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PROTECTIVE AND HISTOLOGICAL EFFECTS OF KUMQUAT (*CITRUS JAPONICA*) EXTRACT IN RATS INJECTED WITH CARBON TETRACHLORIDE (CCl4)

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ABSTRACT

Kumquat or Japanese orange, the smallest tree of the citrus family, grows and gives the best and largest production and the sweetest of fruits in warmer regions. The kumquat has protective importance and a great treat. Several vital metabolic processes, including the delivery and digestion of nutrients, are carried out by the liver., thus we focused on This investigation sought to determine the histological and protective impacts of Kumquat extract in rats given injections of carbon tetrachloride (CCl4). The experiment was conducted in a cage for animals. Before the experiment began, all of the rats were given a baseline diet for a week. The rats were then separated into 5 groups, each with 6 rats. The first group was fed a basal diet exclusively for 28 days as a control negative (C -ve) normal group of rats. The rats of the remaining groups (n = 24 rats) were given injections of Ccl_4. The groups were split into four groups, with 3 groups receiving ethanol extracts of kumquats in various levels (150, 200, and 250 mg/kg), and one group serving as a control that had the illness but didn't follow the experimental diet. All hepatic rats given different diets exhibited substantial mean value declines as contrasted with the control (+) group, according to the data. As contrasted with the healthy group, group 5 had the best therapy for serum ALP to cause hepatopathy (normal rats). This research advised consuming kumquats, which may be had every day.

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Introduction

Infections, excessive alcohol intake, toxic substances, and autoimmune illnesses are the primary causes of liver damage [1]. The liver plays a number of functions in the metabolism of carbohydrates, including gluconeogenesis (the process of creating glucose from lactate, glycerol, or certain amino acids.) glycogenolysis (the process of turning glucose from glycogen), and Glycogenesis (the process of converting glucose into glycogen) [2]. Kumquats are small evergreen trees or shrubs that produce tangy fruits with edible skins that are rich in vitamin C, vitamin A, and potassium. A healthy kumquat tree may give hundreds or even thousands of fruits per year. The flowers are fragrant and pretty. Kumquats are popular ornamental plants. The fruits can be used in various dishes, including salads, or made into marmalade [3]. Kumquats, or Citrus japonica, are indigenous to South Asia and the Asia-Pacific area. They are mostly known for their raw fruits but have also been utilized for their healing and restorative properties [4]. Kumquats provide a respectable level of dietary fiber. The enzymes produced in our stomachs do not break down fiber as it moves through the gastrointestinal system. Also, they ferment in the digestive system and serve as food for the good bacteria in the intestines. They improve immunity and intestinal health overall. Moreover, the soluble fiber included in these fruits may lessen diarrhea [5]. Because of the existence of terpenoids and flavonoids, the kumquat peel is often sweet and edible and has a distinctive scent [6]. Poncirin, a flavonoid found in kumquats, may have a substantial role in avoiding obesity. According to research, Poncirin may lower the possibility of gaining weight by inhibiting the body from producing new fat cells [7]. Flavonoids have been associated with a lower risk of contracting several chronic illnesses [8]. C. japonica has been utilized for its therapeutic and medicinal properties in addition to being primarily utilized for its raw fruits (kumquats). The antiphlogistic, antivirus, carminative, deodorant, and expectorant qualities of C. japonica have been

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acknowledged [9]. The active components in kumquat fruit extract have the ability to have both anti-inflammatory and antioxidant actions on hepatocyte membranes. Moreover, kumquat lessens the damaging impacts of free radicals [10].

Aim of Study
Knowledge of the protective and histological effects of Kumquat (*Citrus japonica*) extract in rats given carbon tetrachloride (CCl4) injections.

Materials and Methods

Materials

Preparation of Kumquat Powder
Kumquats that had just been picked were washed with running water to get rid of any dirt or soil remnants. The Kumquats were divided into half, the seeds were manually removed, and the pieces were then sliced. The Kumquat slices were dried in an electric draught oven overnight at 45°C (AFOS Dryer, England). The dried kumquats were ground in a Multiquick System BRAUN Company produced in Germany grinder to pass through a 60-mesh screen, then placed in an airtight container and kept chilled until use.

Preparation of Kumquat Extracts
In an identical-temperature water bath that is shaking, 50 grams of dried powdered kumquat were isolated for 1 hour with 850 mL of deionized hot water (80°C) and cold water. They were also extracted for 1 hour with 500 mL of 70% ethanol. There was a 100-rpm shaking rate. Whatman No. 1 filter paper was used to filter the extract. The recovered residue was extracted twice using the same method. Three of the obtained filtrates were then put into a 250 mL flask and dried at 40 °C using a rotating vacuum evaporator. Each flask received an appropriate amount of deionized water, methanol, and ethanol to dissolve the filtrate. The resultant solutions were transferred into screw-top brown bottles and kept at _18 _C until usage. as described by [11].

Experiraental Animals
For the investigation, thirty (30) male albino Sprague Dawley rats weighing 150±10g were employed.

Used Chemicals
Ccl4 was purchased as a 10% liquid solution from El-Gomhoryia Company for Chemical Industries in Cairo, Egypt. According to Passmore and Eastwood (1986), it was distributed as a dangerous chemical substance for liver poisoning in white plastic bottles, each holding one liter [12].

