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The Acute Toxicity of Rutin in Mice

Zeena Muhammad Hamid [*] and Hayder B. Sahib[*,1]

[*] Department of Pharmacology, College of Pharmacy, Al-N ahrain University.

Abstract

Acute toxicity is a step to evaluate the toxicity of a substance. Rutin is one of the flavonoid compounds with a variety of pharmacological effects. The aim of the study is to calculate the lethal dose that affect fifty percent of the mice used in the experiment (LD50). Thirty Swiss albino male and 30 non-pregnant female mice have been divided equally and randomly into 5 treated groups and one control group (n=5) Rutin has been administered with concentrations 5, 2.5.1.25,0.625 and 0.312 g/kg administered as a single dose intraperitoneally (IP) while the control group received 1% DMSO (IP). Animals were observed for any morbidity and mortality for 14 days. After 14 days the animal blood collected for biochemical and hematological analysis then all animals are euthanized for histopathological evaluation. The results showed the LD50 was 1.51 g/kg for male mice while for female mice was1.49 g/kg. No significant changes were observed at dose of 1.25glkg (female) and 0.625, 0.312 glkg (both sexes) in body weight measurements and in biochemical or hematological assays. Moreover no significant histopathological changes were reported compared to control.. It can be concluded that Rutin is practically a non-toxic substance.

Keywords: Acute toxicity, Rutin, LD50%, Intraperitoneally, histopathology, DMSO, biochemical and hematological assay.

دراسة السمية الحادة للروتين في الفئران

زينة محمد حامد * و حيدر بهاء صاحب*[،١]

*وزارة الصحة والبيئة، دائرة مدينة الطب، بغداد، العراق .

**فرع الادوية والسموم، كلية الصيدلة، جامعة بغداد، العراق

الخلاصة

تعد السمية الحادة خطوة لتقييم سمية مادة ما ، ويعتبر الروتين أحد مركبات الفلافونويد ذات التأثيرات الدوائية المتنوعة ، وتهدف الدراسة إلى حساب الجرعة المميتة ٥٠٪ وتقييم سمية الروتين في الفئران. تم تقسيم الفئران البيضاء السويسرية ٣٠ ذكر و ٣٠ اناث غير حوامل بالتساوي وبشكل عشوائي إلى ٥ مجموعات مُعالجة ومجموعة سيطرة واحدة (ن = ٥) بتركيز ٥،٢,٥,١,٢٥،٠,٦٢٥ و ٠,٣١٢ جم / كجم جرعه واحده ، داخل الصفاق ثم التغيرات السريريه والوفيات تسجل لمده ١٤ يوم. بعد ال١٤ يوم يتم جمع الدم لاجراء التحليلات الباوكيمائيه والدمويه ومن ثم يتم قتل جميع الحيوانات لجمع الاعظاء الحيويه وارسالها لاختصاص امراض انسجه. كانت النتيجة لذكور الفئران البيضاء السويسرية LD50% 1.51 جم / كجم بينما كانت للاناث LD50%1.49 جم / كجم ووزن الجسم والمقايسة البيوكيميائية والدمية ، أظهر الفحص التشريحي المرضي أنه عند الجرعة ١,٢٥ جم / كجم (للإناث) و ٠,٦٢٥ ، ٠,٣١٢ جم / كجم لكلا الجنسين لا يوجد فرق معنوي ($p>0.05$) مع مجموعة التحكم. لذلك ، يعتبر الروتين مادة غير سامة عمليًا مع أقل تأثير ضار ملحوظ بجرعة ٠,٣١٢ جم / كجم لكلا الجنسين.

الكلمات المفتاحية : السمية الحادة ، روتين LD50% ، داخل الصفاق ، التشريح المرضي ، فحص الكيمياء الحيوية والدم DMSO .

Introduction

In last years, compounds that are derived from herbal or natural origin widely take the world interest as a complementary supplement in many diseases [(1)] Rutin, is one of the flavonol compounds its chemical structure consists of aglycon part (quercetin) that is linked to glycoside moiety (rhamnose or called rutinose sugar part) [(2)] a study conducted in 1936 on citrus fruits and listed as one of vitamins (vitamin P) [(3)] Rutin is present in many vegetables, fruits, plant such as Ruta Graveolens, Eucalyptus spp. , Sophora Japonica and buckwheat [(4,5)]. Rutin has many biological and pharmacological effects such as anti-inflammatory, antioxidant, anti-angiogenic [(6,7)] moreover it has been found effective in many chronic diseases such as Diabetes Mellitus, hypertension, inflammatory bowel syndrome, hyperlipidemia, and Alzheimer's disease[(8-11)]. The aim of this study is to investigate the acute toxicity of Rutin that can kill 50%of treated male and female Swiss albino mice intraperitoneally at different concentrations over 14 days.

Materials and Methods

Acute toxicity study was done according to the guidelines of the World Health Organization (WHO) [(12)] .Thirty male and 30 non-pregnant female Swiss albino mice with an average weight between 25-30 g (purchased from the National center for drug control and research) that is randomly and equally divided into five treated groups and one control group (n=5) for each male and female mice groups.

[1]Corresponding author E-mail: haider_bahaa@yahoo.com
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All mice have been kept in cages for 1 week in the Pharmacology laboratory at Al-Nahrain University/Collage of Pharmacy; where the study is done for environmental accommodation and all conditions such as temperature, food, and water have been adjusted. Rutin (CAS number 153-18-4 /USP has been purchased from Sigma-Aldrich) prepared freshly of 5, 2.5, 1.25, 0.625 and 0.312 g/kg. [13,14] different doses of rutin have been injected intraperitoneally (IP) as a single dose according to the bodyweight while control group has been received 1%DMSO as a negative control. The animals were observed for any sign of mortality or morbidity for 15, 30, 60. 120 and 240min for the first 24hrs after the administration of rutin; and the observation last for 14 days [13,15]. Weighting all mice at zero (before injection), seven and 14 days after administration of Rutin. [16] after 14 days, blood was collect from the jugular vein [17,18] The collected blood was centrifuged at 4000 rpm for 10 min for biochemical analysis such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), bilirubin, creatinine, urea, and albumin serum level. While for hematological analysis blood collected in a tube containing anticoagulant such as Ethylenediaminetetraacetic acid (EDTA) for measuring White Blood Cell (WBC) count and hemoglobin(HGB) level (19)(20). After blood collection, all animals have been euthanized by cervical dislocation to collect vital organs such as (liver, kidney, heart, lung and sex organs)to make histopathological examinations after preserving vital organs in 4% formalin [21,22].

