Median lethal Dose and Acute Toxicity of Rosa canina L: In-Vivo Study

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Abstract:

Rosa canina belongs to rosacea family. *Rosa canina* has a high concentration of phyto-constituents such as flavonoids, carotenoids, triterpene and vitamins as vitamin C, E, and A *Rosa canina* have an anti-inflammatory and antioxidant effects. The antioxidant effect belongs to presence of large quantities of phytochemicals such as flavonoids and polyphenols. *Rosa canina* has been used for arthritis gout, osteoarthritis, urinary tract disorder, diabetes, inflammation and cancer. After grinding the leaves of Rosa canina, they are extracted by using ethanol solvent (cold extraction method), then the extract concentrated by rotary evaporator at 40 c° and leaving it to dry. Median lethal dose (LD50) has been examined on 84 mice (male and female) divided into seven groups, each one contains 12 (6 male and 6 female). The animals were monitored for signs and any behavior changes after administration of *Rosa canina* ethanol extract. Acute toxicity study was done on 20 rats (male and female for fourteen days. The weight of animals was taken at day 0, 7, and 14. At day fourteen, relative organ weight as well as histopathological examination for (heart, liver, spleen, kidney, lung, abdominal stomach, testes and ovaries) were taken. In addition to the serum biochemical tests for) blood glucose, urea, creatinine, ALT, AST and total bilirubin (were done at day fourteen. The result of this study, indicated that lethal dose 50 was 16.527 gram/kilogram. Acute toxicity study, revealed that there is no significant difference between relative organ weight of controlled and treated groups for all organs that were selected and mentioned above. In addition, there is no significant difference between serum biochemical tests for both controlled and treated groups. Finally, no changes have been found between controlled and treated groups regarding histopathology examination due to the p value was $P > 0.05$.

Conclusion

According to the presented study, ethanol extract of *Rosa canina* showed wide range of safety depending on the result of lethal dose 50 (16.527 g/kg). Therefore, the extract considered nontoxic. No cytotoxic effect appeared by using ethanol extract of *Rosa canina* in acute toxicity study. This belongs to the results obtained, which include no significant difference between control and treated male and female rats in biochemistry tests and histopathological examination.

Key words: Median lethal dose, Acute toxicity, *Rosa canina*.

متوسط الجرعة القاتلة والسمية الحادة لنبتة النسرين(روزا كانينا): دراسة داخل الجسم الحي

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الخلاصة:
تنتمي روزا كانينا (النسرين) الى عائلة الورديات التي تمتلك مجموعة واسعة من المكونات النباتية مثل الفلافنويد والكاروتينات، التيربينات الثلاثية والفيتامينات مثل فيتامين ج، ه، أ. روزا كانينا تمتلك فعالية مضادة للألتهاب ومضادة للأكسدة. الفعالية المضادة للأكسدة تعود أيضا لوجود كميات كبيرة من المواد الكيميائية النباتية مثل مركبات الفلافنويد والمركبات المتعددة الفينول. تستخدم الروزا كانينا في التهاب المفاصل والنقرس، وهشاشة العظام واضطراب المسالك البولية والسكري والالتهابات والسرطان. تم اجراء اختبار الجرعة القاتلة ل 50 % على 84 فأر (ذكور واناث). قسمت الى سبع مجموعات، كل مجموعة تحتوي على 12 (6 ذكور و 6 اناث). تم رصد الحيوانات بحثا عن علامات واي تغييرات سلوكية بعد تناولها مستخلص الايثانول للروزا كانينا. تم اجراء دراسة السمية الحادة على 20 جرذ (ذكور واناث) لمدة 14 يوم. تم اخذ وزن الحيوانات في اليوم(0و7و14) ، وزن العضو النسبي وفحص الانسجة لكل من القلب، الكبد، الطحال، الكلى، الرئة، المعدة، الخصيتين والمبيضين بالإضافة الى مصل الاختبارات البايوكيميائية لكلوكوز الدم، اليوريا، الكرياتنين، وانزيمات الكبد والبليروبين الكلي. تم القيام به في اليوم الرابع عشر. وكانت نتيجة الدراسة ان الجرعة المميتة ل 50 % من الحيوانات المختبرية هي 16.527 غرام / كيلوغرام. دراسة السمية الحادة، لا يوجد فرق كبير بين وزن العضو النسبي للمجموعات المعالجة وغير المعالجة لجميع الأعضاء التي تم اختيارها. إضافة الى ذلك لا يوجد فرق واضح بين الاختبارات البايوكيميائية في مصل الدم لكلا المجموعتين المعالجة وغير المعالجة. أخيرا لا يوجد أي تغيرات بين المجموعة المسيطر عليها والمجموعة المعالجة فيما يتعلق بفحص الانسجة المرضية كما هو موضح في قيمة الاحتمالية اكبرمن 0.05.

