room temperature for 48 hours. After soaking, the mixture was filtered through several layers of medical gauze, followed by filtration using Whitman No. 1 filter paper The filtrate was transferred into clean, sterile glass petri dishes and allowed to dry at room temperature for 2-3 days. Once dried, the extract was scraped off using a clean, sterile knife. The resulting dry powder was weighed and stored in clean, airtight plastic containers until use. This preparation is referred to as the dry aqueous extract [5].

2. Chemical Detection

• **Phenolic Detection:** The phenolic compound with the chemical formula C20H14O4 acts as an indicator, being colorless in an acidic medium and pink in water. To prepare the bromine water, use a 100 ml glass bottle with a screw cap. Add 50 ml of distilled water to the bottle. Carefully pour the vapors from a liquid bromine bottle into the water bottle, ensuring that the vapors, which are red and heavier than air, mix with the water. Cover both bottles and shake the water bottle to mix the contents thoroughly. The bromine will dissolve easily, turning the solution yellow. This process should be repeated at least once more. Bromine water should be orange in color when properly diluted, allowing for clear visibility 1994 [1].
• **Tannin Detection:** To determine the presence of tannins, use a lead acetate solution. This method involves adding the lead acetate solution to the sample and observing any changes that indicate the presence of tannins.
• **Alkaloid Detection:** For the detection of alkaloids, use Gold (III) chloride (auric chloride, AuCl3). In the presence of acid, this reagent produces a crystallized yellow precipitate of hydrochloric acid, indicating the presence of alkaloids.
• **Mayer's Reagent for Pan-Alkaloid Detection:** Mayer's reagent, a solution of potassium mercury iodide, is prepared by dissolving mercuric chloride (HgCl2) and potassium iodide (KI) in water. This reagent reacts with all types of alkaloids, producing precipitates that confirm their presence [4].

Each of these methods provides a reliable means of detecting specific secondary compounds in the plant extract.

2.1. Detection of Reducing Sugars

Benedict's Test: Benedict's reagent, a solution composed of copper (II) sulfate, sodium citrate, and sodium carbonate dissolved in water, is used to detect reducing sugars in a solution, such as glucose. The presence of reducing sugars in high concentration is indicated by the formation of a red precipitate. A yellow color indicates a low concentration of reducing sugars [2].

2.2. Animal Housing for Experimental Study

For the experimental study, 20 white male mice were obtained and housed in cages equipped with water containers, sawdust bedding, and protein food sources. The animal house of the Faculty of Science, Department of Biological Sciences, University of Kufa, maintained stable environmental variables such as temperature, humidity, sunlight, and general conditions to ensure consistency and reliability in the study [2].

The FTIR spectrum of isolated amygdalin revealed the presence of various functional groups at different wavenumbers:
• 3404.15 and 3402.43 cm^{-1}: Indicates the presence of alcohols, amines, and amides with single bonds.
• 3300.20 and 3082.25 cm^{-1}: Indicates the presence of alkanes.
• 2931.80 and 2875.88 cm^{-1}: Indicates the presence of acids and alkynes.
• 2200 cm^{-1}: This specific region is associated with the nitrile group with a triple bond (N≡C), indicating the presence of cyanide. The peak in this region rises sharply above the rest, confirming the presence of cyanide.
• 1652.64, 1548.84, 1421.54, 1315.45, 1111, and 1072.42 cm^{-1}: Indicates the presence of esters, ethers, and alcohols with C-O bonds.
• 1600 cm^{-1}: Indicates the presence of an aromatic group.
These findings provide a detailed chemical profile of the isolated amygdalin, confirming its various functional groups and the presence of cyanide.

3. Result

Figure 2 shows the healthy tissue of a rat after treatment. We can observe the testicular cells of the healthy rat: The sterol cells are as described above control, Clear-cut and the process of spermatogenesis is present unlike what is described above in both doses the membrane is blurred features, damaged and the process of spermatogenesis does not occur In the healthy kidney tissue (control), we notice that the renal tubules and Bowman's capsule are in their natural states.

Figure 4 In the white rat liver tissue, as shown above, we observe in groups 1 and 2 the occurrence of bleeding in the area of the blood vessels and the presence of spaces. As for the third group, bleeding occurred in the cells and blood vessels, but to a lesser extent than in both

