

Protective and Histological Effects of Kumquat (*Citrus japonica*) Extract Against Carbon Tetrachloride (CCl₄)-Induced Liver Damage in Rats

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ABSTRACT

Kumquat, also known as Japanese orange, is recognized as the smallest species in the citrus family. This plant thrives in warmer climates, where it produces an abundant yield of sweet fruits. Beyond its nutritional value, kumquat is regarded for its protective health benefits. Considering the essential role of the liver in executing various metabolic activities, such as nutrient absorption and processing, the current study was designed to investigate the protective and histological effects of kumquat (*Citrus japonica*) extract in rats subjected to carbon tetrachloride (CCl₄) toxicity. This experimental investigation was conducted under controlled laboratory conditions using animal cages. Initially, all rats underwent a one-week period of adaptation during which they were maintained on a standard basal diet. Following this, the animals were divided into five separate groups, each comprising six rats. The first group served as the negative control (C -ve), receiving only the basal diet for 28 days without exposure to CCl₄. The remaining 24 rats were administered CCl₄ injections to induce hepatic injury and were subsequently divided into four experimental groups. Among these, three groups were treated with different doses of kumquat ethanol extract — specifically 150, 200, and 250 mg/kg body weight — while the fourth group represented the positive control, receiving CCl₄ without any dietary intervention. The results showed that rats exposed to different concentrations of kumquat extract exhibited significant reductions in the mean values of hepatic markers when compared to the positive control group. Notably, group 5, which received the highest kumquat extract dose, showed the most pronounced improvement in serum alkaline phosphatase (ALP) levels, approaching values observed in healthy (normal) rats. These findings suggest that regular consumption of kumquat may have protective benefits for liver health and may be recommended as a part of the daily diet.

Keywords: Histological effects, Hepatic patients, Kumquat, Protective effect

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Introduction

Liver damage is commonly triggered by several factors, such as infections, chronic alcohol consumption, exposure to toxic agents, and autoimmune disorders [1]. The liver is essential for various metabolic pathways, particularly in carbohydrate metabolism. It is involved in processes like gluconeogenesis — the generation of glucose from non-carbohydrate sources such as lactate, glycerol, or specific amino acids — glycogenolysis, where glucose is released from stored glycogen, and glycogenesis, the storage of glucose in the form of glycogen [2].

Kumquats, scientifically known as *Citrus japonica*, are small evergreen shrubs or trees renowned for producing citrus fruits with edible peels, rich in vitamin C, vitamin A, and potassium. Under favorable conditions, a single healthy kumquat tree can yield hundreds to thousands of fruits annually. Their fragrant flowers add to their ornamental appeal, making them popular decorative plants. Furthermore, kumquats are used in various culinary preparations, such as salads or processed into marmalades [3].

Originating from South Asia and the Asia-Pacific regions, *C. japonica* is well known for its consumption in fresh form but is also traditionally recognized for its therapeutic and restorative uses [4]. These fruits offer a good

source of dietary fiber, which is not digested by enzymes within the human gastrointestinal tract. Instead, the fiber undergoes fermentation in the gut, promoting the growth of beneficial intestinal bacteria and supporting digestive health. Additionally, the soluble fiber present in kumquats may contribute to reducing the incidence of diarrhea [5].

The peel of kumquats is characterized by its pleasant sweetness and distinctive aroma due to the presence of terpenoids and flavonoids [6]. One notable flavonoid, Poncirin, found in kumquats, has been reported to potentially prevent obesity by inhibiting the formation of new adipose cells, thereby reducing fat accumulation [7]. Numerous studies have indicated that flavonoids are linked to a decreased risk of various chronic diseases [8].

Besides its nutritional value, *C. japonica* has long been utilized in traditional medicine for its therapeutic benefits. The plant is recognized for its anti-inflammatory, antiviral, carminative, deodorizing, and expectorant properties [9]. The bioactive compounds present in kumquat extract exhibit significant antioxidant and anti-inflammatory effects on hepatocyte cell membranes, aiding in the reduction of oxidative stress. Additionally, kumquat consumption may mitigate the harmful effects induced by free radicals within the body [10].

Aim of the study

This research was designed to investigate the protective and histological effects of kumquat (*C. japonica*) extract in rats exposed to liver injury induced by carbon tetrachloride (CCl₄) injections.