الاستنتاج:
حسب الدراسة المقدمة، اظهر مستخلص الايثانول للروزا كانينا نطاقا واسعا من الأمان اعتمادا على نتيجة الجرعة المميتة (16.527 غرام لكل كيلو غرام). لذلك يعتبر المستخلص غير سام. لم يظهر أي تأثير سام للخلايا باستخدام مستخلص الايثانول من نبات روزا كانينا في دراسة السمية الحادة. تنتمي هذه الى النتائج التي تم الحصول عليها والتي لا تتضمن فروق ذات ذات دلالة إحصائية بين ذكور واناث الجرذان المعالجة والغير معالجة في اختبارات الكيمياء الحيوية والفحص النسيجي المرضي.

الكلمات المفتاحية: الجرعة القاتلة المتوسطة، السمية الحادة،النسرين (روزا كانينا).

Introduction

Rosa canina belong to rosacea family, many of them are characterized by appearance of stipules above the leaf. The flowers contain five-part sets. The fruit is known as Rose hip (not true fruits). The latin name was canis means dog because of its root could be utilized as treatment for the mad dog bite. The name of it also belong to hooked prickles that found on the stem that are similar to dog's canines [1]. Identification of median lethal dose (lethal dose fifty) *of Rosa* canina is important to avoid lethal dose and in the development of natural plant with high level of safety and lower adverse effects. *R. canina* has been widely used in treatment of several diseases such as osteoarthritis, gout, diabetes, diarrhea, inflammatory disorder, renal disturbances, rheumatoid arthritis and skin disorders· [2]. *Rosa canina* has strong anti-oxidant effect due to high content of vitamin C, A and E. phytochemical constituents such as poly phenolic compounds especially flavonoids, terpenoids and carotenoids. Therefore used effectively in cancer treatment. All these active ingredients are responsible for *Rosa canina* pharmacological action· *Rosa canina* is considered beneficial in diarrhea and dysentery, also used as an expectorant. The bioactive compounds that are found in *Rosa canina* which responsible for pharmacological action of *Rosa canina* are flavonoids, carotenoids, unsaturated fatty acid and galactolipid. Vitamins, triterpene and dietary fibers. [3-7]. figure (1) represent the picture about *Rosa canina.*

Figure (1): *Rosa canina* (Rose hip)

Aim of the study: to investigate the lethal dose fifty and acute toxicity of *Rosa canina* in both mouse and rat models.

Materials and methods

The plant purchased from Zaiona and Graiat, the leaves were collected, washed, dried and grinded by electrical grinder. 44.58 gram of the leaves powder was used for the extraction of *R. canina* by using ethanol solvent through cold extraction method. Rotary evaporator was used at 40 c° to concentrate the extracted material. 1.07 gram used for LD50 test. 1041gram of the leaves powder was used for extraction by using ethanol solvent for acute toxicity study, 25 gram was used as extracted plant in acute toxicity study.

1-Eighty-four male and female mice, weight of them 20-25 gram were used in lethal dose 50.

