Original Article:
Acute Toxicity of Quercetin From Onion Skin in Mice

Nathan Isaac Dibal[1*], Sani Hyedima Garba[1], Tamunotonye Watson Jacks[1]

1. Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri, Maiduguri, Nigeria.

*** Corresponding Author:**
Nathan Isaac Dibal, MSc.
Address: Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri, Maiduguri, Nigeria.
Phone: +23 (480) 69088308
E-mail: nathandibal@gmail.com

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ABSTRACT

Background: Quercetin is the most abundant flavonoid molecule, is widely distributed in the plant kingdom, and has a wide range of uses.

Objectives: This study aimed to determine the LD_{50} of quercetin from onion (Allium cepa) skin (QOS) and its effect on the livers and kidneys of mice.

Methods: This study consisted of two phases. In phase one, 9 mice BALB/c were divided into three groups of three mice each. The mice in each group received QOS at 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively, and were monitored for 24 h for any signs of toxicity or mortality. In phase two, three mice were divided into three groups of one mouse each. Each mouse received QOS at 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg. The mice were observed for 24 h for any signs of toxicity or mortality.

Results: A significant increase was observed in serum albumin, total protein, and aspartate aminotransferase (AST) levels in mice that received 10 mg/kg, 100 mg/kg, and 1000 mg/kg QOS. A significant decrease in serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), cholesterol, creatinine, urea, and the electrolyte was noticed in mice that received QOS at 10 mg/kg, 100 mg/kg, and 1000 mg/kg when compared with the control group. The livers of mice that received 1600 mg/kg and 2900 mg/kg QOS showed hemorrhage and enlarged sinusoids along with a distortion of the renal tubule and aggregation of lymphocytes within the kidneys.

Conclusion: The LD_{50} of QOS was 3807 mg/kg in mice. QOS above 1000 mg/kg led to a distortion of the hepatocytes and renal tubule with an increase in serum AST, ALT, and creatinine, suggesting that QOS could be toxic at 1000 mg/kg and above.

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Introduction

Flavonoids are classified into 13 different categories; they serve as chemo-preventers in foods and play an antioxidant role by preventing the rancidity development in lipids before consumption (or during the digestion processes) [1]. Quercetin is the most abundant flavonoid molecule, is widely distributed in the plant kingdom, and has a wide range of uses. Most of the dietary intake of the quercetin-type flavonol is quercetin glycosides [2]. The aglycone form of quercetin is not as abundant as the flavonol glycoside form. Two of the most important food sources of the aglycone form of quercetin are onions and shallots [3]. Quercetin in shallot flesh is about 99.2% quercetin glucosides and 0.8% quercetin aglycone, while the dry shallot skin consists of 83.3% quercetin aglycone and 16.7% quercetin glucosides [3]. The flesh of onions mostly contain quercetin glucosides, with only trace amounts of quercetin aglycone (while the skin and outermost layers of onion have much more quercetin aglycone) [4]. Flavonoids are generally found at higher concentrations in the outer layers of fruits and vegetables [5]; therefore, peeling results in their great loss. After peeling, red onions contain 79% of the original total content of quercetin-4'-glucoside and only 27% of the anthocyanins [6].Onion has more quercetin (300 mg/kg) than blackcurrants (40 mg/kg), broccoli, black grapes and apple (30 mg/kg) [7]. The total content of quercetin is higher in the upper part of an onion as compared with the lower part [8].

Many studies have reported the preventive and therapeutic role of quercetin from different fruits and vegetables on various organs and tissues of the body in humans, cell lines, and animal models. Quercetin was reported to protect the liver, kidney, and heart of Wistar rats from doxorubicin-induced toxicity [9]. However, rats fed with 2000 mg/kg body weight of synthetic quercetin were reported to have developed renal adenoma [10]. This suggests that quercetin could be toxic at higher doses or with a prolonged period of consumption. This study aimed to determine the LD_{50} of Quercetin from Onion Skin (QOS) and its effect on the livers and kidneys of mice.

Materials and Methods

Onion (*Allium cepa*) skin is the thin and dry covering of onion that peels off when onions are kept. The onion skin was purchased from Gamboru market Maiduguri, Nigeria. Quercetin was extracted from onion skin according to a modified method of Horbowicz [11]. The onion skin was ground into powder; 50 g was dissolved in 2.5 L of cold ethyl acetate for 24 h with constant shaking. It was filtered and the solvent was evaporated in a rotary evaporator at 40°C. The weight of residue after evaporation of the solvent was used to calculate the yield (in %).