Materials and Methods

Materials

Kumquat powder preparation

Fresh kumquats were thoroughly cleaned under running water to eliminate any dirt or debris. The fruits were then sliced in half, with seeds carefully removed, and the remaining pieces were cut into thin slices. These slices were placed in an electric air-drying oven and dried overnight at a temperature of 45 °C (AFOS Dryer, England). Once completely dried, the kumquats were ground using a Multiquick System BRAUN grinder (Germany) fitted with a 60-mesh sieve. The resulting powder was stored in an airtight container and kept refrigerated until further use.

Kumquat extract preparation

To prepare the extract, 50 grams of the dried kumquat powder were placed in a shaking water bath, where they were extracted with 850 mL of deionized water at 80 °C for one hour, followed by an extraction with cold water for another hour. A second extraction was performed using 500 mL of 70% ethanol, maintaining a shaking speed of 100 rpm for one hour. The resulting liquid was filtered through Whatman No. 1 filter paper. The residue was subjected to two additional extractions using the same procedure. The filtered extracts were combined in a 250 mL flask and dried using a rotary vacuum evaporator at 40 °C. After evaporation, appropriate amounts of deionized water, methanol, and ethanol were added to dissolve the extract. The prepared solutions were transferred to brown screw-cap bottles and stored at -18 °C until they were ready for use, as described by Lou *et al.* [11].

Experimental animals

Thirty male albino Sprague Dawley rats, each with an average weight of 150 ± 10 g, were selected for the experiment.

Chemicals used

The CCl₄ used in the study was obtained in the form of a 10% liquid solution from El-Gomhoryia Company for Chemical Industries, Cairo, Egypt. It was provided in one-liter white plastic bottles, as a known hazardous substance for inducing liver toxicity, following Passmore and Eastwood [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), KI (1.6 mg), ZnCl₂ (0.5 mg), CaHPO₄·2H₂O (150 mg), K₂HPO₄ (645 mg), NaCl (334 mg), MgSO₄·2H₂O (204 mg), Fe (C₆H₅O₇)·26H₂O (55 mg), MnSO₄·4H₂O (10 mg), and CuSO₄·5H₂O (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 tetrachloride (CCl₄)

CCl₄, a toxic substance used to induce liver damage, was procured from El-Gomhoryia Chemical Industries in Cairo, Egypt, as a 10% liquid solution. According to Passmore and Eastwood [12], it was sold in white plastic containers, each holding one liter, and was known for its hazardous properties, specifically related to liver poisoning [12]. Additionally, paraffin oil was used for dilution during the induction process.

Rats

For the experiment, thirty male Sprague-Dawley albino rats, aged 14–16 weeks and weighing 150–160 g, were obtained from the Laboratory of Animal. The animals were housed in plastic cages equipped with metal tops, adhering to strict sanitary protocols. They were allowed a 7-day acclimatization period during which they received a standard diet. To prevent spillage and contamination, specialized feeding cups were used, and water was administered ad libitum via a narrow-mouthed bottle with a securely attached metal tube sealed with rubber tubing. The rats were kept on a 12-hour light-dark cycle throughout this period.

Induction of liver toxicity

Following the method outlined by Jayasekhar *et al.* [16], CCl₄ was administered to the rats in paraffin oil (50% V/V) via subcutaneous injection at a dose of 2 ml/kg body weight, twice a week, for two weeks. To assess the extent of liver damage, retro-orbital blood samples were collected after each injection for analysis of liver function.

Experimental design and group allocation

The rats were divided into five groups of six animals each, with the following assignments:

- Group 1: Control negative (Control -ve) rats were fed a standard diet for 28 days with no treatment.
- Group 2: Control positive (Control +ve) rats, which were subjected to liver damage through CCl₄ administration but received no treatment.
- Group 3: Rats with liver damage in this group were treated daily with 150 mg/kg of kumquat ethanol extract.
- Group 4: Rats with liver damage in this group received 200 mg/kg of kumquat ethanol extract orally each day.
- Group 5: The final treatment group received 250 mg/kg of kumquat ethanol extract daily.

Biological assessment

Daily food intake was monitored throughout the study, and body weight was recorded weekly. Various parameters, including body weight gain percentage (B.W.G.%), food efficiency ratio (FER), and organ weights, were evaluated using the approach described in Chapman *et al.* [17].