Statistical analysis

The results were introduced as (as means_+SD standard deviation) one-way analysis of variance (ANOVA) and SSPS statistical software version (20) has been used in statistical analysis of data and the level of significance was set as P>0.05a significant, highly significant [23].

Results

After administration of rutin at different concentration IP, the percentage of mortality of both male and female mice at dose 5 and 2.5g/kg were 100% and male at dose 1.25 also 100% while for female at the same dose were 60% other next doses there is variation in the percentage of mortality between male and female mice where at dose 0.625 g/kg the percentage of mortality for both sex were 60% while at dose 0.312 g/kg the percentage were 0% and 20% for male and female respectively. However, the sign observed can be summarized in table (1) and table (2). Where (+), (++) and (+++) represent a degree of acute toxicity as slight, moderate and severe respectively.

Table 1.Sign observed after rutin administration to male mice

dose gm/kg	T/M	The period at sign observed	sign of toxicity	No.of mice dead at this period
5	5/5	5 mint_15 mint	Clonic convulsion, loss of gait, miosis, muscular fasciculation , tachycardia, dyspnea, lacrimation, death(+++)	2
		15mint_4hrs	hypoactivity, asthenia, abdominal contract, diarrhea, atypical locomotion, back limbs failling, bardycardia, dyspnea, death(++)	2
		4hrs_6hrs	bradycardia, atypical locomation, piloerection, asthenia ,dyspnea and death(+)	1
2.5	5/5	5 mint_15 mint	clonic convulsion, loss of gait, miosis, muscular fasculation, tachycardia, dyspnea ,lacrimation, death(+++)	2
		15mint_4hrs	hypoactivity, asthenia ,abdominal contract, diarrhea, atypical locomotion ,back limbs failling , bardycardia, dyspnea, death(++)	1
		4hrs_6hrs	bradycardia,atypical locomation,piloerection,asthenia,dyspnea and death(+)	1
		24-48 hrs.	Hypoactivity	1
1.25	5/5	5min_15 min	clonic convulsion, loss of gait,miosis,muscular fasculation,tachycardia,dyspnea,lacrimation,death(++)	0
		15mint_4hrs	hypoactivity, asthenia, abdominal contract ,diarrhea, atypical locomotion, back limbs failling, bardycardia, dyspnea, death(++)	0
		4hrs_6hrs	bradycardia, atypical locomotion, piloerection, asthenia, dyspnea, and death(+)	1
		24-48 hrs.	hypoactivity, sunken eye	4

Continued table 1.

dose gm/kg	T/M	The period at sign observed	sign of toxicity	No.of mice dead at this period
0.625	5/3	10_15 mint	loss of gait, muscular fasciculation, miosis, tachycardia (+)	0
		15mint_4hrs	asthenia, atypical locomotion(back limbsfalling, abdominal control, hypoactivity, hyperventilation ++	0
		6 _24 hrs	asthenia, hypoactivity, piloerection, atypical locomotion (back limbs failing) and pale food pads, and pale ear	2
		24-48 hr	hypoactivity, sunken eye	1
0.312	5/0	1_6 hr	tachycardia, hypoactivity, stool in the anus	0
		24 hrs_14 days	no sign of toxicity, no death	0

Table 2. Sign observed after rutin administration to female mice:

dose gm/kg	T/M	The period at sign observed	sign of toxicity	No.of mice dead at this period
5	5/5	5min-15 min	clonic convulsion, loss of gait, miosis ,muscular fasculation, tachycardia, dyspnea, lacrimation, death(+++)	2
		15min-4 hrs	hypoactivity, asthenia, abdominal contract, diarrhea, atypical locomotion, back limbs failing, bradycardia, dyspnea, death(++)	1
		4hrs-6hrs	Bradycardia, atypical locomotion, piloerection, asthenia, dyspnea, and death(+)	1
2.5	5/5	5 mint_15 mint	Clonic convulsion, loss of gait, miosis, muscular fasciculation, tachycardia, dyspnea, lacrimation, death(+++)	2
		15mint_4hrs	hypoactivity, asthenia, abdominal contract, diarrhea, atypical locomotion, back limbs failling,bardycardia, dyspnea, death(++)	1
		4hrs_6hrs	bradycardia, atypical locomotion, piloerection, asthenia, dyspnea, and death(+)	1
		24-48 hrs	hypoactivity	1
1.25	5/3	5min_15 min	clonic convulsion, loss of gait, miosis, muscular fasciculation, tachycardia, dyspnea, lacrimation , death(+++)	0
		15mint_4hrs	Hypoactivity ,asthenia ,abdominal contract, diarrhea, atypical locomotion , back limbs failing , bardycardia , dyspnea, death(++)	1
		4hrs_6hrs	bradycardia,atypical locomation, piloerection, asthenia, dyspnea and death(+)	1
		6-24hrs	hypoactivity, bluish mucous membrane of the mouth, and tail	1
0.625	5/3	10_15 mint	loss of gait, muscular fasculation,miosis,tachycardia (+)	0
		15mint_4hrs	asthenia, atypical locomotion(back limbs falling, abdominal control, hypoactivity, hyperventilation ++	0
		6 _24 hrs	asthenia, hypoactivity, piloerection, atypical locomotion (back limbs failing) and pale food pads, and pale ear	2
		24-48 hrs	Hypoactivity	1
0.312	5/1	1_6 hr	tachycardia, hypoactivity, stool in the anus	0
		24 hrs_48hr	hypoactivity, bluish mucous membrane of the mouth, and tail	1
		48hr-14 days	no sign of toxicity	0

From the calculation of both percentage of mortality and dose giving for both male and female mice, a Dose-response curve has been drawn to calculate LD50% as shown in figure (1) and (2) for male and female respectively where lethal dose was 1.51 g/kg for male mice while female mice were 1.49 g/kg.