Methods

Biological Experiment

Rats' Normal Diet
- Casein (10%), maize oil (10%), salt mixture (4%), vitamin combination (1%), choline chloride (0.2%), cellulose (5%), methionine (0.3%), and corn starch (69.5%) were the main components of the basal diet [13].
- CaCO3 (600 mg), Kl (1.6 mg), Zncl2 (0.5 mg), Ca HPO4. 2H2O (150 mg), K2 HPO4 (645 mg), Nacl (334 mg), MgSO4.2H2O (204 mg), Fe (C6H5O7) 26H2O (55 mg), MnSO4.4H2O (10 mg), and Cu SO4. 5H2O (0.06 mg) were all included in the test's baseline diet [14].
- Vitamin D (100 Iu), Folic acid (0.02 mg), Niacin (4.00 mg), Choline chloride (200 mg), Inositol (24 mg), Para-aminobenzoic acid (0.02 mg), Vitamin K (0.50 Iu), Thiamin (0.50 mg), Vitamin E (10 Iu), Vitamin A (200 Iu), Calcium pantothenic acid (0.40 mg), Pyridoxine (1.00 mg), and Vitamin B12 (0.02 mg) were all included in the test's baseline diet [15].

Carbon tetra Chloride (Ccl4)
From the El-Gomhoryia Company for Chemical Industries in Cairo, Egypt, Ccl4 was bought as a 10% liquid solution. According to Passmore and Eastwood (1986), it was distributed as a dangerous chemical substance for liver poisoning in white plastic bottles, each holding one liter [12]. At the same time, paraffin oil from the pharmacy is added for dilution throughout the induction.

Rats
The Laboratory of Animal received adult male Sprague-Dawley strain albino rats weighing 150-160 g B.Wt. at 14–16 weeks of age. The animals were housed under rigorous hygiene guidelines in plastic cages with metallic stainless coverings. Rats were provided with the baseline diet for seven days prior to the experiment's start to help them adjust. In order to prevent food

loss and contamination, rats were fed their diets in specialized non-spattering feeding cups. A narrow-mouth bottle with a metallic tube that was firmly fastened at its mouth by a piece of rubber tubing served as the means of ad libitum water distribution. Animals were housed for 7 days before the commencement of the experiment for acclimatization under a 12-hour light/12-hour dark cycle.

Liver Intoxication Induction in Rats

According to the procedure outlined by Jayasekhar *et al*. (1997) thirty (30) male albino rats were given Ccl4 in paraffin oil 50% V/V (2 ml/kg b. wt) was subcutaneously injected twice weekly for two weeks [16]. Retro-orbital blood samples were taken after the injection of Ccl4 to establish the existence of liver damage and determine liver function.

Animal Groups and Experimental Design

The rats were split up into 5 groups of six rats each. The following are the rat groups:

- Group (1): During 28 days, normal rats were given a baseline diet as the Control negative (Control -group) without any therapy.
- Group (2): Rats with liver poisoning were maintained in the Control + group for 28 days on a baseline diet without receiving any therapy.
- Group (3): Rats with liver toxicity were given 150 mg/kg of kumquat ethanol extract orally every day.
- Group (4): Rats with liver toxicity were given 200 mg/kg of kumquat ethanol extract orally every day.
- Group (5): Rats with liver toxicity were given an oral dosage of kumquat ethanol extract (250 mg/kg) every day.

Biological Assessment

Every day of the 28-day trial, feed consumption was recorded, and every week, body weight was determined. Body weight increase (B.W. G.%), food efficiency ratio (FER), and also organ weights were calculated as regard [17].

Blood Sampling

At the conclusion of the trial, after 12 hours of fasting, blood samples were taken. Blood was drawn into a dry, clean centrifuge tube using the retro-orbital technique using microcapillary glass tubes, and it was then allowed to clot for 30 minutes at room temperature in a water bath (37 oC). The serum portion of the blood was separated from the blood by centrifuging it for 10 minutes at 3000 rpm to determine the blood's glucose content. The remaining blood was then carefully aspirated, transferred to clean, tight-fitting plastic tubes, and stored frozen at (-20°C) for analysis. The liver, heart, kidney, and spleen were removed, cleaned in saline solution, and then weighed down and preserved in 10% formalin solution using the techniques outlined by [18].

Organs

The organs were taken out, weighed, and then the liver, lungs, kidney, heart, and spleen were removed. Following the procedures outlined by Drury and Wallington (1980), livers and kidneys were preserved in 10%, v/v formalin solution [18].

$$\text{Percentage Relative organ weight} = (\text{Fresh organ weight}/\text{final weight}) \times 100 \quad (1)$$

according to [19].

Biological Evaluation

Food consumption (intake), body weight gain *percentage* (BWG%), and FER as reported by [17]. Use the subsequent equation.

$$\text{BWG\%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (2)$$

$$\text{FER} = \frac{\text{Gain in body weight (g/day)}}{\text{Food Intake (g/day)}} \quad (3)$$

$$\text{Relative weight of organs} = \frac{\text{Organ's weight}}{\text{Animal body weight}} \times 100 \quad (4)$$

Biochemical Analysis

The Measurement of Liver Enzyme Activity

- *Aspartate Aminotransferase (AST) Activity Estimation:* By employing specialized kits and a spectrophotometer, the AST enzyme was measured (BioMerieux) [20].