Blood sampling

After the experiment, following a 12-hour fasting period, blood samples were collected via retro-orbital bleeding. Blood was allowed to clot in a water bath set to 37 °C for 30 minutes, then centrifuged for 10 minutes at 3000 rpm to separate the serum. The serum was analyzed for glucose levels, while the remaining blood was transferred to clean tubes and stored at -20 °C for further analysis. The liver, heart, kidneys, and spleen were excised, washed with saline, weighed, and preserved in 10% formalin for histological examination, as outlined by Drury and Wallington [18].

Organ collection and preservation

After euthanasia, the liver, lungs, kidneys, heart, and spleen were harvested and weighed. These organs were preserved in a 10% formalin solution for histopathological analysis, following the guidelines provided by Drury and Wallington [18].

Calculation of relative organ weight

The relative weight of each organ was determined using the formula [19]:

$$\text{Relative Organ Weight (\%)} = (\text{Organ weight} / \text{Final body weight}) \times 100 \quad (1)$$

Biological evaluation

The food intake, body weight gain percentage (BWG (%)), and food efficiency ratio (FER) were calculated following the procedures described by Chapman *et al.* [17].

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

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

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

Biochemical evaluation

Assessment of liver enzyme activity

- Aspartate aminotransferase (AST) activity: The AST enzyme activity was quantified using specialized kits in conjunction with a spectrophotometer (BioMérieux) [20].
- Serum alanine aminotransferase (ALT) measurement: The colorimetric method was employed to assess the activity of the ALT enzyme [20].
- Serum alkaline phosphatase (ALP) measurement: The activity of ALP was measured using a colorimetric approach, based on established techniques [21].

Lipid profile evaluation

- Total cholesterol in serum: The total cholesterol content was calculated using the method proposed by Ratliff and Hall [22].
- Triglycerides: Triglyceride levels were determined using the enzymatic colorimetric method as outlined by Jacobs and Van Denmark (1960) [23].
- HDL measurement: HDL levels were determined based on the methodology provided by Jacobs and Van Denmark [23].
- VLDL and LDL determination: VLDL and LDL cholesterol levels were quantified using the procedure described by Lee and Nieman [24].

Kidney function assessment

- Creatinine Levels: Creatinine was measured using Henry's (1974) kinetic method [25].
- Urea Concentration: Urea was quantified following the enzymatic method developed by Patton and Crouch [26].
- Uric Acid Measurement: Uric acid levels were determined using the procedures established by Patton and Crouch (1977), Barham and Trinde (1972), and Faulkner and King (1976).

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA), with post-hoc testing performed using the least significant difference (LSD) method as described by Snedecor and Cochran [27].

Results and Discussion

The study aimed to investigate the protective and histological effects of *C. japonica* extract in rats administered with CCl₄ injections.

Biological alterations

Table 1 presents the average daily weight gain (g/day/rat) of rats with liver damage on various diets. Notably, the average weight gain (BWG) of the control (+) group was lower than that of the control (-) group, showing values of 0.11 ± 0.02 and 0.75 ± 0.11 , respectively. This difference between the groups was statistically significant. When compared to the control (+) group, the median weight gain values for the hepatic rats receiving different concentrations of kumquat ethanol extract did not show significant differences, with corresponding values of 0.59 ± 0.01 , 0.54 ± 0.02 , and 0.59 ± 0.01 . Additionally, there was no significant variation in weight gain between rats in groups 3 and 5.

Table 1 also illustrates the average daily food intake (g/day/rat) for rats with liver damage. The control (+) group showed a lower food intake (11.5 ± 0.1) compared to the control (-) group (15.75 ± 0.2). This difference was statistically significant, with the control (-) group consuming 27% more food than the control (+) group. Among the groups, the highest food intake was observed in group 4 (hepatic rats fed 200 mg/kg kumquat ethanol extract), which was significantly higher than the control (-) group. Hepatic rats fed varying amounts of kumquat ethanol extract showed notable differences in food intake compared to the control (+) group.

The median food efficiency ratio (FER) values for the liver rats on different diets are also presented in the same table. The control (+) group had a significantly lower FER value (0.0010 ± 0.01) compared to the control (-) group (0.048 ± 0.04), showing a 340% decrease in the control (+) group. The median FER values for the hepatic rats on the kumquat extract diets were 0.044 ± 0.04 , 0.037 ± 0.04 , and 0.025 ± 0.042 for the 150 mg/kg, 200 mg/kg, and 250 mg/kg doses, respectively. When compared to the control (-) group, group 3 (150 mg/kg kumquat ethanol extract) exhibited the best FER values.