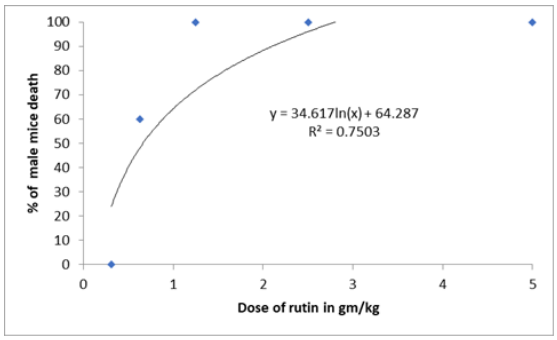

Figure 1. dose-response curve of rutin in male mice.

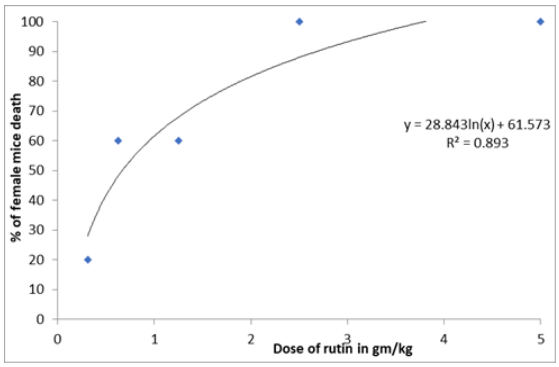

Figure 2.Dose-Response curve of rutin in female mice.

By measuring the body weight at day 0, 7 and 14 to all treated and control group the bodyweight tend to increase gradually with time to all treated and control groups of male and female mice, However, There is no significant difference ($p>0.05$) in body weight changes by comparing treated male and female mice groups that have been received 1.25, 0.625 and 0.312g/kg of rutin with the control group at day 0, 7 and 14 as shown in figure (3) and (4) of treated and control male and female mice respectively.

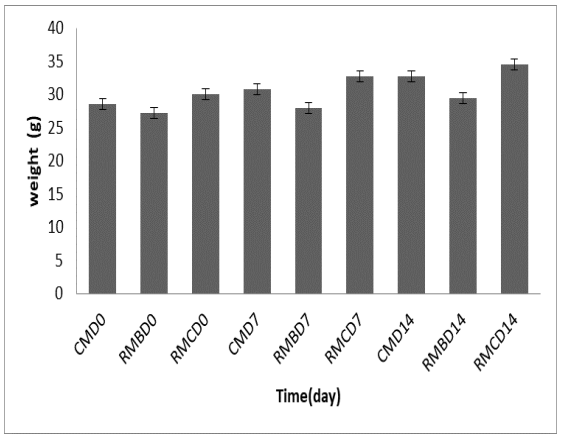

Figure 3. Body weight changes of treated groups of male mice receiving 0.625 g/kg and 0.312 g/kg of rutin at day 0, 7 and 14 where CMD0, RMBD0, RMCD0, CMD7, RMBD7, RMCD7, CMD14, RMBD14, and MCD14 represent control group, group of mice receive 0.625 g/kg 0.312 g/kg at day 0, control group, group of mice receive 0.625 g/kg, 0.312 g/kg at day 7, control group, group of mice receive 0.625 g/kg, 0.312 g/kg at day 14 respectively.

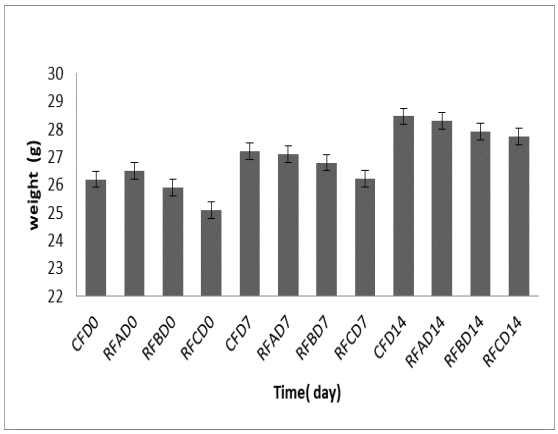

Figure 4.Body weight changes between control and treated groups of female mice receiving 1.25, 0.625 and 0.312 g/kg of rutin at day 0, 7 and 14 where CFD0, RFAD0, RFBD0 , RFCD0, CFD7, RFAD7, RFBD7,RFCD7, CFD14, DFA14, RFBD14, RFCD14 represent control group, group of mice receive 1.25 g/kg, 0.625g/kg 0.312 g/kg at day 0, control group, group of mice receive 1.25 g/kg, 0.625g/kg 0.312 g/kg at day 7, control group, group of mice receive 1.25 g/kg, 0.625g/kg 0.312 g/kg at day 14 respectively.

Biochemical and hematological analysis of treated mice that have been received 1.25, 0.625 and 0.312g/kg for both sex revealed that there is no significant difference ($p> 0.05$) when comparing with control groups. As shown in table (3).

Table 3. serum and blood profile of treated male mice receiving 0.652, 0.312 g/kg rutin and female mice receiving 1.25, 0.652, 0.312 g/kg and control mice groups.