- *Measuring the Level of Serum Alanine Aminotransferase (ALT):* The colorimetric method was used to test the ALT enzyme's activity [20].
- *Measuring the Level of Serum Alkaline Phosphatase:* Alkaline phosphatase (ALP) measurement method based on ALP colorimetric measurement [21].

Determination of Lipid Profile

- *Determination of Total Cholesterol in Serum:* Ratliff and Hall's (1973) method was used to calculate total cholesterol [22].
- *Determination of Triglycerides:* Triglyceride enzymatic colorimetric measurement was carried out in accordance with Jacobs and Van Denmark (1960) [23].
- *Determination of HDL:* According to Jacobs and Van Denmark's (1960) methodology, HDL was determined [23].
- *VLDL and LDL Determination:* The technique developed by Lee and Nieman (1996) was utilized to determine VLDL and LDL levels [24].
- *Evaluation of Kidney Functioning*
  - *Creatinine Determination:* Creatinine was determined utilizing Henry's (1974) kinetic technique [25].
  - *Urea Determination:* Using Patton and Crouch's (1977) enzymatic approach, urea was determined [26].
  - *Uric Acid Determination:* Uric acid was measured utilizing the technique outlined by Patton and Crouch (1977), Barham and Trinde (1972), and Faulkner and King (1976), respectively.

Statistical Analysis

The statistical analysis was performed utilizing the one-way categorization. Analysis of variation (ANOVA) and Least Substantial Difference (LSD) as per [27].

Results and Discussion

The purpose of the research is to ascertain the protective and histological effects of Kumquat) Citrus japonica) extract in rats given Ccl4 injections.

Biological Changes

Table 1 shows the average rate of weight growth (g/day/rat) in liver rats fed on different diets. It was noticeable that the control (+) group's median BWG value was lower than the control (-) group's, coming in at (0.11 +0.02 and 0.75 +0.11, respectively), indicating a substantial variation from the control (+) group. As contrasted with the control (+) group, the median values of all the hepatic rats fed on varied concentrations of ethanol extract kumquat exhibited no statistically significant changes. 0.59 +0.01, 0.54 +0.02, and 0.59 +0.01 were the corresponding values. Rats fed in groups 3 and 5 did not vary significantly from one another.

Data in **Table 1** indicate the average amount of feed consumed daily by rats with liver disease (g/day/rat). Results showed that the control (+) group's average value of (F.I) was less than that of the control (-) group, at (11.5+0.1 and 15.75+0.2, respectively. This variation was statistically substantial, with the control (-) group's increase in (F.I) being +27% more than that of the control (+). statistically, the best FI was noted for group 4 (hepatic rats fed on (200 mg/kg Ethanol Extract kumquat) as contrasted with control (-) group. A1I hepatointoxicated rats fed on different amounts of ethanol extract kumquat revealed substantial changes in average value as contrasted with control (+). The median FER value of liver-fed rats on various diets is shown in the same Table. Results reveal that the control (+) group's median FER value was smaller than the control (-) group's, at (0.0010 ±0.01 and 0.048 ±0.04, respectively), demonstrating a substantial variation with the control (-) group's percent drop of 340% when contrasted with the control (+). The median values of all the hepatic rats fed on varied diets significantly decreased as contrasted with the control (+) group. The results for kumquats at 150, 200, and 250 mg/kg of ethanol extract were (0.044± 0.04,0.037 ±0.04, and 0.025 ±0.042), respectively. As contrasted with the control (-) group, group 3 (150 mg/kg ethanol extract kumquat) had the best FER in terms of numbers. These findings support those of Wu *et al.* (2018) revealed that Antioxidant, anti-inflammatory, and immune-stimulating qualities are all present in kumquats [28]. They may assist with gastrointestinal issues and aid in keeping a healthy weight because of their high fiber content.

Table 1. Shows the (BWG g), (FER), and (FI g/d) for control (-), control (+), and other various groups of hepatitis rats fed on different levels of ethanol extract kumquat

Variable \ Groups	B. W. G. (g)	F. I. (g)	F. E. R.
G1 Control (-)	0.75 ^{a} ±0.01	15.75 ^{a} ±0.2	0.048 ^{a} ±0.04
Variation in control (+) group%	0.00	0.00	0.00
G2 Control (+)	0.11 ^{f} ±0.002	11.5 ^{e} ±0.1	0.010 ^{d} ±0.001
Change of control (+) group%	390.9	27.3	340
G3 Ethanol Extract kumquat (150 mg/kg)	0.59 ^{a} ±0.01	13.5 ^{d}±0.05	0.044 ^{b}±0.04

Change of control (+) group%	436.4	17.04	270
G4 Ethanol Extract kumquat (200 mg/kg)	0.54 [b] ±0.02	14.75 [b] ±0. 1	0.037 [d] ±0.04
Change of control (+) group%	390.9	27.3	270
G5 Ethanol Extract kumquat (250 mg/kg)	0.59 [a] ±0.01	14 [c] ±0.2	0.025 [c] ±0.042
Change of control (+) group%	436.4	21.7	430