These results align with those of Wu *et al.* [28], who demonstrated that kumquats possess antioxidant, anti-inflammatory, and immune-boosting properties. Their high fiber content may contribute to gastrointestinal health and aid in maintaining a healthy weight.

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 of kumquat

Variable	Groups	B. W. G. (g)	F. I. (g)	F. E. R.
G1 control (-)		$0.75^a \pm 0.01$	$15.75^a \pm 0.2$	$0.048^a \pm 0.04$
Variation in control (+) group (%)		0.00	0.00	0.00
G2 control (+)		$0.11^f \pm 0.002$	$11.5^e \pm 0.1$	$0.010^d \pm 0.001$
Change of control (+) group (%)		390.9	27.3	340
G3 ethanol extract of kumquat (150 mg/kg)		$0.59^a \pm 0.01$	$13.5^d \pm 0.05$	$0.044^b \pm 0.04$
Change of control (+) group (%)		436.4	17.04	270
G4 ethanol extract of kumquat (200 mg/kg)		$0.54^b \pm 0.02$	$14.75^b \pm 0.1$	$0.037^d \pm 0.04$
Change of control (+) group (%)		390.9	27.3	270
G5 ethanol extract of kumquat (250 mg/kg)		$0.59^a \pm 0.01$	$14^c \pm 0.2$	$0.025^c \pm 0.042$
Change of control (+) group (%)		436.4	21.7	430

Impact of various levels of ethanol extract of kumquat on relative organ weight (g/100g B.W.) of hepatic rats

Table 2 illustrates the average liver weight (g) of rats with healthy livers fed various diets. Compared to the control (-) group, the control (+) group had a higher average liver weight (4.3 ± 0.02 vs. 3.7 ± 0.1 g), reflecting a significant difference, with a 13.9% decrease in the control (-) group. The liver weight of rats on different diets showed noticeable reductions when compared to the control (+) group, with changes ranging from -2.3% to -9.3%. However, no significant differences were found in liver weight among the diet groups. Among the groups, the highest liver weight was observed in group 5 (rats fed 250 mg/kg ethanol kumquat extract).

Regarding heart weight, **Table 2** reveals that the control (+) group had a higher average heart weight (0.7 ± 0.01 g/100 g) than the control (-) group (0.4 ± 0.02 g/100 g), indicating a substantial difference, with a 42.8% reduction in the control (-) group. The percentage decreases across the groups varied from -14.2% to -35.7%. Significant differences were noted between the control (+) group and all the other groups, with group 4 (rats fed 200 mg/kg kumquat ethanol extract) showing the highest heart weight.

For kidney weight, the control (+) group had a greater average value (1.3 ± 0.03 g) than the control (-) group (0.7 ± 0.03 g), a notable difference with a 46.1% decrease in the control (-) group. Percentage reductions ranged from -7.6% to -30.7% in other groups, with no significant differences observed between groups 3 and 5. Group 5 exhibited the highest kidney weight compared to the control (+) group.

Lung weight data from **Table 2** indicate that the control (+) group had a higher average lung weight (0.8 ± 0.02 g) than the control (-) group (0.7 ± 0.01 g), with a 12.5% decrease in the control (-) group. All hepatic rats on various diets showed significant differences in lung weight compared to the control (+) group. However, groups 3, 4, and 5 did not show much variation from one another.

Finally, the spleen weight data in **Table 2** show that the control (+) group had a higher spleen weight (0.6 ± 0.02 g) compared to the control (-) group (0.4 ± 0.01 g), reflecting a substantial difference with a 33.3% reduction in the control (-) group. All hepatic rats on different diets exhibited significant changes in spleen weight compared to the control (+) group, with groups 1, 3, and 5 showing similar spleen weights.

These findings align with those of Zakay *et al.*, who conducted histopathological studies on liver tissues, showing non-toxic effects from methanolic Sambucus. Similar studies were also conducted by Schmitzer *et al.* [29].