Serum and blood profile	Cm (mean± SD)	CF (mean± SD)	Male 0.625 (mean± SD)	Male 0.312 (mean± SD)	Female 1.25 (mean± SD)	Female 0.625(mean± SD)	Female 0.312(mean ± SD)
AST U/L	263.7±8.34	216.25±6.57	266.55±9.12	264.6±1.27	219.5±10.60	217.5±9.19	217±4.24
ALT U/L	53.7±3.81	60±2.82	57±4.24	55±4.24	64.5±4.94	62.5±0.70	61.5±3.53
ALP U/L	74±8.48	68±2.82	77±4.24	76.5±10.60	71±4.24	69±5.65	68.8±1.83
Bilirubin T mg/dl	0.1±0.14	0.1±0.14	0.15±0.07	0.05±0.07	0.2±0.14	0.15±0.07	0.1±0.14
Albumin g/dL	3.05±0.21	3.2±0.14	3.1±0.14	3± 0.28	3.15±0.21	3.05±0.35	2.9±0.28
Creatinine mg/dl	0.4±0.14	0.4±0.28	0.5±0.14	0.45±0.35	0.55±0.35	0.5±0.14	0.45±0.07
Urea mg/dl	30.5±2.12	31.45±2.75	31.5±3.53	31±1.41	32.6±1.27	31.6±2.68	31.5±2.12
WBC $\times 10^9$/L	6±0.28	4.95±0.35	6.45±0.77	6.3±0.28	5.95±0.07	5.2±0.28	5.05±0.35
HGB g/dl	14.25±0.77	13.7±0.84	13.9±0.14	14±1.41	13.35±0.49	13.4±0.56	13.5±0.70

The results of Histopathological examination of vital organs after staining with Hematoxylin and eosin (H and E) stain revealed there are no significant histopathological changes in tissue ($p>0.05$) of rutin treated mice at dose 1.25, 0.625 and 0.312 g/kg for male and female mice when compare with the tissue of control group as shown in the following figures:

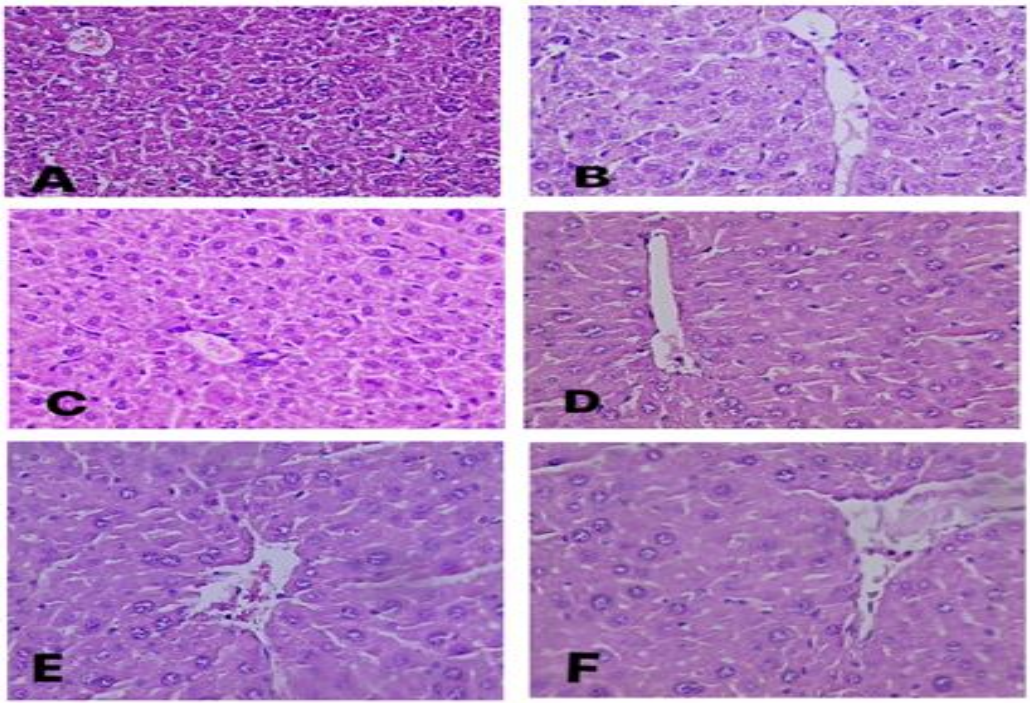

Figure 5. represent section of liver x400 that stain with hematoxyline and eosin stain (H&E) where section of liver look like normal histological structure apperance in which central vein and the hepatocyte cell arranged as thread around it. There are no fibrosis or hepatocyte inflammation or necrosis where A,B,C,D,E,F represent groups of female that have been 1.25 , 0.625, 0.312 g/kg of rutin ,control group, groups male mice reciving 0.625,0.312 g/kg rutin respectively.no significant difference when compare all male and female mice treated at all dose with control dose.

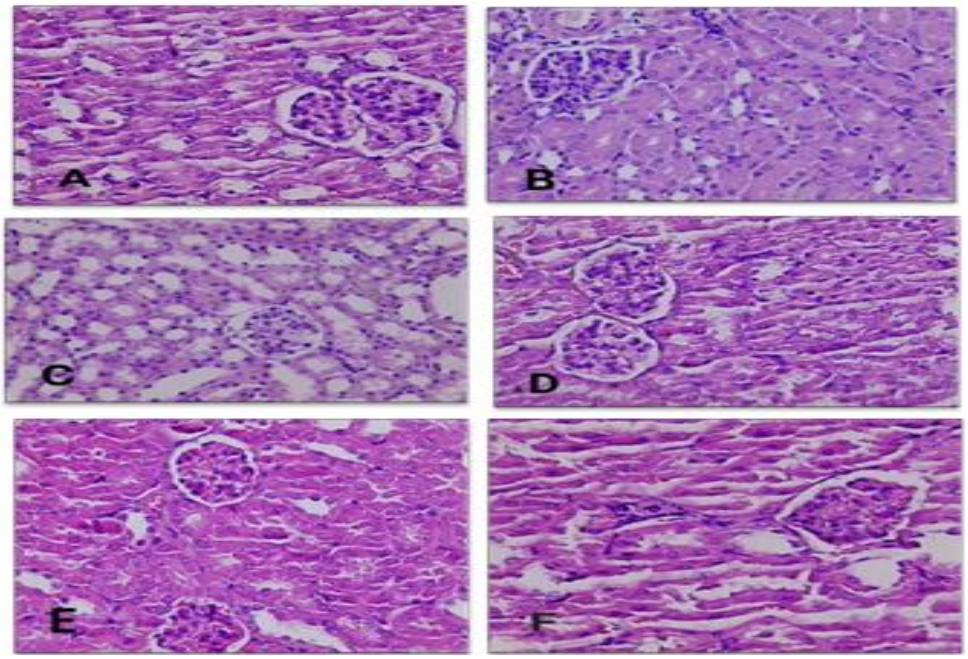

Figure 6. represent section of kidney x400 that stain with hematoxyline and eosin stain (H&E) that show there is no infilteration of inflammatory cell in interstitial space , normal tubular vascular and glomerular A,B,C,D,E,F represent groups of female that have been receiving 1.25 , 0.625, 0.312 g/kg of rutin ,control group, groups male mice reciving 0.625,0.312 g/kg rutin respectively. no significant difference when compare all male and female mice treated at all dose with control dose.