2-Twenty rats (male & female) were utilized in acute toxicity experiment for fourteen days. The rats that are used in laboratory apparently healthy young adult. Female must be nulliparous and not pregnant. At dosing of each animal must be between 8 and 12 weeks. The weight of tested animals should be within the range ± 20 % of the average weight of each formerly dosed animals. [8]

Median lethal dose (Lethal dose fifty (LD50)

The lethal dose known as the dose that killed about 50 % of experimental animals in the test. Weight of them approximately 20 grams for each one. Each group contains twelve treated animals (six male and six female), in addition to twelve control group (6 male and 6 female). All animals are dealed with the same conditions in the laboratory such as food and water. After food overnight deprivation, the treated groups of animals received ethanol extract orally. The doses of *R. canina* that are used in this experiment were 1, 2.5, 5, 10, 15, and 20 gram per kilogram that were dissolved in 2 % of tween 80. The control group were administered 0.3 ml solution of 2 % tween 80 by oral route. During the first three days after administration of *R. canina* extract, the animals were observed for changes in the signs and symptoms, general behavior, death and physiological changes. [8,9]

Acute toxicity study

According to the world health organization (2000) as well as an economic cooperation and development organization, the acute toxicity study was evaluated. Randomly twenty rats were taken and grouped in to two groups (ten for each group). The first group considered control group (five male and five female) while the second group considered treated group (five male and five female). Treated group administered ethanol extract of *R. canina* orally as a single dose 5 gram for each kilogram of body weight. The control groups were given 3 ml water. The experiment duration fourteen days after administration of ethanol, extract of *R. canina*. The parameter that was observed are mortality, the body weight of rats, relative organ

weight as well as biochemistry tests and histopathology investigation. [9]

1-Mortality: the observation of animals daily about any behavior change and mortality

2-Body weight: the body weight was measured at days zero, seven and fourteen during experimental period by using sensitive balance.

3-Relative organ weight: all the rats were euthanized under ketamine and xylazine anesthesia on day fourteen of experiment. Several organs were measured their weight in gram such as heart, liver, lung, spleen, kidney, stomach and sex organs (testes and ovaries). Then the relative organ weight was measured according to this formula. [9]

Absolute organ weight in gram / body weight of rat on this day in gram * 100

Result

Median lethal dose (Lethal dose fifty)

After administration of a single dose of sequential dilution of *Rosa canina*. Ethanol extract of *R.canina* was administered to each group that was divided in to six groups (male mice and female mice). Each one administered a concentration while the control group was administered the tween solution. Depending on the concentration that was administered, the results were taken in the table (1) and figure (2).

Table (1): lethal dose fifty of *R. canina* ethanol extract tested on male and female mice

Groups	Dose in gram/ kilogram	Number of male and female mice	Number of dead male and female mice	Accumulative %	
Control group	0	6 males 6 females	0	0	
Group 1	1	6 mal 6 females	0	0	
Group 2	2.5	6 males 6 females	0	0	
Group 3	5	6 males 6 females	0	0	
Group 4	10	6 males 6 females	1 1	16%	
Group 5	15	6 males 6 females	3 1	50%	16%
Group 6	20	6 males 6 females	4 4	66%	66%

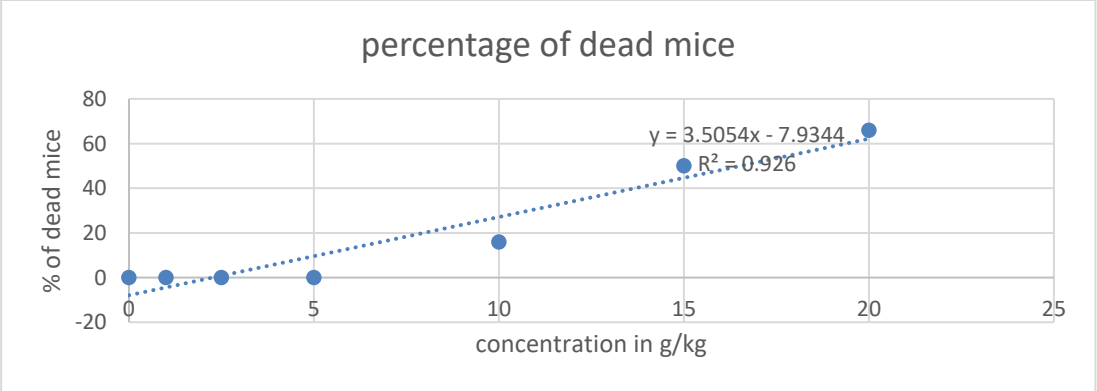


Figure (2): lethal dose 50 of *R. canina* ethanol extract on male and female mice groups