Table 2. Impact of various levels of ethanol extract of kumquat on the relative organ weights (g/100g) of hepatitis rats

Parameters	Groups	Liver (g/100g)	Spleen (g/100g)	Lungs (g/100g)	Heart (g/100g)	Kidneys (g/100g)
G1 control (-)		$3.78^g \pm 0.01$	$0.4^c \pm 0.01$	$0.7^c \pm 0.01$	$0.4^f \pm 0.02$	$0.7^f \pm 0.03$
Change of control (+) group (%)		-13.95	-33.3	-12.5	-42.86	-46.15
G2 control (+)		$4.3^a \pm 0.002$	$0.6^a \pm 0.02$	$0.8^a \pm 0.02a$	$0.7^a \pm 0.01$	$1.3^a \pm 0.05$
Control (+) group change (%)		0	0	0	0	0
G3 ethanol extract of kumquat (150 mg/kg)		$4.1^c \pm 0.01$	$0.4^c \pm 0.02$	$0.65^d \pm 0.1$	$0.5^d \pm 0.02$	$1^e \pm 0.01$
Change of control (+) group (%)		-4.65	-33.3	-18.75	-28.57	-23.08
G4 ethanol extract of kumquat (200 mg/kg)		$4.2^b \pm 0.01$	$0.55^b \pm 0.01$	$0.65^d \pm 0.2$	$0.45^e \pm 0.01$	$1.2^b \pm 0.02$
Change of control (+) group (%)		-2.33	-8.3	-18.75	-35.71	-7.69
G5 ethanol extract of kumquat (250 mg/kg)		$3.9^c \pm 0.02$	$0.5^c \pm 0.01$	$0.8^a \pm 0.01$	$0.6^b \pm 0.03$	$0.9^d \pm 0.02$
Change of control (+) group (%)		-9.30	-16.7	0	-14.2	-30.7

Impact of different levels of ethanol extract of kumquat on the lipid profile in hepatic rats

Table 3 illustrates the average serum total cholesterol (TC) levels (mg/dl) in hepatic rats subjected to various dietary regimens. The results show that the control (+) group had significantly higher TC levels (278 ± 0.4 mg/dl) compared to the control (-) group (145 ± 0.3 mg/dl), representing a notable 47.8% reduction. All hepatic rats on different diets exhibited substantial decreases in TC values compared to the control (+) group, with the groups receiving 150, 200, and 250 mg/kg ethanol extract of kumquat showing respective serum TC values of 146.3 ± 0.2 , 150.2 ± 0.3 , and 149.5 ± 0.5 mg/dl. The percent reductions in TC were -47.39%, -45.9%, and -46.2%, with group 3 (150 mg/kg kumquat ethanol extract) showing the most favorable results.

Furthermore, **Table 3** presents the average serum triglyceride (TG) levels (mg/dl) in rats with liver dysfunction after being administered various doses of kumquat ethanol extract. The control (+) group displayed higher TG levels (180.6 ± 0.4 mg/dl) than the control (-) group (97 ± 0.1 mg/dl), reflecting a 46.06% drop. All hepatic rats fed different concentrations of kumquat extract showed significant reductions in TG levels, with percentage declines ranging from -33.4% to -36.5%. Among the groups, those fed 150 mg/kg of kumquat ethanol extract (group 3) showed the best results in reducing TG levels.

Table 3 also reveals the average serum HDL cholesterol (HDL-c) levels (mg/dl) in rats fed various diets. The control (+) group had lower HDL-c values (25.3 ± 0.2 mg/dl) compared to the control (-) group (51.8 ± 0.2 mg/dl), demonstrating a significant increase in HDL-c in the latter group by +104.7%. Compared to the control (+) group, all hepatic rats fed different diets exhibited significant increases in HDL-c levels, with percentage increases of +80.2%, +66%, and +73.9%. Among these, the group receiving 150 mg/kg ethanol extract of kumquat (group 3) had the most pronounced improvement in HDL-c.

In terms of serum LDL cholesterol (LDL-c) levels, **Table 3** shows that the control (+) group had significantly higher levels (216.7 ± 0.3 mg/dl) compared to the control (-) group (74 ± 1 mg/dl), reflecting a substantial

reduction of -96.6%. All hepatic rats fed different diets displayed significant decreases in LDL-c levels compared to the control (+) group.

Table 3 further presents the average serum very low-density lipoprotein cholesterol (VLDL-c) levels (mg/dl). The control (+) group had higher VLDL-c levels (36.1 ± 0.1 mg/dl) compared to the control (-) group (19.5 ± 0.5 mg/dl), with a 46.6% reduction in the control (-) group. The percentage reductions in VLDL-c for rats fed 150, 200, and 250 mg/kg kumquat ethanol extract were -36.01%, -33.5%, and -35.1%, respectively. Rats in groups 3 and 5 did not show significant differences from one another, with group 3 (150 mg/kg ethanol extract) showing the most effective results.