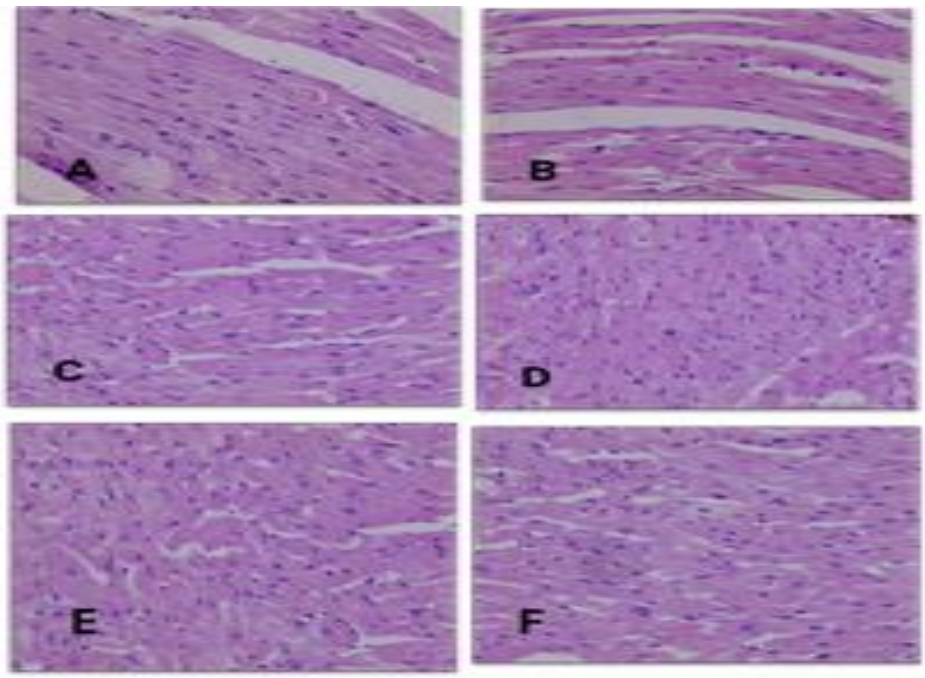

Figure7. represent section of heart x 400 that stain with hematoxyline and eosin stain (H&E) that show normal structure of heart with no infilteration of inflammatory cell in perivascular or interstitial regions , no injury or necrosis, where A,B,C,D,E,F represent groups of female that have been receiving 1.25 , 0.625, 0.312 g/kg of rutin ,control group, groups male mice reciving 0.625,0.312 g/kg rutin respectively. no significant difference when compare all male and female mice treated at all dose with control dose.

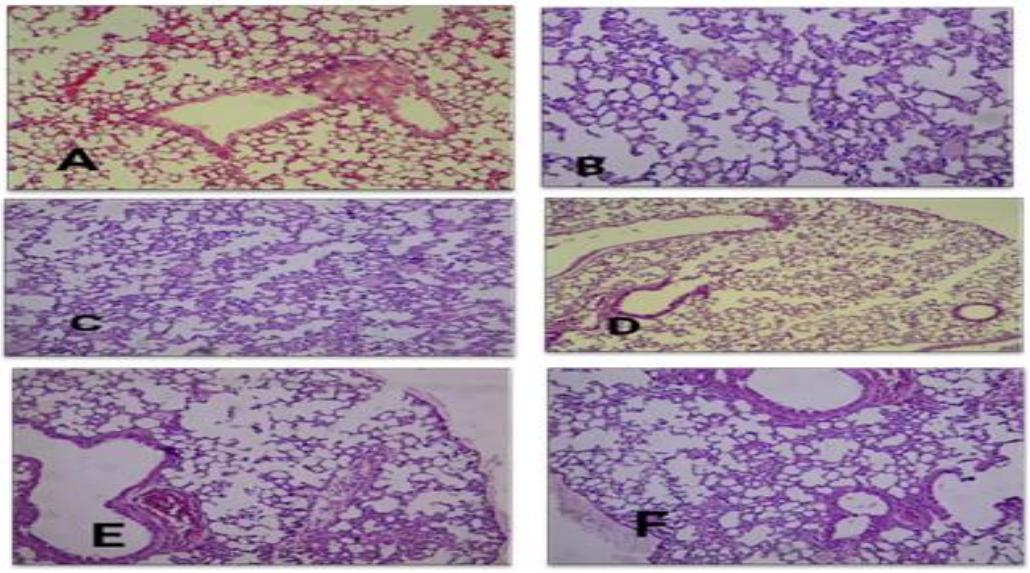

Figure 8.Represent section of lung x10 that stain with hematoxyline and eosin stain (H&E) that show normal structure of lung that consist of normal alveoli, no damage in the alveolar septa or alveolar cell necrosis , there is no inflammatory cell or edema in interstitial space or intra-alveolar region, there is no congestion or hemorrhage in capillary or alveolar duct where A,B,C,D,E,F represent groups of female that have been receiving 1.25 , 0.625, 0.312 g/kg of rutin ,control group, groups male mice receiving 0.625,0.312 g/kg rutin respectively. .no significant difference when compare all male and female mice treated at all dose with control dose.