LD50 of *R. canina* ethanol extract was measured by using the linear regression equation (y= 3.5054x-7.9344)
Y= the percentage of inhibition
X= the extract concentration
LD50 = 16.527 gram/kg.
Acute toxicity study of *R. canina* ethanol extract
After administration of single dose of 5 gram for each kilogram of ethanolic extract of *R. canina* to the experimental animals, there is no sign of toxicity or death rates were discovered for the duration of fourteen days of acute toxicity test period. The changes in body weight were taken and recorded at day zero, seven and fourteen. At day fourteen as remembred in table (2), the relative organ weights were taken, drawing of blood samples from the heart was done at day 14 from both treated and control groups. Finally, histo pathological examination was done from selected organs in the acute toxicity study.

Table (2): Animals weight (males and females) during experiment days.

Time / day	Wight (gram)			
	control male group	Treated male group	Control female group	Treated female group
Day zero	232.8±175.024	217.4±133.777	236.6±19.919	220.8± 4.086
Day seven	244±136.859	227.6±135.818	241±17.776	238.6±35.203
Day fourteen	257.2±98.598	240.6±102.466	243±11.510	241.8±21.076

Relative organ weight in the acute toxicity study
The results appeared no significant change showed between treated and control male organs. (P >0.05) according to tables (3 and 4) respectively.

Table (3): relative organ weights of control and treated male rats receiving *R. canina* ethanol extract after fourteen days of an acute toxicity study.

Male organs treated with R. canina ethanol extract (5 g/kg (and their controls			
Organs	Control group	R. canina group 5g/kg	P value
Heart	0.833±0.208	0.766±0.251	0.742
Liver	7.166±2.753	6.933±2.900	0.924
Spleen	0.866±0.230	0.833±0.288	0.883
Kidney	2.8±721	2.6±0.529	0.718
Lung	2.666±0.577	2.566±0.665	0.854
Stomach	2.833±0.288	2.733±0.246	0.672
Testes	4.3±0.264	4.166±0.288	0.587

Table (4): relative organ weights of control and treated female rats receiving *R. canina* ethanol extract after fourteen days of an acute toxicity study.

Female organs treated 5 g/kg RC ethanol extract and their controls			
Organ	Control group	R. canina group 5g/kg	P value
Heart	0.766±0.152	0.666±0.208	0.539
Liver	7.766±1.078	7.716±1.032	0.957
Spleen	0.823±0.265	0.716±0.246	0.637
Kidney	1.466±0.550	1.436±0.487	0.947
Lung	1.706±0.300	1.606±0.368	0.734
Stomach	2.363±0.505	2.213±0.500	0.733
Ovaries	0.276±0.107	0.246±0.136	0.780

Measurement the Serum biochemical tests for rats in acute toxicity study

After administration 5 gram/kg of *R.canina* Ethanol extract,

The results obtained from serum biochemical tests for treated rats that compared to their controls. There was found as no significant alteration between treated and control groups. (P> 0.05)

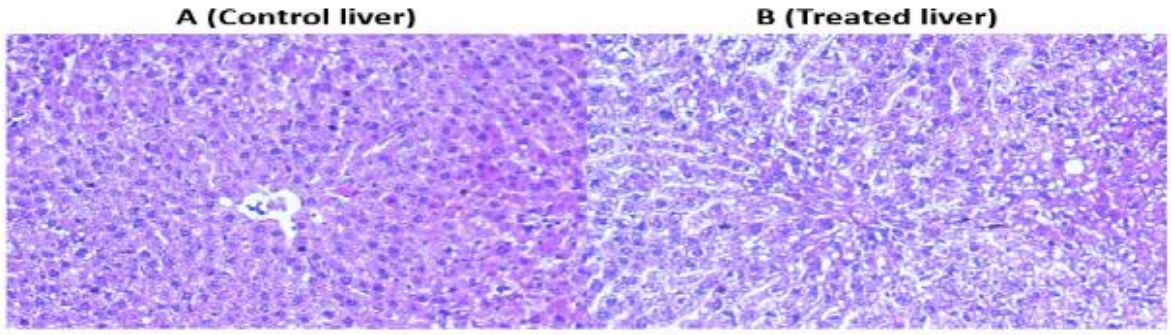
The results presented in the table (5).