Ollitrault *et al.* [30] found that cholesterol accumulation can lead to arterial blockages, contributing to heart disease, high blood pressure, and stroke. However, research suggests that kumquats, with their flavonoid, fiber, and vitamin C and A content, may help reduce fat accumulation in the arteries, thereby lowering the risk of heart disease. Additionally, the fiber in kumquats can aid in cholesterol excretion through the feces, contributing to a reduction in overall cholesterol levels.

Table 3. Impact of various levels of ethanol extract of kumquat on serum levels of TC, TG, HDLc, LDLc, and VLDLc of the hepatic rat

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

Impact of different levels of ethanol extract of kumquat on liver function in hepatic rats

Table 4 presents the average serum levels of serum glutamate oxaloacetate transaminase (GOT) (AST) (U/L) in rats with liver damage. It is clear that the control (+) group had higher GOT (AST) levels (155 ± 1 U/L) compared to the control (-) group (48 ± 0.2 U/L), with a significant 69.03% reduction in the latter group. All the hepatic rats across different dietary treatments showed significant decreases in GOT levels compared to the control (+) group, with percentage reductions ranging from -56.2% to -59.4%. Among these, group 3 (150 mg/kg ethanol extract of kumquat) demonstrated the best results in reducing GOT activity.

In terms of serum glutamate pyruvate transaminase (GPT) (ALT) levels (U/L), the control (+) group's average value was 75 ± 1 U/L, while the control (-) group had a much lower value at 35 ± 0.2 U/L, representing a 53.3% drop. When compared with the control (+) group, all rats with liver impairment, regardless of their diet, showed significant decreases in ALT levels. The percentage reductions varied between 33.3% and 40%, with group 3 (150 mg/kg ethanol extract of kumquat) again showing the best therapeutic effect.

For alkaline phosphatase (ALP) levels, the control (+) group had a higher median value (150 ± 2 U/L) compared to the control (-) group (111 ± 1 U/L), indicating a 26% reduction in the latter group. When comparing the hepatic rats across different diets to the control (+) group, substantial declines in ALP levels were observed. Group 5 (250 mg/kg ethanol extract of kumquat) exhibited the most significant improvement in serum ALP activity.

Table 5 supports our findings and is consistent with the work of Yasuda *et al.* [31], who reported that kumquats are rich in vitamins A and C, both of which are potent antioxidants. These vitamins help counteract the damage caused by free radicals, which can cause cellular damage when present in excess. The antioxidant properties of kumquats may therefore help reduce oxidative stress caused by these free radicals, improving overall liver function.

Similarly, Zhu *et al.* [32] demonstrated that kumquats are an excellent source of vitamin C. This vitamin is known for its antioxidant capacity and ability to reduce oxidative stress from free radicals. Excessive free radicals, which

can result from normal metabolic processes or environmental toxin exposure, can damage cells and tissues, potentially leading to serious health issues such as diabetes, heart disease, cancer, and Alzheimer's disease.

Table 4. Impact of various levels of ethanol extract of kumquat on the 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 of 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 of 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 of 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 of kumquat on kidney function in hepatic rats

Table 5 reveals a significant reduction in the serum urea concentration (mg/dl) of rats with liver damage fed different diets. The control (+) group exhibited higher urea levels (48.1 ± 0.1 mg/dl) compared to the control (-) group (22.6 ± 0.1 mg/dl), resulting in a notable drop of 53.02% in the latter. Hepatic rats on various kumquat ethanol extract doses showed marked declines in urea levels, with percentage reductions ranging from -26.8% to -40.8%. Among the groups, the 150 mg/kg kumquat extract (group 3) was most effective, suggesting its potential to counteract kidney dysfunction induced by CCl₄.

For serum creatinine, the control (+) group had higher levels (1.4 ± 0.5 mg/dl) compared to the control (-) group (0.76 ± 0.1 mg/dl), with a decrease of 45.7% in the control (-) group. All hepatic rats showed considerable reductions in creatinine when compared to the control (+) group. The percentage reductions for groups 3, 4, and 5 were -38.6%, -32.9%, and -32.9%, respectively, with no significant difference between groups 4, 5 and the healthy rats.