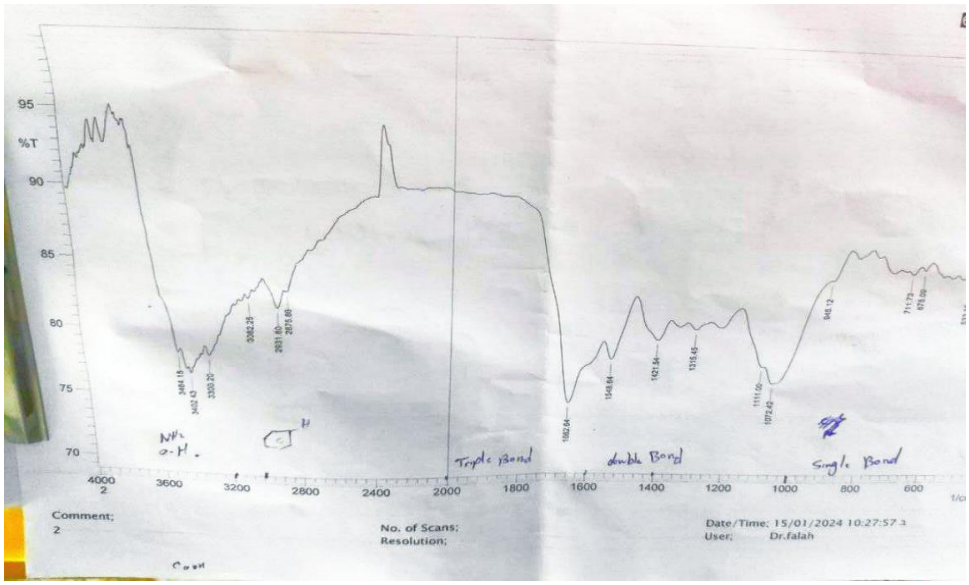

Figure 1: The FTIR spectrum of isolated amygdalin revealed the presence of various functional groups at different wavenumbers

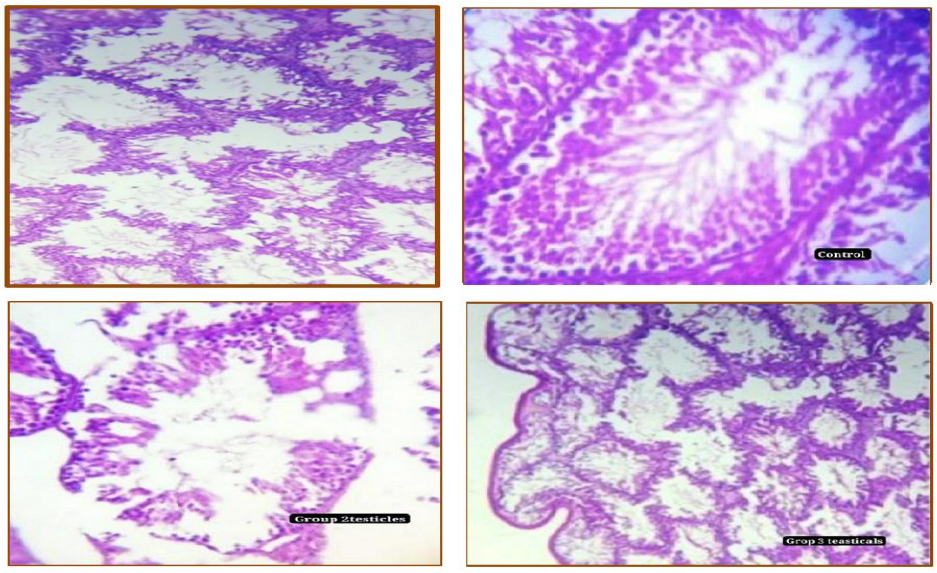

Figure 2: shows the healthy tissue of a rat after treatment

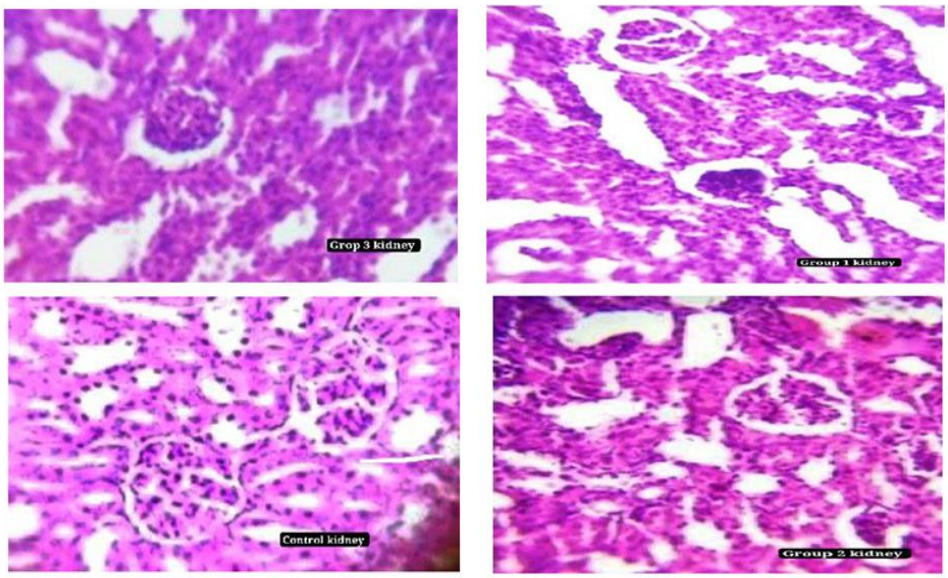

Figure 3: In the first and second groups, there are large spaces between the renal tubules and also inside Bowman's capsule. As for the third group, we observe the presence of fewer spaces and less damage compared to the first and second groups due to the resting period of three weeks before