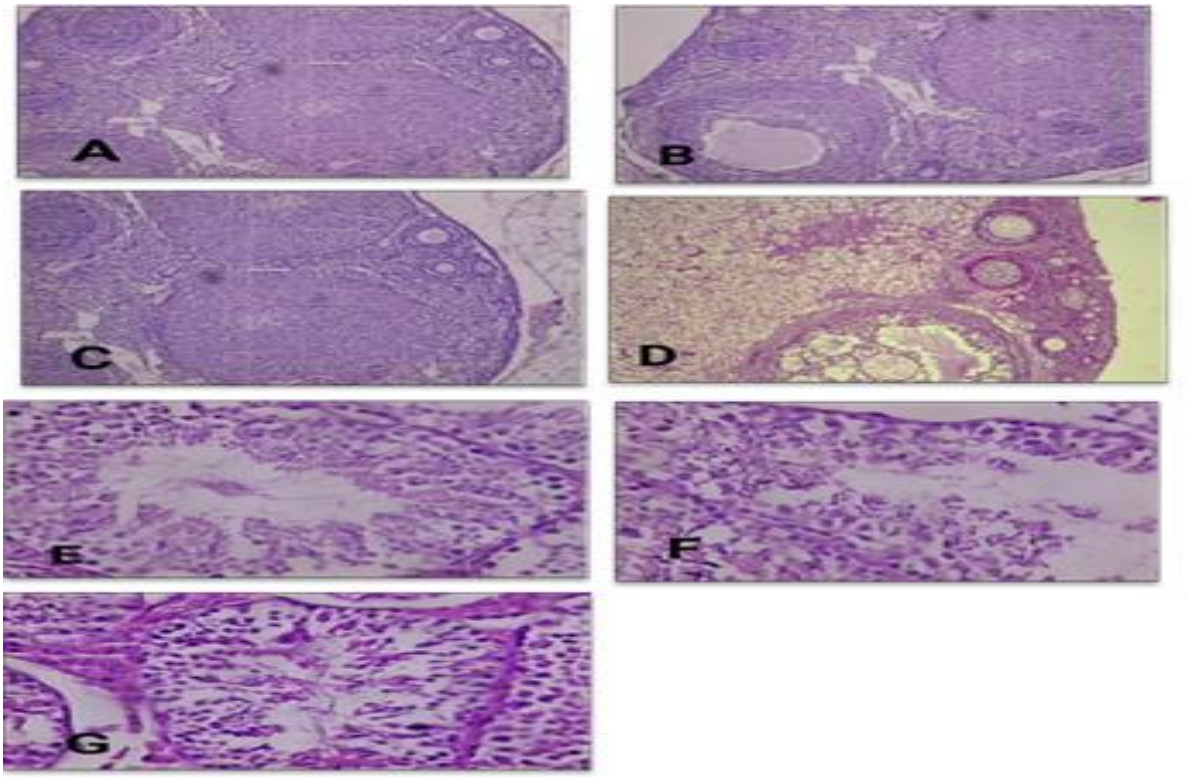

Figure 9. represent Section of testis and ovary where testis show normal structure of seminiferous tubules that inside it consist of numerous sperm and normal spermatogenesis, no atrophy in leyding cells or tubules or injury of basement membrane of tubules. While ovary consist of normal primary follicle and graafian follicle with no change and normal ovarian stroma A,B,C,D,E,F,G represent groups of female that have been receiving 1.25 , 0.625, 0.312 g/kg of rutin ,control group female,control group male, groups male mice receiving 0.625,0.312 g/kg rutin respectively. No significant difference when compare all male and female mice treated at all dose with control dose.

Discussion

The capability of a substance to make harmful, toxic effect or even death after single short exposure can be named acute toxicity study [11]. In the present study, male and female Swiss Albino injected as a single dose intraperitoneally at a different concentration, (5 and 2.5 g/kg), of rutin showed high mortality rate (100%). This may be due to that Rutin have the ability to inhibit Acetylcholinesterase (AChE) in dose dependent manner [24] this inhibition done due to OH group of rutin can bind to the anionic site in the AChE and inhibit its activity [25]. This inhibitory activity of cholinesterase (Acetylcholinesterase and buterylesterase) had been studied in vitro and in vivo [26,27]. The increase Acetylcholine activity at its receptors (nicotinic and muscarinic) may lead to cholinergic crisis that is show in tables 1 and 2 such as Clonic convulsion, loss of gait, miosis, muscular fasculation, tachycardia, dyspnea, lacrimation, urination that finally lead to respiratory failure , cyanosis and death[28,29] subsequent dose show significant difference in the mortality rate and survival rate between male and female mice this may be due to sex effect where cytochrome P450activity in female higher than in male [11] or may be due to IL-6 level , after its stimulated synthesis by any stimulus , its level will sustained in circulation in male mice more than female mice [30]. The sign of toxicity in lower dose is less extensive and resolve step by step with time [31]. The LD50 of Rutin in male 1.51 g/kg while to female 1.49 g/kg .

Body weight of animals have been used as a good indicator of the side effects of the drugs or chemical on the animal [32]. Reduction in body weight of the mice when administered certain drugs or material over a certain time may be indicated of the harmful effect of that drugs [33]while there is no significant difference in body weight gain from normal diet compare with the control group with good water and food consumption may indicate drug did not change the metabolic events of the tested treated mice groups and not affect growth rate [34]. It is concluded that mice treated with rutin do not show a significant difference in body weight compared with the control group.

AST, ALT, ALP use as an indicator of liver dysfunction or damage due to various causes such as a toxin, drugs, viral that increase in the serum level[35]. ALT is an enzyme specific to the liver while AST is an enzyme present in other tissue such as heart, muscle [36]. Increase level of ALP may be due to bile duct obstruction, cholestasis. hepatitis [37]. Albumin and bilirubin is another marker for liver function[38]. Decrease level of albumin can be account to low diet intake, toxicity inside the body, increase catabolism and kidney problem [39] while increase level of it can be cleared mostly as high protein diet [40]. Creatinine and urea consider as a marker of kidney function [32].