Similarly, the control (+) group had higher serum uric acid (UA) levels (7.9 ± 0.1 mg/dl) than the control (-) group (3.1 ± 0.1 mg/dl), representing a 60.7% reduction in the latter. Hepatic rats on all kumquat doses showed notable decreases in UA levels, ranging from -40.5% to -48.1%. The group receiving 150 mg/kg of kumquat ethanol extract (group 3) exhibited the most significant improvement in UA levels.

Table 5. Impact of various levels of ethanol extract of kumquat on the 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 of 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 of 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 of 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 different levels of ethanol extract of kumquat on serum glucose (mg/dl) in hepatic rats

Table 6 presents the blood glucose concentrations (mg/dl) in hepatic rats consuming different diets. The control (+) group had significantly higher glucose levels compared to the control (-) group, with respective values of

201.6 ± 1.5 and 88.5 ± 0.2, showing a marked difference and a reduction of -56.1% in the control (-) group. All rats in the hepatic groups exhibited notable reductions in their average glucose levels compared to the control (+) group, with percentage reductions ranging from 13.6% to 30.6%. Group 3 showed the most favorable results when compared to the control (+) group.

AGM Plants-Ornamental [33] highlighted the importance of controlling blood glucose levels in diabetics to prevent complications like retinopathy and foot ulcers. To avoid abrupt increases in blood sugar, individuals with diabetes often prefer foods with a low glycemic index. Studies have shown that kumquats, due to their minimal sugar content, are well-suited for diabetics and have a low glycemic index [34]. Additionally, the high fiber content in kumquats may further help reduce the likelihood of post-meal blood sugar spikes.

Table 6. Impact of various levels of ethanol extract of kumquat on the 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 of kumquat (150 mg/kg)	140 ^d ± 0.44
	Change of control (+) group (%)	-30.6
	G4 ethanol extract of kumquat (200 mg/kg)	161 ^c ± 0.1
	Change of control (+) group (%)	-20.14
	G5 ethanol extract of kumquat (250 mg/kg)	174 ^b ± 0.88
	Change of control (+) group (%)	-13.69

Histopathological results

Histopathological examination of life

Microscopic examination of the liver in the untreated control group (group 1, control (-)) revealed no significant histological changes (**Figure 1**). However, rats from group 2 (control positive) exhibited signs of central vein congestion and sporadic hepatocyte necrosis in their liver tissues (**Figure 2**). In contrast, rats from group 3, which were fed 150 mg/kg of ethanol extract of kumquat, showed no histopathological abnormalities, with their liver resembling the appearance of round black eggplant peel (**Figure 3**). Similarly, rats in group 4, which received 200 mg/kg of the kumquat extract, also displayed no histological changes, with liver tissue resembling round black eggplant pulp (**Figure 4**). Finally, rats in group 5, fed 250 mg/kg of ethanol extract of kumquat, also showed no histopathological alterations in their liver, similar to the previous groups (**Figure 5**).

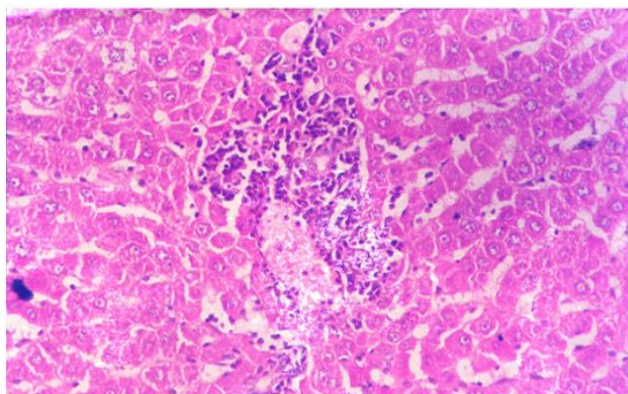


Figure 1. Typical histologic structure of the central vein (CV) and hepatic parenchymal cells (HCs) may be seen in the control rat's liver (H and E × 100).