Table (5): the tests of serum biochemical for rats receiving 5g/kg as a single dose of *R. canina* ethanol extract versus their controls.

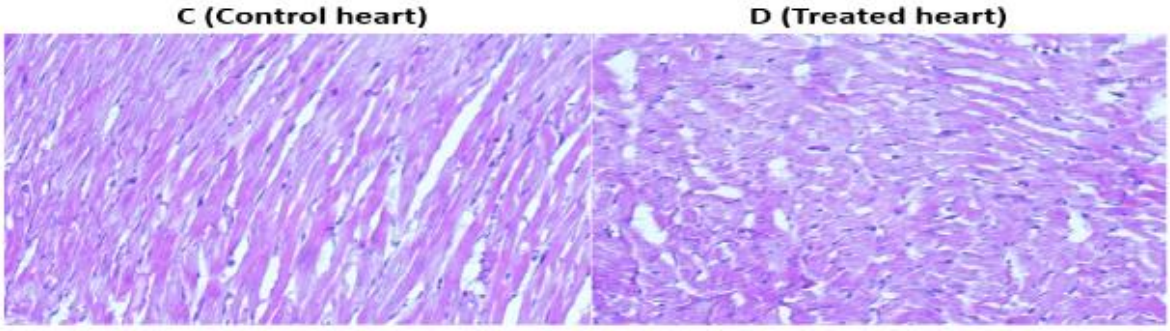
Biochemical tests	Male rats			Female rats		
	Control group	treated group	P value	Control group	Treated group	P value
Blood sugar mg/d	191±11.53	192.6±6.42	0.885	185±10.01	183±11.26	0.474
Blood urea mg/dl	30±9.53	26.66±7.50	0.693	34.66±5.50	31.66±8.62	0.272
S. creatinine mg/dl	0.44±0.13	0.45±0.27	0.968	0.36±0.12	0.33±0.07	0.669
ALT u/l	63.66±23.50	67.66±2.51	0.815	42.33±4.4	43.66±4.16	0.800
AST u/l	101.66±20.2	108.33±21.5	0.081	80.33±13.79	81.66±12.58	0.547
Total bilirubin mg/dl	0.5±0.4	0.46±0.30	0.667	0.233±0.152	0.266±0.208	0.874

Histopathology examination for certain organs in acute toxicity study

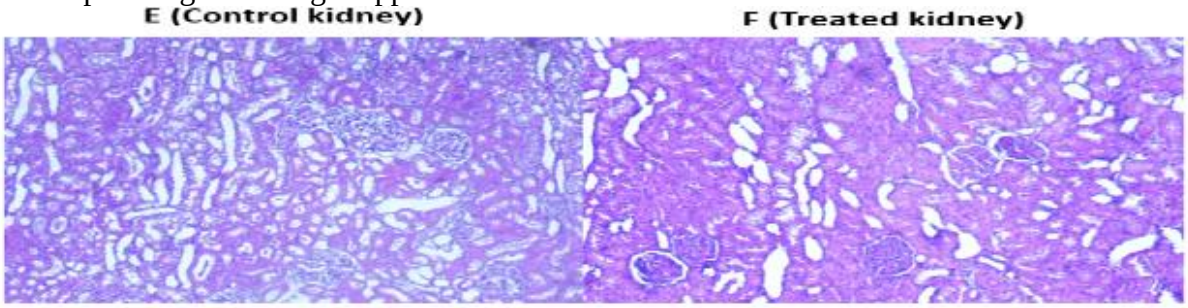
The histopathological examination of certain organs in the acute toxicity test in rats appeared no significant change through comparison between both control and treated groups of male and female rats. Figure (3).