Twelve BALB/c mice were used for the study. They were kept in the animal house of the Department of Biochemistry, University of Maiduguri, for 2 wk to acclimatize before and after the commencement of the experiment. They were fed with grower mash (Vital Feed, Grand Cereal Jos, Nigeria) and water ad libitum. The research was approved by the Department of Human Anatomy Ethics Committee (Code: UM/HA/PGR18.19-09900); it was conducted following the ARRIVE Guidelines (Animal Research Reporting of in Vivo Experiment) [12].

Oral toxicity was conducted according to the method described by Lorke [13]. In the first phase, 9 mice were divided into three groups of 3 mice each. The mice in each group received QOS at 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. The mice were monitored for 24 h for any signs of toxicity or mortality. In the second phase, 3 mice were divided into 3 groups of one mouse each. Mouse in each group received QOS at 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg. The mice were observed for 24 h for any sign of toxicity (lacrimation, salivation, tremor, lethargy, and diarrhea) or mortality. The LD_{50} was calculated using the formula: $LD_{50}= \sqrt{DO \times D100}$, where D0=Highest dose that gave no mortality and D100=lowest dose that produced mortality.

The mice were weighed every day for 14 days after administration. They were euthanized on the day 14. Blood samples of each mouse were collected through a cardiac puncture in a plain bottle. The blood was centrifuged at 5000 rpm and the levels of AST, ALT, albumin, total protein concentration, cholesterol, creatinine, urea, and electrolyte were determined. The livers and kidneys were dissected, fixed in 10% formalin, and processed for light microscopy. All values were expressed as Mean±SEM and were analyzed with InStat3 Graph pad. One-way analysis of variance (ANOVA) was used to determine the difference between and within groups and $P<0.05$ was considered statistically significant.

Results

The quercetin obtained from onion skin is a brownish yellow substance, soluble in methanol, ethanol, Tween 80, and Tween 20 with a clear yellow suspension but insoluble in cold, warm, and hot water. The weight of quercetin obtained after evaporating the solvent (ethyl

Table 1. Liver function of mice that received a single dose of quercetin from onion skin

Doses	Mean±SEM				
	(g/dL)		(IU/L)		(g/dL)
	ALB	ALP	ALT	AST	TP
0 mg/kg	2.22±0.55*	34.59±4.81*	79.33±19.92*	79.67±16.79a*	6.04±2.14*ab
10 mg/kg	4.43±0.41a	20.60±0.56a	53.33±4.37a	315.00±28.58*	21.31±2.63*
100 mg/kg	4.79±0.46*	31.61±5.29b	85.00±7.02b	206.33±34.84a	16.37±1.88a
1,000 mg/kg	3.71±0.76b	33.39±3.77c	100.00±11.15c	113.67±45.96b	18.84±2.41b

a, b, c, *: P<0.05 (n=3);

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SEM: Standard Error of Mean; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TP: Total Protein.

Table 2. Kidney function of mice that received a single dose of quercetin from onion skin

Doses	Mean±SEM						
	(mg/dL)			(mmol/L)		(mg/dL)	
	Cholesterol	Creatinine	Urea	Potassium	Sodium	Bicarbonate	Chloride
0 mg/kg	253.93±33.13*	0.87±0.03*	157.26±4.04*	28.47±9.88*	117.66±5.08*	86.00±3.51*	28.67±1.20*
10 mg/kg	339.71±47.84a	0.73±0.09a	107.74±10.57*	33.16±1.25a	137.14±12.92a	86.00±4.36 a	29.67±2.67 a
100 mg/kg	323.90±35.82b	0.93±0.09b	149.63±7.61a	23.27±0.47 b	115.68±5.63 b	84.67±8.29 b	28.67±0.88 b
1,000 mg/kg	248.90±37.95c	1.20±0.10*	162.33±6.49b	40.56±0.76 c	107.02±6.46 c	80.33±4.67 c	29.67±0.88 c

a, b, c, *: P<0.05 (n=3);

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SEM: Standard Error of Mean.

acetate) was 4.2 g per 50 g of onion skin used. The yield of quercetin=4.2/50×100=8.4%.

The mice that received QOS at 10 mg/kg and 100 mg/kg showed no signs of toxicity; they were hyperactive for 3 days after administration. The mice that received QOS at 1000 mg/kg only showed body weakness for about 5 h. The mouse that received QOS at 1600 mg/kg and 2900 mg/kg exhibit some signs of toxicity such as erected hair, body weakness, and loss of appetite within the first three days after administration while the mouse that received QOS at 5000 mg/kg had erected hair, bulged eye, together with body weakness and loss of appetite. The mouse died 22 h after the administration of onion skin quercetin. $LD_{50}=\sqrt{DO\times D100}$, where D0=2900 mg/kg and D100=5000 mg/kg. Therefore, $LD_{50}=\sqrt{2900\times5000}$ mg/kg= mg/kg =$\sqrt{14,500000}$ 3807 mg/kg.