Impact of Various Levels of Ethanol Extract Kumquat on Relative Organ Weight (g/l00g B, W) of Hepatic Rats

Table 2 shows the average liver weight (g) of rats with healthy livers that were given different diets. As contrasted with the control (-) group, the control (+) group's average liver(g.) value was greater at (4.3+0.02, 3.7+0.1), indicating a substantial variation with a percent drop of 13.9 between the two groups. Rats with diverse diets in their livers revealed substantial drops in mean values when contrasted with the control (+) group. Reduced percentages varied from -2.3% to -9.3%. Rats fed to all groups had no statistically substantial changes from the control (+) group. As contrasted with the control (+) group, group 5 (hepatic rats fed on ethanol extract kumquat 250 mg/kg) had the best liver weight measurements.

Also, **Table 2** It can be seen that the control (+) group's median heart (g/l00g) value was greater than the control (-) group's, at (0.7+0.01 & 0. 4+0.02, respectively. This difference is noteworthy since the control (-) group's percent drop from the control (+) group was -42.8. For groups, the percentage declines varied from 14.2 to -35.7%. Rats fed on all groups had distinct variation from group 2 (control +) in a substantial way. As contrasted with the control (+) group, group 4 (hepatic rats fed on ethanol extracts kumquat 200mg/kg) had the best heart weight in terms of numbers. Also, **Table 2** indicates the average kidney weight (g%) of rats with liver disease fed on various diets. It was apparent that the control (+) group's median kidney (g%) value was larger than the control (-) group's, at (1.3±0.03 & 0.7±0.03, respectively, demonstrating a substantial variation with the control (+) group's percent reduction of -46.1. The range of declines in percentage terms was -7.6 to -30.7%. Rats fed in groups 3,5 had no discernible changes among them. As contrasted with the control (+) group, group 5 showed the best kidney weight. **Table 2** shows the average weight in grams of the lungs in hepatic rats fed on varied diets. It could be shown that the control (+) group's median value of lungs (g%) was greater than the control (-) group's, being (0.8±0.02 & 0.7±0.01, respectively, suggesting a substantial variation with the control (-) group's percent decline of -12.5% as contrasted with the control (+) group. Except for group (+), all hepatic rats fed on varied diets had substantial variations in median values from the control (+) group (5). Rats fed in groups 3, 4, and 5 didn't vary much from one another.

Table 2 shows the average spleen weight (g%) of rats with impaired liver function fed on varied diets. The control (+) group's median spleen weight (g.) was greater than the control (-) group's, at (0.6 ±0.02 & 0.4 ±0.01 respectively, suggesting a substantial variation with the control (-) group's percent drop of 33.3 contrasted with the control (+) group. As contrasted with the control (+) group, all hepatic rats fed on diverse diets had substantial mean value changes. Rats fed in groups 1, 3, and 5 didn't vary much from one another. Our findings from **Table 2** are comparable to those of Zakay *et al*. (2004) who found that Histopathological studies in liver rat tissues demonstrated that methanolic Sambucus was non-toxic. Schmitzer *et al*. (2010) and Kong, (2019) worked on similar projects for hepatic rats [29].

Table 2. Impact of Various Levels of Ethanol Extract Kumquat on Relative Organs Weight (g/100g) of hepatitis rats

Parameters \ **Groups**	**Liver g/100g**	**Spleen g/100g**	**Lungs g/100g**	**Heart g/100g**	**Kidneys g/100g**
G1 Control (-)	3.78 [g] ±0.01	0.4 [c] ±0.01	0.7 [c] ±0.01	0.4 [f] ±0.02	0.7 [f] ±0.03
Change of control (+) group%	-13.95	-33.3	-12.5	-42.86	-46.15
G2 Control (+)	4.3 [a] ±0.002	0.6 [a] ±0.02	0.8 [a] ±0.02a	0.7 [a] ±0.01	1.3 [a] ±0.05
control (+) group Change %	0	0	0	0	0
G3 Ethanol Extract kumquat (150 mg/kg)	[c] ±0.01 14.	0. 4 [c] ±0.02	0.65 [d] ±0.1	0.5 [d] ±0.02	1 [e] ±0.01
Change of control (+) group%	-4.65	-33.3	-18.75	-28.57	-23.08
G4 Ethanol Extract kumquat (200 mg/kg)	4.2 [b] ±0.01	0.55 [b] ±0.01	0.65 [d] ±0. 2	0.45 [e] ±0.01	1.2 [b] ±0.02
Change of control (+) group%	-2.33	-8.3	-18.75	-35.71	-7.69
G5 Ethanol Extract kumquat (250 mg/kg)	3.9 [c] ±0.02	0.5 [c] ±0.01	0.8 [a] ±0.01	0.6 [b] ±0.03	0.9 [d] ±0.02
Change of control (+) group%	-9.30	-16.7	0	-14.2	-30.7

Impact of Various Levels of Ethanol Extract Kumquat on the Lipid Profile of Hepatic Rat

Table 3 shows the average serum (TC) (mg/dl) level in hepatic rats fed on various diets. It was evident that the control (+) group's average value of (TC) was greater than the control (-) group's, coming in at (278 +/- 0.4 & 145 +/- 0.3, respectively), indicating a substantial variation with a percent drop of -47.8% between the two groups. Comparing all of the hepatic animals to the control (+) group, the mean values all showed substantial drops. In terms of (150, 200, and 250 mg/kg ethanol extract kumquat), the results were (146.3±0.2, 150.2±0.3, and 149.5±0.5) mg/dl, respectively. The corresponding percent drops were -47.39, -45.9, and -46.2. As contrasted with the control (+) group, group 3 (hepatic rats fed on ethanol extract kumquat 150 mg/kg) had improved serum (TC).