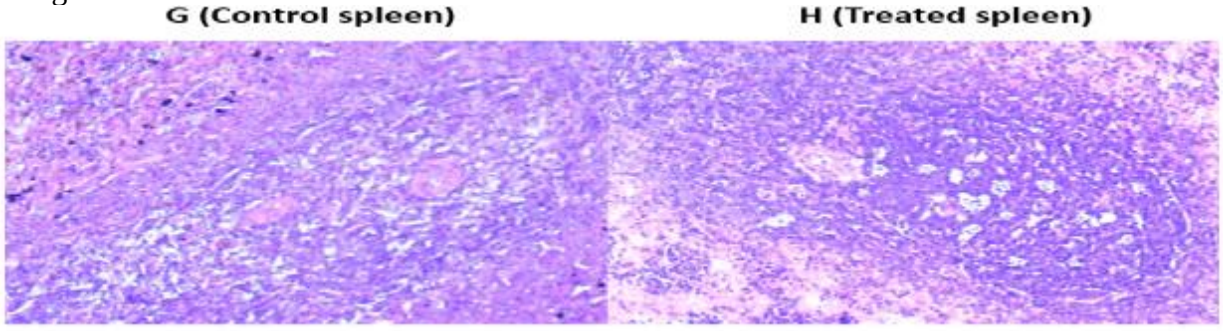
*In image A and B appeared normal hepatic lobules, there is no congestion of portal veins, no necrosis and no notable pathology.



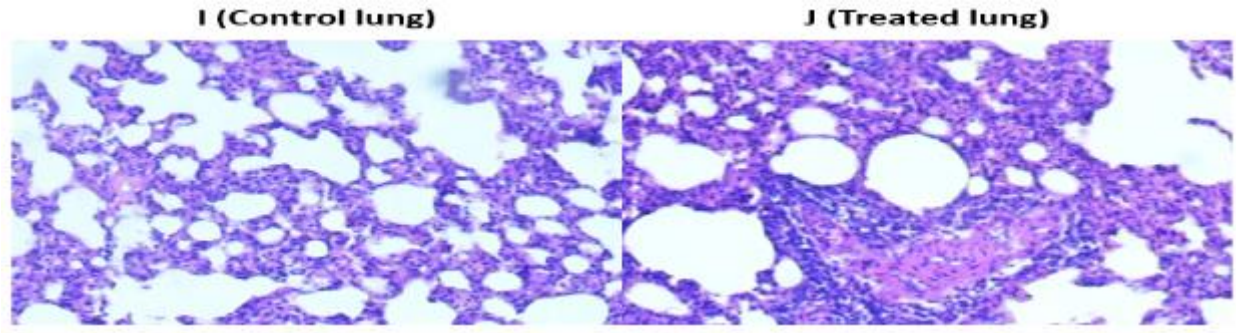
*In images, C and D show normal cardiac muscle associated with centrally located nucleus and no pathological changes appeared.



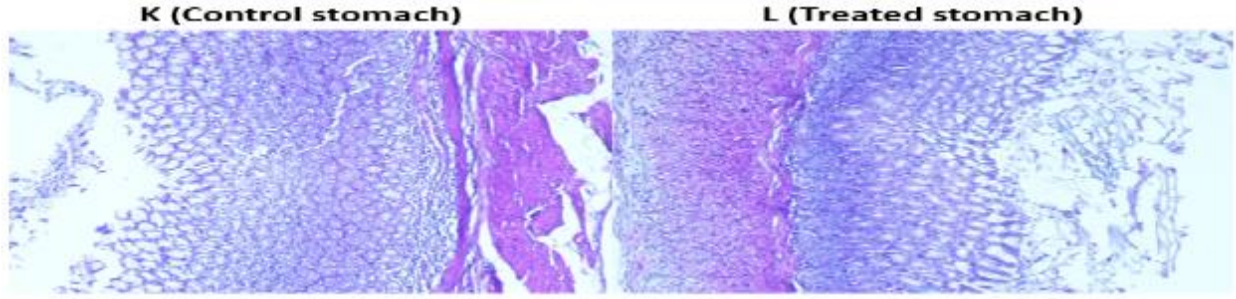
*In images E and F we see normal glomerular and tubular tissue and there is no pathological changes in both cortex and Medulla.