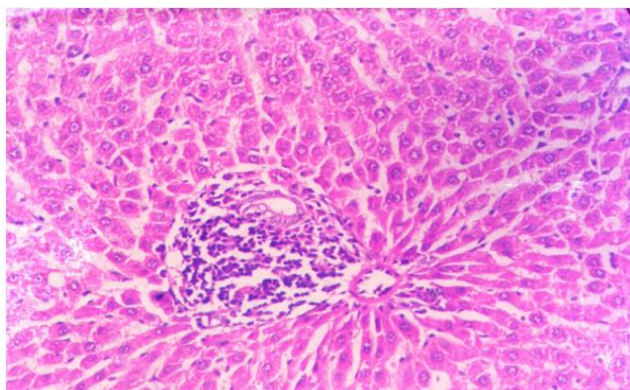


Figure 2. Rat liver from the control (+) group displaying occasional hepatocyte necrosis and central vein congestion (H and E $\times$ 200)

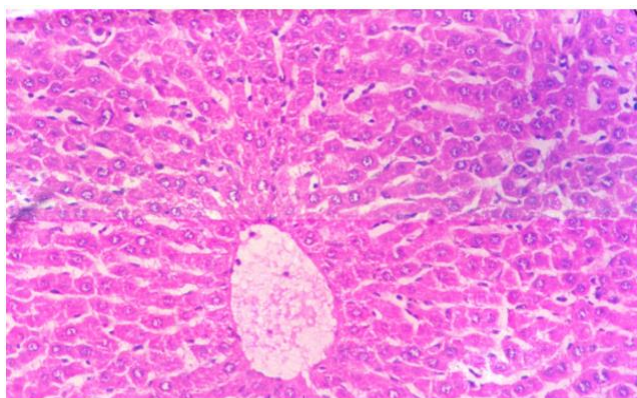


Figure 3. Rat liver from a batch of rounded, black eggplant peels exhibited no histological alterations (H and E $\times$ 200)

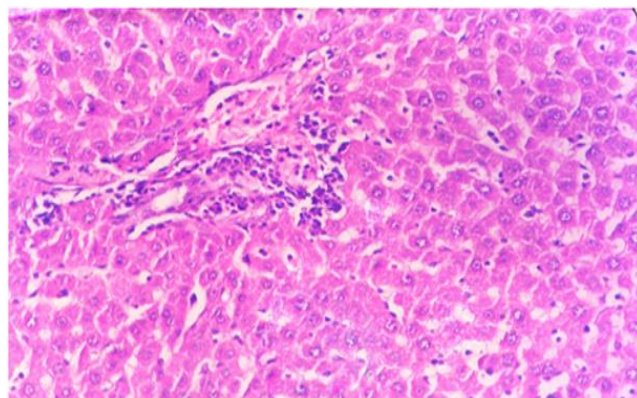


Figure 4. Rat liver from a batch of round, black eggplant pulp shows no histological modifications (H and E $\times$ 200)

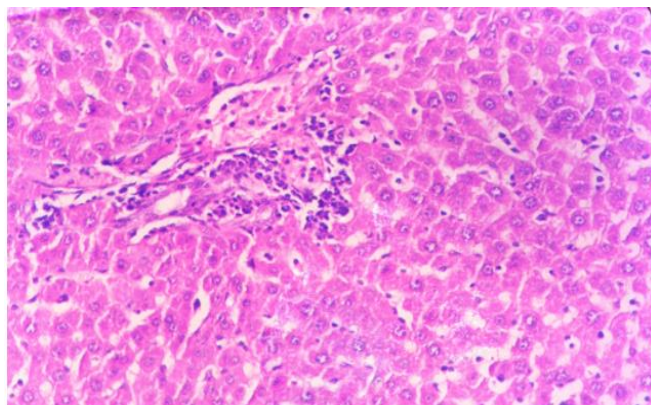


Figure 5. Rat liver from a batch of round, black eggplant pulp shows no histological modifications (H and E $\times$ 200)

Conclusion

The findings of this study reveal that kumquats are rich in vitamin C, a well-known antioxidant. This vitamin plays a crucial role in reducing oxidative stress caused by free radicals. Free radicals are by-products generated through normal metabolic processes and environmental toxins, and their accumulation can damage cells and organs. Such damage has been linked to various health conditions, including diabetes, cardiovascular diseases, liver disorders, Alzheimer's disease, and others.

Recommendations

1. The results suggest that the high vitamin C content in kumquats may help reduce the likelihood of liver diseases by minimizing the generation of free radicals in the body.
2. It is recommended to incorporate kumquats into the daily diet.
3. As a rich source of antioxidants, kumquats offer health benefits when consumed, whether in food or used for medicinal purposes.
4. Community education on the nutritional benefits of kumquats should be encouraged, promoting their daily consumption.

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