Also, **Table 3** shows the average serum (TG) (mg/dl) concentration of rats with impaired liver function given varying doses of kumquat ethanol extract. It was clear that the control (+) group's average value of (T.G.) was greater than the control (-) group, at (180.6± 0.4 mg/dl and 97± 0.1 mg/dl, respectively. This variance indicated a substantial variation with the control (-) group's percent drop from the control (+) group of -46.06%. As contrasted with the control (+) group, all hepatic rats fed on varied concentrations of ethanol extract kumquat showed considerable decreases in median values. For groups, the percentage declines ranged from -33.4 to -36.5%. As contrasted with the control (-) group, the group (3) of liver-damaged rats fed on ethanol extract kumquat 150 mg/kg showed the greatest results.

Table 3 indicates the average amount of serum HDL-c (mg/dl) in liver-fed rats on various diets. It was noted that the control (+) group's median value of (HDL c) was higher than the control (-) group's, coming in at (25.3.54 ±0.2 vs. 51.8± 0.2), indicating a substantial variation with the control (+) group's percent gain of +104.7%. As contrasted with the control (+) group, all hepatic rats fed on different diets showed considerable elevations in median values. For the various groups, the percentage gains were (+80.2, +66, and +73.9). As contrasted with the control (-) group, the group 3 hepatic rats given the ethanol extract kumquat 150 mg/kg showed better serum (HDLc).

Table 2 demonstrates the average serum (LDL c) (mg/dl) level in hepatic rats fed on various diets. It was noticeable that the control (+) group's median value of (LDL c) was greater than the control (-) group's, coming in at (216.7 ±0.3 vs. 74 ±1), suggesting a substantial variation with a percent drop of -96.6% between the two groups. All hepatic rats given various meals showed substantial mean value declines as contrasted with the control (+) group.

Data from **Table 3** shows the average level of serum (VLDL-c) (mg/dl) in liver-fed rats on various diets. It could be seen that the control (+) group's mean value of (VLDL c) was greater than the control (-) group's, coming in at (36.1 ±0.1 vs. 19.5 ±0.5, respectively, and that there was a considerable variation in the control (-) group's percent drop from the control (+) group at 46.6%. The percentage reductions for the groups (150, 200, and 250 mg/kg ethanol extract kumquat) were correspondingly -36.01, -33.5, and -35.1%. Rats fed in groups 3, 5, or both did not vary significantly from one another. The Group 3 therapy (150 mg/kg ethanol extract) was noted as the most effective.

Ollitrault *et al*. (2020) reported that because of the buildup of cholesterol in the body, heart disease results in artery obstruction [30]. Heart failure, excessive blood pressure, and stroke may arise from it. Yet, research indicates that kumquats' flavonoids, fiber, vitamin C, and A content may lessen the buildup of fat in the arteries. It thus lowers the overall chance of developing heart disease.

By enhancing its excretion via feces, the fiber included in kumquats may aid in lowering cholesterol levels.

Table 3. Impact of various levels of Ethanol Extract kumquat on serum levels of TC, TG, HDLc, LDLc, and VLDLc of hepatic rat

Parameters \ Groups	TC (mg/dL)	TG (mg/dL)	HDLc. (mg/dL)	VLDLc. (mg/dL)	LDLc. (mg/dL)
G1 Control (-)	145 ^{f}±0.3	97.4 ^{g} ±0.1	51.8 ^{a}±0.2	19.5 ^{f} ±0.5	74 ^{f}±0.1
Change of control (+) group%	-47.8	-46.07	104.7	-46.6	-96.6
G2 Control (+)	278 ^{a} ±0.4	180.6 ^{a} ±0.4	25.3 ^{f}±0.2	36.1 ^{a} ±0.1	216.7 ^{a} ±0.3
Control (+) group change %	0	0	0	0	0
G3 Ethanol Extract kumquat (150 mg/kg)	146.3 ^{e} ±0.2	115.5 ^{c} ±0.25	45.6 ^{b} ±0.2	23.1 ^{d} ±0.2	77.6 ^{c} ±0.4
Change of control (+) group%	-47.37	-36.05	80.2	-36.01	-96.4
G4 Ethanol Extract kumquat (200 mg/kg)	150.2 ^{c} ±0.3	120.2 ^{c}±0.2	42 ^{d} ±0.1	24 ^{c} ±0.2	84.2 ^{c}±0.3
Change of control (+) group%	-60.0	-33.4	66	-33.5	-96.1
G5 Ethanol Extract kumquat (250 mg/kg)	149.5 ^{d} ±0.5	117.1 ^{d}±0.1	44 ^{c} ±0.5	23.4 ^{d}±0.1	82.1 ^{d} ±0.5
Change of control (+) group%	-46.2	-35.2	73.9	-35.2	-96.2