According to Table 1, there was no significant change in the serum levels of ALP and ALT between the control mice and mice that received a single dose of QOS at 10 mg/kg, 100 mg/kg, and 1000 mg/kg (P>0.05). A significant increase was noticed in AST and total protein levels in mice that received QOS at 10 mg/kg when compared with the control (P<0.05). For albumin and total protein, the control mice have a significantly lower level when compared with mice that received QOS at 100 mg/kg (Table 1). Even though there was an increase in the levels of albumin, ALP, ALT, AST, and total protein in mice that received 1000 mg/kg of QOS when compared to that of the control mice, the only significant difference was seen in total protein concentration at P<0.05 (Table 1).

Based on Table 2, the serum levels of cholesterol, potassium, sodium, bicarbonate, and chloride were not significantly changed in mice that received 10 mg/kg, 100 mg/kg, and 1000 mg/kg QOS when compared with the control mice (P>0.05). There was a significant increase in the serum levels of creatinine in mice that received 1000 mg/kg QOS when compared with the control at P<0.05 (Table 2). Significant reduction in the serum urea level was noticed in mice that received 10 mg/kg QOS as compared with the control at P<0.05 (Table 2).

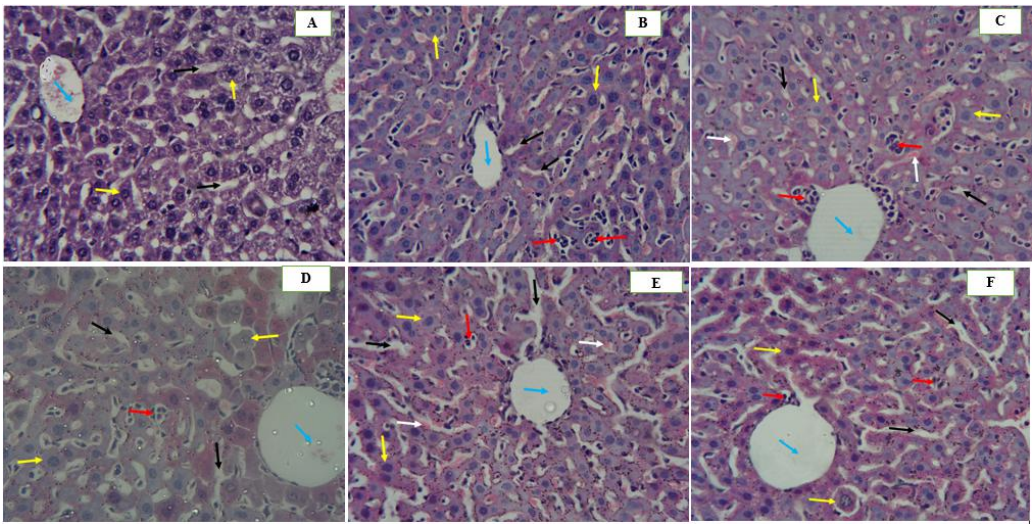

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Figure 1. Photomicrograph of the livers of the control mice

In A and those of mice that received quercetin from onion skin at 10 mg/kg; in B: 100 mg/kg; in C: 1000 mg/kg; in D: 1600 mg/kg; in E and 2900 mg/kg; in F: Respectively; showing hepatocytes (yellow arrows), sinusoids (black arrows), central vein (blue arrows), hemorrhage (white arrows) and lymphocytes (red arrows). H&E staining, magnificationx100.

Photomicrograph of the livers of the control mice showed normal central vein, hepatocytes, and sinusoids (Figure 1A). Photomicrograph of the livers of mice that received a single dose of QOS at 10mg/kg showed a normal central vein, hepatocytes, and sinusoids (Figure 1B). The livers of mice that received a single dose of QOS at 100mg/kg also showed normal central vein, hepatocytes, and sinusoids with an aggregation of lymphocytes (Figure 1C). The livers of mice that received 1000 mg/kg and 1600 mg/kg QOS showed enlarged sinusoids, tissue hemorrhage, and aggregation of lymphocytes (Figure 1D & E). While the livers of mice that received a single dose of OSQ at 2900 mg/kg showed tissue hemorrhage and enlarged sinusoids (Figure 1F).