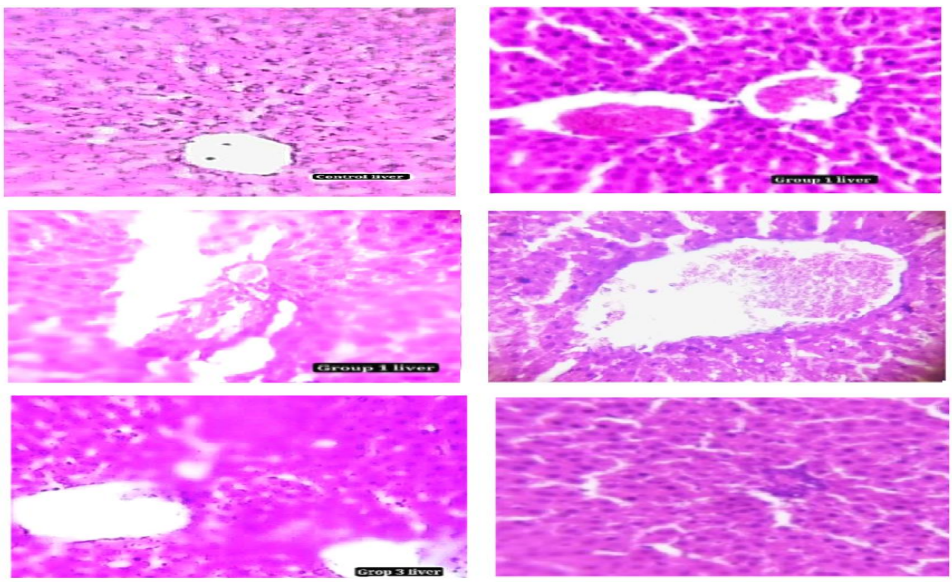

Figure 4: In the white rat liver tissue

groups 1 and 2 due to the rest period taken by the rat after the extract was administered. The rest period was three weeks, indicating that hepatic cells, once the treatment is stopped, will regenerate and repair themselves.

4. Discussion

The results confirm that apple seeds contain a variety of secondary compounds, including toxic compounds and phytochemicals such as phenols, alkaloids, glycosides, amygdalin, and terpenes. Some of these compounds function as antioxidants, which are chemicals that mitigate or prevent the effects of free radicals by donating electrons without becoming reactive themselves. This donation of electrons neutralizes the free radicals, causing them to cease their harmful reactions. However, not all antioxidants can neutralize all free radicals due to the unique chemical properties of each type of free radical.

Free radicals are single, unpaired electrons that orbit within atomic or molecular spaces. These molecules contribute to the bonding of atoms by being attracted to each other, leading to atomic interactions. When oxygen molecules split and become singular, they transform into unstable free radicals that seek other molecules to bond with, a process known as oxidative stress. The danger of oxidative stress lies in free oxygen atoms reaching the genetic material (DNA) within cells, potentially altering its structure and inducing mutations that can transform cells into malignant cancer cells capable of uncontrolled growth and multiplication.

The FTIR analysis of raw apple seed extract revealed the presence of alkyne, alkene, and carboxyl groups. Notably, a sharp peak at 2200 cm^{-1} indicated the presence of cyanide (CN). These findings highlight the potential toxicity of apple seed extract, emphasizing the importance of considering the equivalent toxicity to ensure safe treatment dosages.

4.1. Measuring the Activity of Cancer Cells and Evaluating the Effect of the Extract

Cancer cells were extracted from deep freezing and cultured in Falcon tubes under anaerobic conditions for 24 hours. Different concentrations of apple seed extract were added to each Falcon tube, and they were incubated under anaerobic conditions for another 24 hours. Following incubation, the cells were transferred to a microtiter plate containing wells. MTT dye (Methylthiazolyldiphenyl-tetrazolium bromide), which determines cell viability by converting to formazan through enzymes produced by living cells, was added to each well. After three hours, the cells were washed to remove excess dye. DMSO (Dimethyl Sulfoxide), an organic solvent, was then added to dissolve the cells and convert the stored MTT dye into a measurable product using an ELISA device.

4.2. Potential Future Applications

The findings suggest that it may be possible to develop an anticancer treatment from apple seed extract due to its demonstrated efficacy. Individuals with a hereditary predisposition to cancer or those seeking to prevent cancer could potentially take this extract in studied pharmaceutical proportions for one year to inhibit the growth of foreign or cancerous cells.

Based on this study, it is not recommended to consume apple seeds unless they are of pharmaceutical grade. If consumption is desired for preventive purposes, it should not exceed 30 seeds per year to minimize the risk of toxicity.

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