Impact of Various Levels of Ethanol Extract Kumquat on Liver Function of Hepatic Rats

Data from **Table 4** demonstrates the average value of the liver-fed rats' serum (GOT) (AST) (U/L) levels. It was apparent that the control (+) group's median value for (GOT) (AST) was greater than that of the control (-) group, coming in at (155 ±1 and 48 ±0.2, respectively. This difference was substantial, with the control (-) group's percent drop from the control (+) group being -69.03%. Comparing all of the hepatic animals to the control (+) group, the mean values all showed substantial drops. For all categories, the percentage drops varied from (-56.2 to -59.4%). In terms of (GOT) activity, serum (GPT) (U/L), it was found that group 3 (150 mg/kg ethanol extract kumquat%) provided the best treatment. The average value of (GPT)(ALT) of the control (+) group were (75 ±1& 35± 0.2) respectively, showing substantial variation with percent of reduction -53.3% of the control (-) group when contrasted with the control (+) group. As contrasted with the control (+) group, all hepatic rats fed on varied diets showed considerable decreases in median values. For all categories, the percentage drops varied from 33.3% to 40%. It was shown that the control (+) group's median value of (ALP) was greater than the control (-) group's, coming in at (150 ±2) and (111 ±1), respectively. This difference suggested a substantial difference, with the control (-) group's percent

drop from the control (+) group being -26%. As contrasted with the control (+) group, all hepatic rats fed on varied diets revealed substantial declines in median values. Group 5 had the most effective therapy for serum ALP.

Table 5 shows that Our results are in line with those of Yasuda *et al*. (2015) revealed that Vitamins A and C, two powerful antioxidants, are abundant in kumquats [31]. When present in excess, free radicals may harm cells. Kumquats contain antioxidants that may lessen the oxidative stress brought on by free radicals in our bodies. So, drink some kumquat juice to rid your body of pollutants.

Zhu *et al*. (2022) found that Kumquats contain high levels of vitamin C [32]. The ability of vitamin C to act as an antioxidant is widely established. They also aid in lowering oxidative stress brought on by free radicals. Free radicals are waste products that the body produces as a result of regular metabolic processes, exposure to toxins from the environment, etc. Cells and organs may get harmed by an excess of free radicals in the body. These might lead to diabetes, heart disorders, cancer, Alzheimer's illness, and many other conditions.

Table 4. Impact of various levels of Ethanol Extract kumquat on liver function of hepatic rats

Parameters \ Groups	AST (U/ L)	ALT (U/ L)	ALP (U/ L)
G1 Control (-)	48 ^{g}±0.2	35 ^{g} ±0.1	111 ^{f}±1
Change of control (+) group%	-69.0	-53.33	-26
G2 Control (+)	155^{a} ±1	75 ^{a} ±0.4	150 ^{a}±2
Control (+) group change %	0.00	0.00	0.00
G3 Ethanol Extract kumquat (150 mg/kg)	59 ^{c} ±0.39	45 ^{c}±0.3	129 ^{d} ±0.05
Change of control (+) group%	-61.9	-40.0	-14
G4 Ethanol Extract kumquat (200 mg/kg)	67.9 ^{c} ±0.23	47 ^{d}±0.5	134.9^{c} ±0
Change of control (+) group%	-56.2	-37.3	-10.7
G5 Ethanol Extract kumquat (250 mg/kg)	63 ^{d} ±0.5	50.6 ^{c}±0.5	126^{c} ±0.7
Change of control (+) group%	-59.4	-33.3	-16

Impact of Various Levels of Ethanol Extract Kumquat on Kidney Function of Hepatic Rats

Based on **Table 5**'s findings, the average serum (Urea) concentration (mg/dl) of liver-fed rats on varied diets. A substantial variation with a percent drop of -53.02% of the control (-) group as contrasted with the control (+) group could be seen in that the average values of uric acid of the control (+) group was greater than the control (-) group, being (48.1 ±0.1 & 22.6 ±0.1) mg/dl, respectively. In comparison with the control (+) group, all hepatic rats fed on various diets had significant average value decreases. From -26.8 to -40.8 percent of declines were recorded. As contrasted with the control (+) group, group 3 (150 mg/kg ethanol extract kumquat%) was shown to be the most effective therapy. It is plausible that the kumquat ethanol extract might reverse the alterations in renal function brought on by the injection of CC14 into rats. Regarding serum creatinine, it was found that the control (+) group's median value of creatinine was greater than the control (-) group's, coming in at (1.4± 0.5 & 0.76 ±0.1, respectively), demonstrating the substantial variation with the control (-) group's percent reduction of -45.7% when contrasted with the control (+) group. Comparing all of the hepatic animals to the control (+) group, the mean values all showed substantial drops. For groups 3, 4, and 5, the percentage declines were (-38.6, -32.9, and -32.9), respectively. Rats fed on groups 4, 5, and exhibited no discernible changes from healthy rats.