Photomicrograph of the kidneys of control mice showed normal glomeruli and convoluted tubules (Figure 2A). Photomicrograph of the kidneys of mice that received a single dose of OSQ at 10 mg/kg and 100 mg/kg showed normal glomeruli and convoluted tubules (Figure 2 B & C). The kidneys of mice that received QOS at 1000 mg/kg and 1600 mg/kg showed distorted convoluted tubules, tissue hemorrhage, and aggregation of lymphocytes (Figure 2 D & E). While the kidneys of mice that received QOS at 2900 mg/kg showed distorted convoluted tubules, enlarged Bowman's space, and tissue hemorrhage (Figure 2F).

Discussion

Quercetin from onion skin is a brownish-yellow substance; this is in agreement with an earlier report of quercetin as a yellow substance [11, 14] and golden brown substance [15]. The quercetin extracted from onion skin was insoluble in both cold and hot water; this is in agreement with studies that reported quercetin as lipophilic with poor solubility 0.1648mg/ml in water [16, 17]. The yield of quercetin extracted from onion skin is 4.2 g per 50 g (8.4%). This is in agreement with an earlier report of quercetin having low yield, quercetin in *Trigonella foenum-graecum* leaf using ethyl acetate as a solvent was reported to be 5.64 [18], while the yield of quercetin in onion skin using cold ethyl acetate ranges between 3.57 and 4.56 [11]. The yield of quercetin extracted with 60% ethanol using conventional extraction, microwave-assisted extraction, and ultrasound-assisted extraction were 3.42±0.30 mg/g, 4.75±0.15 mg/g, and 3.76±0.38. mg/g, respectively, [19], while the yield of quercetin from *Allium cepa* was reported to be 0.1% [20].

The signs of toxicity that were observed in mice that received QOS at 1600 mg/kg and 2900 mg/kg is an indication that quercetin might exert a mild toxic effect at these doses. The mortality that was recorded in mice that received 5000 mg/kg QOS showed that, even with the numerous beneficial health effects of quercetin, it could be toxic at 5000 mg/kg or with continuous consumption leading to an accumulated dose of up to 5000 mg/kg. Lucida et al. [21] reported no mortality in mice that received a single dose of quercetin at 1000 mg/kg and 1600 mg/kg. While some studies reported low oral LD_{50} of quercetin in mice such as 160 mg/kg and [22], 575 mg/kg [23], others reported high LD_{50} of more than 1600 mg/kg in mice [21]. The range of lethal quercetin doses may vary among species and strain of animals and

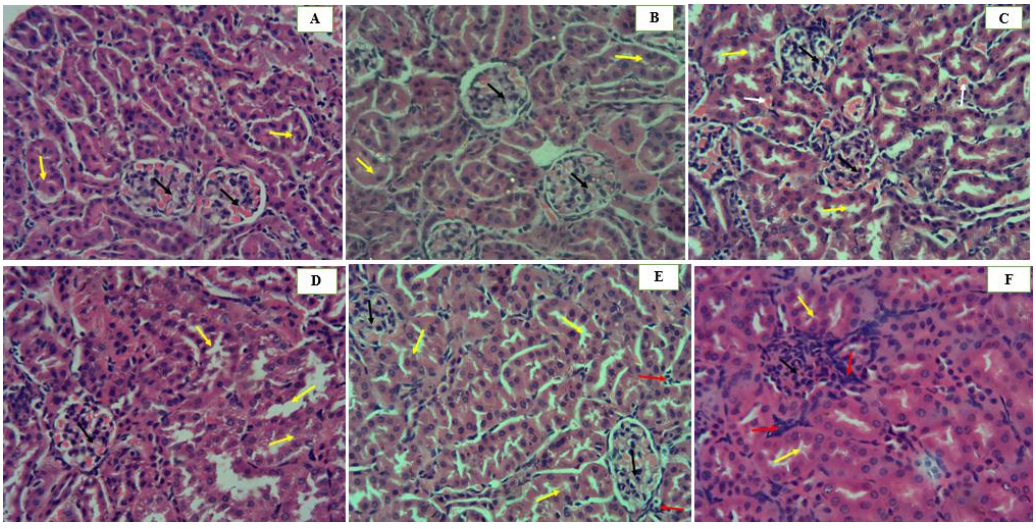

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Figure 2. Photomicrograph of the kidneys of the control mice

In A, and those of mice that received quercetin from onion skin at 10 mg/kg in B 100 mg/kg in C: 1000 mg/kg; in D: 1600 mg/kg; in E and 2900 mg/kg in F, respectively; showing glomeruli (black arrows), renal tubules (yellow arrows), hemorrhage (white arrows) and lymphocytes (red arrows); H&E staining and magnificationx100.

might be dependent on the source of quercetin; that is why different LD_{50} are reported with the consumption of quercetin from different sources in the same species. Sex, age, protocol, test chemical source, and route of administration may also affect the lethal dose of a particular compound within a single strain [24, 25].