Hematological analysis was done to evaluate the risk of hematological changes due to it considers important and sensitive parameter where toxic material or drugs targeting on it that lead to detect the toxicity of drugs and physiopathological alteration in a biological system[34,10] alteration in hematological analysis such as hemoglobin (HGB) level may indicate toxicity that induces hemorrhage and hemolysis[41] or dysfunction of renal system While white blood cell(WBC) increase level of it may indicate there is an activation of the immune system due to either injury of liver, kidney or other tissue or infection[42]

Rutin treated groups in male and female in doses (1.25, 0.625, and 0.312 g/ kg) show no significant difference when compare with the control group and all results of the biochemical analysis revealed it lie in the normal range that is confirmed by histopathological examination for (liver, kidney, heart, lung and sex organs) that show no significant changes of treated tissue when compare with control tissue groups[43]. Other studies show that rutin when administer orally for 13 weeks to rat was safe till 2000 mg/kg [44] while other study show when administered orally to rats as a single(acute toxicity study) and repeated dose(subacute toxicity study) is safe till 5000 mg/kg [45]. In the present study, the lowest observed adverse effect LOAE was seen at dose 0.312 g/kg and according to" Loomis and Hayes classification" is classified as a practically non-toxic substance [43] .

Conclusion

Acute toxicity study of rutin revealed that it is a practically non-toxic substance

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Reference

1. Huang Y, Feng F, Jiang J, Qiao Y, Wu T, Voglmeir J, et al. Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. Food Chem [Internet]. 2017;221:1400–5. Available from: http://dx.doi.org/10.1016/j.foodchem.2016.11.013
2. MacIkova P, Halouzka V, Hrbac J, Bartak P, Skopalova J. Electrochemical behavior and determination of rutin on modified carbon paste electrodes. Sci World J. 2012;2012(April).
3. Hooresfand Z, Ghanbarzadeh S, Hamishehkar H. Preparation and characterization of rutin-loaded nanophytosomes. Pharm Sci [Internet]. 2015;21(3):145–51. Available from: http://dx.doi.org/10.15171/PS.2015.29
4. Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: A review on extraction,

identification and purification methods, biological activities and approaches to enhance its bioavailability. Trends Food Sci Technol. 2017;67:220–35.

5. Nouri Z, Fakhri S, Nouri K, Wallace CE, Farzaei MH, Bishayee A. Targeting multiple signaling pathways in cancer: The rutin therapeutic approach. Vol. 12, Cancers. 2020. 1–34 p.
6. Gagliardi A, Paolino D, Costa N, Fresta M, Cosco D. Zein- vs PLGA-based nanoparticles containing rutin: A comparative investigation. Mater Sci Eng C [Internet]. 2021;118(September 2020):111538. Available from: https://doi.org/10.1016/j.msec.2020.111538
7. Li B, Yang X. Rutin-loaded cellulose acetate/poly(ethylene oxide) fiber membrane fabricated by electrospinning: A bioactive material. Mater Sci Eng C. 2020;109:1–17.
8. Oyagbemi AA, Bolaji-Alabi FB, Ajibade TO, Adejumobi OA, Ajani OS, Jarikre TA, et al. Novel antihypertensive action of rutin is mediated via inhibition of angiotensin converting enzyme/mineralocorticoid receptor/angiotensin 2 type 1 receptor (ATR1) signaling pathways in uninephrectomized hypertensive rats. J Food Biochem. 2020;44(12):1–14.
9. Nakamura K, Koyama M, Ishida R, Kitahara T, Nakajima T, Aoyama T. Characterization of bioactive agents in five types of marketed sprouts and comparison of their antihypertensive, antihyperlipidemic, and antidiabetic effects in fructose-loaded SHRs. J Food Sci Technol. 2016;53(1):581–90.
10. Tiwari R, Siddiqui MH, Mahmood T, Farooqui A, Bagga P, Ahsan F, et al. An exploratory analysis on the toxicity & safety profile of Polyherbal combination of curcumin, quercetin and rutin. Clin Phytoscience. 2020;6(1).
11. Mukinda JT. Acute and chronic toxicity of the flavonoid- containing plant , Artemisia afra in rodents Keywords. 2005;(December).
12. Bank/WHO U. Non-Clinical Safety. 2002;115.
13. Nair RR. Evaluation of acute and sub-acute oral toxicity of ethanolic root extract of Tetracera akara (Burm. f.) Merr., an ethnomedicinal plant used by the Kani tribe of Kerala. J Tradit Folk Pract. 2018;5(2).
14. Zhao J, Lin DQ, Yao SJ. Adsorption of rutin with a novel β-cyclodextrin polymer adsorbent: Thermodynamic and kinetic study. Carbohydr Polym [Internet]. 2012;90(4):1764–70. Available from: http://dx.doi.org/10.1016/j.carbpol.2012.07.066
15. Féres CAO, Madalosso RC, Rocha OA, Leite JPV, Guimarães TMDP, Toledo VPP, et al. Acute and chronic toxicological studies of Dimorphandra mollis in experimental animals. J Ethnopharmacol. 2006;108(3):450–6.
16. Doshi GM, Pawar MK, Chavda KH. Quantification of rutin and quercetin by HPTLC/HPLC and in vitro immunomodulatory and anticancer activities of Capparis moonii fruits extracts. Int J Basic Clin Pharmacol. 2017;7(1):153.
17. Yakovlevich Golovenko N, Nikolayevna Kovalenko V, Borisovich Larionov V, Semenovich Reder A. Dose and time-dependent acute and subchronic oral toxicity study of propoxazepam in mice and rats. Int J Pharmacol Toxicol. 2020;8(1):1.
18. Hoek-van Den Hil EF, Van Schothorst EM, Van Der Stelt I, Swarts HJM, Venema D, Sailer M, et al. Quercetin decreases high-fat diet induced body weight gain and accumulation of hepatic and circulating lipids in mice. Genes Nutr. 2014;9(5).
19. Da Silva ARH, Moreira LDR, Brum EDS, De Freitas ML, Boligon AA, Athayde ML, et al. Biochemical and hematological effects of acute and sub-acute administration to ethyl acetate fraction from the stem bark Scutia buxifolia Reissek in mice. J Ethnopharmacol [Internet]. 2014;153(3):908–16. Available from: http://dx.doi.org/10.1016/j.jep.2014.03.063
20. Rasekh HR, Nazari P, Kamli-Nejad M, Hosseinzadeh L. Acute and subchronic oral toxicity of Galega officinalis in rats. J Ethnopharmacol. 2008;116(1):21–6.
21. Bedi O, Krishan P. Investigations on acute oral toxicity studies of purpurin by application of OECD guideline 423 in rodents. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(4):565–71.
22. Singh H, Bishen KA, Garg D, Sukhija H, Sharma D, Tomar U. Fixation and Fixatives: Roles and Functions—A Short Review. Dent J Adv Stud. 2019;07(02):051–5.
23. Daniel WW, Cross CL. Biostatistics: a foundation for analysis in the health sciences. Wiley; 2018.
24. Yan X, Chen T, Zhang L, Du H. Study of the interactions of forsythiaside and rutin with acetylcholinesterase (AChE). Int J Biol Macromol [Internet]. 2018;119:1344–52. Available from: https://doi.org/10.1016/j.ijbiomac.2018.07.144
25. Anesti M, Stavropoulou N, Atsopardi K, Lamari FN, Panagopoulos NT, Margarity M. Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice. Epilepsy Behav [Internet]. 2020;102:106632. Available from: https://doi.org/10.1016/j.yebeh.2019.106632
26. Li M, Gao X, Lan M, Liao X, Su F, Fan L, et al. Inhibitory activities of flavonoids from Eupatorium adenophorum against acetylcholinesterase. Pestic Biochem Physiol