Additionally, it was noted that the control (+) group's median U.A values were greater than the control (-) group's, coming in at 7.9 ±0.1 mg/dl and 3.1 ±0.1 mg/dl, respectively. This distinction showed a substantial distinction with the control (-) group's percent decrease from the control (+) group being -60.7%. Comparing all of the hepatic animals to the control (+) group, the median values all showed substantial drops. Reductions ranged in percentage from (-40.5 to -48.10%). As contrasted with the control (+) group of serum U.A., group 3 (150 mg/kg ethanol extract kumquat) showed the greatest superiority.

Table 5. Impact of various levels of Ethanol Extract kumquat o on kidney function of hepatic rats

Parameters \ Groups	Urea (U/ L)	Uric acid (U/ L)	Creatinine (U/L)
G1 Control (-)	22.6 ^{f}±0.1	3.1^{e} ±0.1	0.76 ^{b}±0.01
Change of control (+) group%	-53.02	-60.8	-45.7
G2 Control (+)	48.1^{a} ±0.15	7.9 ^{a} ±0.1	1.4 ^{a}±0.05
control (+) group Change %	0	0	0
G3 Ethanol Extract kumquat (150 mg/kg)	28.5 ^{e} ±0.39	4.1^{d}±0.03	0.86^{b} ±0.05
Change of control (+) group%	-40.8	-48.1	-38.6
G4 Ethanol Extract kumquat (200 mg/kg)	30.7 ^{d} ±0.23	4.5^{c}±0.05	0.94^{b} ±0.005
Change of control (+) group%	-36.2	-43.03	-32.9

G5 Ethanol Extract kumquat (250 mg/kg)	35.2 ^{c} ±0.5	4.6 ^{bc}±0.03	0.94^{b} ±0.005
Change of control (+) group%	-26.8	-40.5	-32.9

Impact of Various Levels of Ethanol Extract Kumquat on Serum Glucose (mg/dl) of Hepatic Rats

Table 6 shows the average blood glucose level (mg/dl) of liver-fed rats on various diets. It was clear that the control (+) group's median glucose value was greater than the control (-) group's, coming in at 201.6± 1.5 and 88.5 ±0.2, respectively, indicating a substantial variance and a percent drop of -56.1% when contrasted with the control (+) group. All hepatic rats given various meals show relevant mean value declines when contrasted with the control (+) group. For all groups, the percentage reductions varied from 13.6% to 30.6%. For group 3, it was determined that it was superior to the control + group.

AGM Plants - Ornamental. (2017) reported That diabetics must keep their blood glucose levels within normal ranges [33]. Diabetes that is not under control may lead to a number of problems, including retinopathy and foot ulcers. As a result, low glycemic index fruits are preferred by diabetics to avoid unexpected rises in blood sugar levels after meals. Varieties of kumquats, and hybrids. (2009) found that Kumquats have little sugar [34]. As a result, they are ideal for diabetics and have a low glycemic index. Also, owing to their high fiber content, kumquats may lessen the likelihood of blood sugar increases after meals.

Table 6. Impact of various levels of Ethanol Extract kumquat in serum glucose of hepatic rats

Parameters / **Groups**	**glucose (mg/dl)**
G1 Control (-)	88.5^{g}±0.20
Change of control (+) group%	-56.1
G2 Control (+)	201.6^{a} ±0.5
control (+) group Change %	0.00
G3 Ethanol Extract kumquat (150 mg/kg)	140 ^{d} ±0.44
Change of control (+) group%	-30.6
G4 Ethanol Extract kumquat (200 mg/kg)	161 ^{c} ±0.1
Change of control (+) group%	-20.14
G5 Ethanol Extract kumquat (250 mg/kg)	174 ^{b}±0.88
Change of control (+) group%	-13.69

Histopathological Results

Histopathological Examination of Life

No histological alterations were seen in the liver of the untreated, group 1 control (-) rat when viewed under a microscope **(Figure 1)**. On the other hand, Rat from group 2 liver (control positive) showing congestion of central vein and necrosis of sporadic hepatocytes **(Figure 2)**. While Rat from group 3 liver (fed Ethanol Extract kumquat (150 mg/kg**)** showed liver round black eggplant peel showing no histopathological alterations **(Figure 3)**. Rat from group 4's liver **(**fed Ethanol Extract kumquat (200 mg/kg) of round black eggplant pulp showing no histopathological alterations **(Figure 4)**. In other hand **Figure 5** Rat from group 5 liver **(**fed Ethanol Extract kumquat (250 mg/kg) of round black eggplant pulp showing no histopathological alterations.

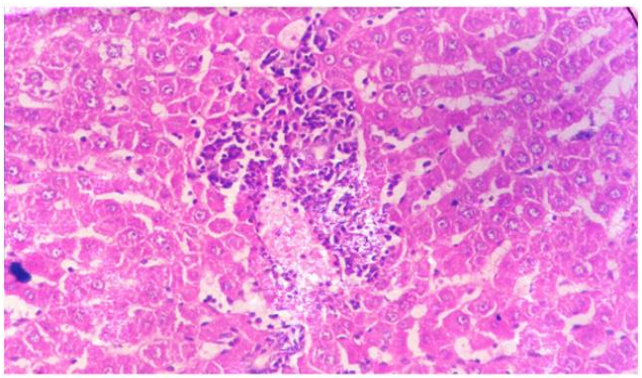

Figure 1. Typical histologic structure of the central vein (CV) and hepatic parenchymal cells (HCs) may be seen in the control rat's liver (H and E × I00).