The Organization for Economic Cooperation and Development (OECD) classify and label compounds based on their oral LD_{50} values as follows: very toxic, ≤5 mg/kg; toxic, >5 mg/kg≤50 mg/kg; harmful, >50 mg/kg≤500 mg/kg; and no label (not classified) >500 mg/kg≤2000 mg/kg [26]. The globally harmonized system (GHS) of classification and labeling chemicals also classified and labeled chemical compounds based on their oral LD_{50} values into 5 categories: category 1 (≤5 mg/kg) and 2 (>5 mg/kg ≤50 mg/kg) are labeled fatal if swallowed, category 3 (>50 mg/kg ≤300 mg/kg) are labeled toxic if swallowed, category 4 (>300 mg/kg ≤ 2000 mg/kg) are labeled harmful if swallowed while category 5 (>2000 mg/kg ≤ 5000 mg/kg and >5000 mg/kg) are considered possibly harmful if swallowed and no label or not classified, respectively.

QOS is a category 5 compound based on the GHS classification in contrast to earlier studies that placed quercetin in category 4 based on the LD_{50}. The LD_{50} test was first developed in 1927 for establishing the toxic potential of compounds, classification and labeling compounds, and to help get a dose of a new biological compound [27, 28]. According to OECD guidelines, to discover the safety and efficacy of a new compound, toxicological studies must be conducted in animal models [29].

Albumin is termed as a negative acute phase reactant and serum albumin declines in almost every disease condition [30]. Low serum albumin level is associated with in-hospital mortality in patients with necrotizing fasciitis [31]. The increase in albumin that was reported in this study indicates that QOS could improve liver function by increasing the production of plasma protein. An increase in serum albumin is probably the effect of increased total protein concentration that was reported in this study because albumin is the most abundant plasma protein produced by the hepatocytes [32]. This might be the reason why there was no mortality in mice that received a single dose of QOS at 10 mg/kg, 100 mg/kg, and 1000 mg/kg. An increase in AST level is an indication of hepatocellular injury. AST was reported to increase by about 25% after 8-24 h exercise, increasing the distance of exercise from 400 m to 1000 m also causes an increase in serum ALT [33, 34]. The increase in serum AST observed in this study is not related to pathological conditions but possible muscle leakage as a result of hyperactivity displayed by the mice that received 10 mg/kg and 100 mg/kg QOS.

Creatinine is produced in the muscles by the non-enzymatic changes of creatinine and phosphocreatine while urea is an organic compound that plays a vital role in the metabolism of nitrogen-containing compounds [35-37]. The increase in creatinine that was observed in mice that received 1000 mg/kg QOS might be the reason for renal tubule distortion. An earlier study reported an increase in

serum creatinine and urea in patients with renal failure [38]. Patients with chronic kidney disease have higher levels of serum creatinine and urea [37]. Quercetin at 50 mg/kg and 100 mg/kg were reported to decrease serum creatinine levels in diabetic mice [39]. Hence, this the reason for the low urea level in mice that received QOS at 10 mg/kg and 100 mg/kg in the present study.

The aggregation of lymphocyte that was noticed in the livers and kidneys of mice that received QOS at 1000 mg/kg and 1600 mg/kg is an indication that 1000 mg/kg and 1600 mg/kg QOS could lead to lymphocytes aggregation as a result of liver and kidney injury that will promote tissue repair. This finding is in agreement with an earlier study that reported an upregulation in the expression of M2 macrophage-related genes (arginase-1, MR, and chitinase 3-like protein 3) suggesting that quercetin could promote macrophage polarization to M2 macrophages and create a pro-chondrogenic microenvironment that increases glycosaminoglycan that will promote cartilage repair [40]. The present study suggests that QOS can initiate lymphocyte aggregation and promoting the repair of damaged liver and kidney tissues.

The LD_{50} of QOS administered through the oral route is 3807 mg/kg in mice. The administration of QOS above 1000 mg/kg led to a distortion of hepatocytes and renal tubule with an increase in serum AST, ALT, and creatinine. This suggests that quercetin could be toxic at doses above 1000 mg/kg. Therefore, care should be taken on the dosage of any form of quercetin.

Ethical Considerations

Compliance with ethical guidelines

The research was approved by the Department of Human Anatomy Ethics Committee (Code: UM/HA/PGR18.19-09900) and conducted following the ARRIVE Guidelines (Animal Research Reporting of in Vivo Experiment).

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Authors' contributions

All authors equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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