[Internet]. 2020;170(February):104701. Available from: https://doi.org/10.1016/j.pestbp.2020.104701

27. Oboh G, Adebayo AA, Ademosun AO, Olowokere OG. Rutin restores neurobehavioral deficits via alterations in cadmium bioavailability in the brain of rats exposed to cadmium. Neurotoxicology [Internet]. 2020;77:12–9. Available from: https://doi.org/10.1016/j.neuro.2019.12.008

28. Khan H, Marya, Amin S, Kamal MA, Patel S. Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. Biomed Pharmacother [Internet]. 2018;101(October 2017):860–70. Available from: https://doi.org/10.1016/j.biopha.2018.03.007

29. Singh R, Firdous S, Sharma P. Neurotoxic effect of cypermethrin and protective role of resveratrol in Wistar rats. Int J Nutr Pharmacol Neurol Dis. 2014;4(2):104.

30. Dewar D, Moore FA, Moore EE, Balogh Z. Postinjury multiple organ failure. Injury. 2009;40(9):912–8.

31. Klaassen CD. Casarett and Doull's toxicology: the basic science of poisons. Vol. 1236. McGraw-Hill New York; 2013.

32. Ibrahim MY, Bustamam A, Abdul H, Azmi T, Ibrahim T, Abdelwahab SI, et al. Evaluation of acute toxicity and the effect of single injected doses of zerumbone on the kidney and liver functions in Sprague Dawley rats. African J Biotechnol. 2010;9(28):4442–50.

33. Petterino C, Argentino-storino A. ARTICLE IN PRESS EXPERIMENTAL AND TOXICOLOGIC Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. 2006;

34. Wang J, Sun F, Tang S, Zhang S, Lv P, Li J, et al. Safety assessment of vitacoxib: Acute and 90-day sub-chronic oral toxicity studies. Regul Toxicol Pharmacol [Internet]. 2017;86:49–58. Available from: http:// dx.doi .org/ 10. 1016 / j. yrtph .2017.02.020

35. Yalçın E, Çavuşoğlu K, Acar A, Yapar K. In vivo protective effects of Ginkgo biloba L. leaf extract against hydrogen peroxide toxicity: cytogenetic and biochemical evaluation. Environ Sci Pollut Res. 2020;27(3):3156–64.

36. Osagie-eweka S, Orhue NEJ. Toxicity Status of Methanol Leaf Extract of Simarouba glauca on Rat Liver Toxicity Status of Methanol Leaf Extract of Simarouba glauca on Rat Liver. 2020;(December 2019).

37. Roy S, Majumdar S, Singh AK, Ghosh B, Ghosh N, Manna S, et al. Synthesis, characterization, antioxidant status, and toxicity study of vanadium–rutin complex in balb/c mice. Biol Trace Elem Res. 2015;166(2):183–200.

38. Gnanamani A, Sudha M, Deepa G, Sudha M, Deivanai K, Sadulla S. Haematological and biochemical effects of polyphenolics in animal models. Chemosphere. 2008;72(9):1321–6.

39. Gryshchenko VA, Minina VS. Marker changes of blood plasma proteinogram in rats with toxic hepatitis. Regul Mech Biosyst. 2020;11(3):360–6.

40. Mutlu E, Keshavarzian A, Mutlu G. Hyperalbuminemia and elevated transaminases associated with high-protein diet. Scand J Gastroenterol. 2006;41(6):759–60.

41. Sangeetha MK, Eazhisai Vallabi D, Sali VK, Thanka J, Vasanthi HR. Sub-acute toxicity profile of a modified resveratrol supplement. Food Chem Toxicol. 2013;59:492–500.

42. Dibal NI, Hyedima Garba S, Watson Jacks T. Acute Toxicity of Quercetin From Onion Skin in Mice. Pharm Biomed Res. 2021;6(4):269–76.

43. Loomis and Hayes. Loomis essential of Toxicology. fourth. California A press, editor. 1996.

44. Hasumura M, Yasuhara K, Tamura T, Imai T, Mitsumori K, Hirose M. Evaluation of the toxicity of enzymatically decomposed rutin with 13-weeks dietary administration to Wistar rats. 2004;42:439–44.

45. Tatsuro S, Toshikazu M, Takahiro N, Koji I. Acute and Subacute Toxicity Studies on Rutin-Rich Tartary Buckwheat Dough in Experimental Animals. 2015;175–81.