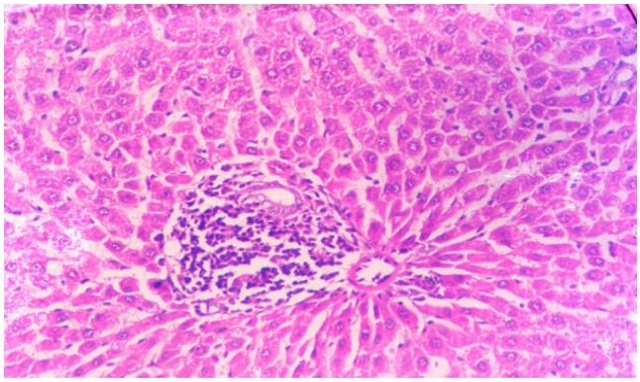

Figure 2. Rat liver from the control (+) group displaying occasional hepatocyte necrosis and central vein congestion. (H and E × 200)

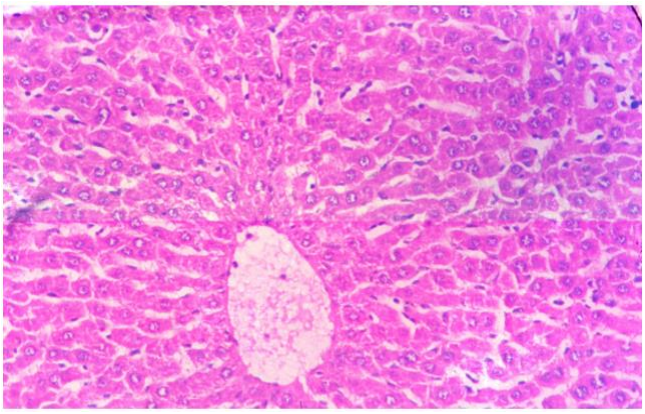

Figure 3. Rat liver from a batch of rounded, black eggplant peels exhibited no histological alterations. (H and E × 200)

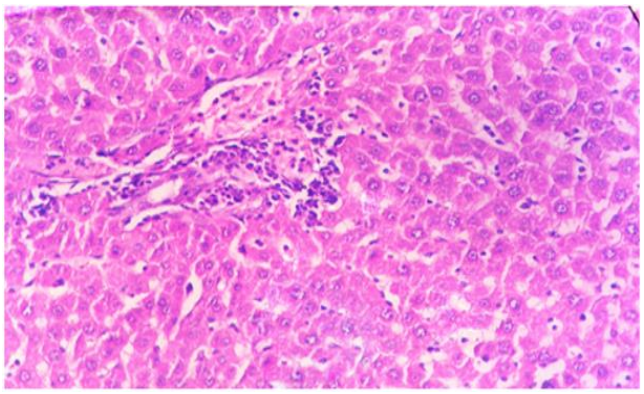

Figure 4. Rat liver from a batch of round, black eggplant pulp shows no histological modifications. (H and E × 200)

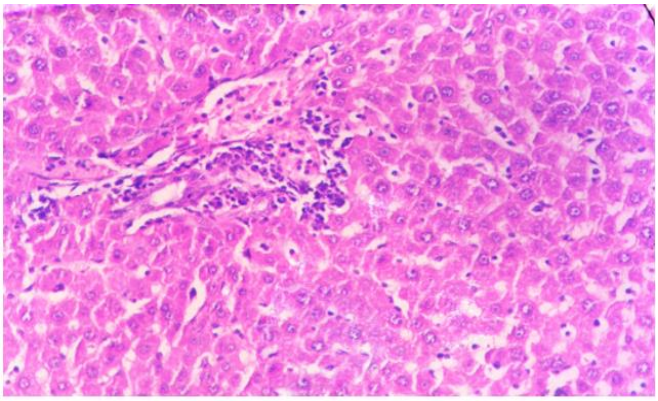

Figure 5. Rat liver from a batch of round, black eggplant pulp shows no histological modifications. (H and E × 200)

Conclusion

The results showed that Kumquats contain high levels of vitamin C. The ability of vitamin C to act as an antioxidant is widely established. They also aid in lowering oxidative stress brought on by free radicals. Free radicals are waste products that the body produces as a result of regular metabolic processes, exposure to toxins from the environment, etc. Cells and organs may get harmed by an excess of free radicals in the body. They might cause diabetes, heart disorder, liver illness, Alzheimer's illness, and a host of other diseases.

Recommendations

1. According to this research, kumquats' vitamin C content may lower the risk of hepatic disease conditions by reducing the body's production of free radicals.
2. Consumption of kumquats, which may be consumed every day.
3. A significant source of antioxidants, kumquats may provide health advantages whether consumed or used in culinary or medicinal applications.
4. It is advised to nutritional education for the community about the nutritional benefits of kumquat and increase its consumption daily.

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Conflict of interest: Therapeutic Nutrition – food sciences

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Ethics statement: The experiment was conducted in the rat laboratories (Animal House), Menoufia University (Egypt), which were equipped and followed the ethics of scientific research while conducting the experiment.

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