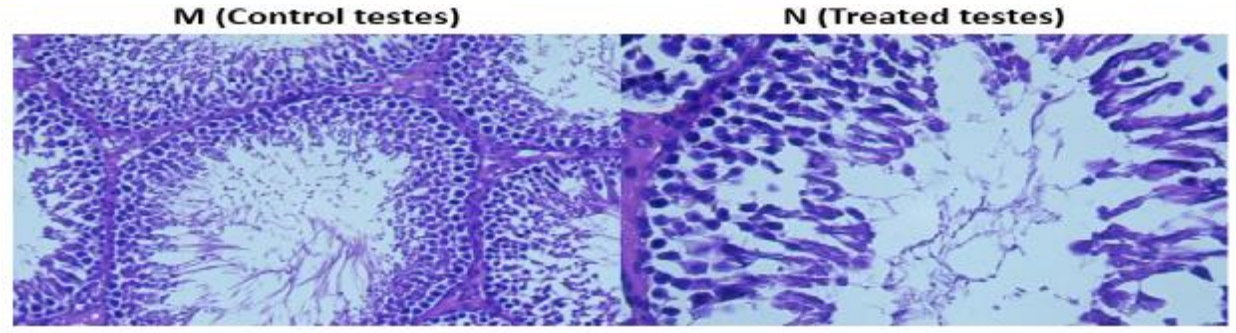
*In images G and H appear normal splenic tissue without notable pathology.



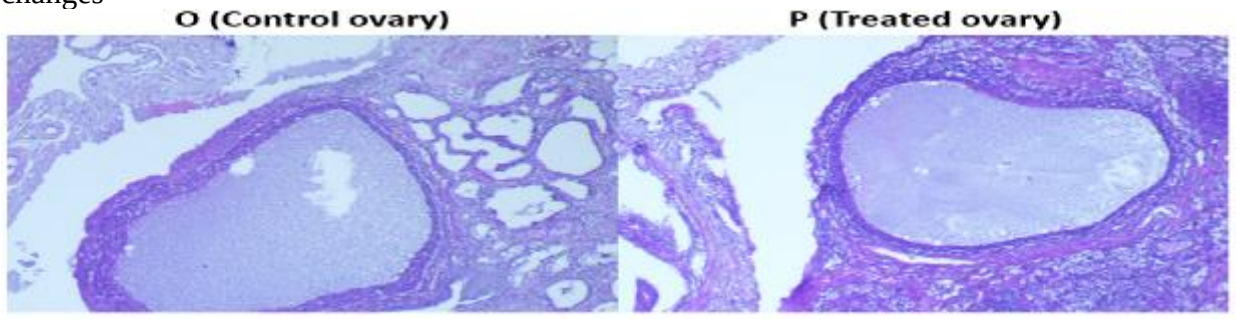
*In images I and J, the lung appeared with normal alveolar walls and there are no pathological changes.



*In images K and L, normal looking stomach tissue without notable pathology.



*In images, M and N demonste normal looking spermatogenesis and there is no distinguished changes



*In images O and P, the ovaries looking normal, the maturation of vesicular follicles was normal. There are no pathological changes in ovarian stroma.

Figure (3): histopathological images of certain organs in acute toxicity test of R. canina ethanol extract. That represent A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, and p that represents control liver, treated liver, control heart, treated heart, control kidney, treated kidney, control spleen, treated spleen, control lung, treated lung, control stomach, treated stomach, control testes, treated testes and control ovary, treated ovary respectively.

Discussion

The median lethal dose fifty of ethanol extract of *Rosa canina*

The extract yield by using ethanol solvent was 2.4g/100 gram of dried powder. The lethal dose fifty (dose that killed fifty percent of tested animals in administered group was determined (16.527 gram/ kilogram). Lethal dose 50 was considered as a parameter for determination of toxicity, identification of adverse effects and clinical signs and symptoms. In addition to identification of the safe dose [10]. LD50 may be used to identify the safe dose about 10 % of lethal dose. According to Kennedy et al, the test sample that have LD50 greater than 5000 mg/kg was considered as nontoxic dose. Therefor LD50 of 16.527 g/kg or 16527 mg /kg was nontoxic. The therapeutic dose about 1652.7 mg/kg that aquel to 10 % of LD50. However, 500 microgram was very effective when used in CAM assay. Therefore, ethanol extract of *R. canina* has wide therapeutic index (wide therapeutic range between toxic and effective dose. In previous studies, the LD50 of methanolic extract of *R. canina* leaves extract in mice that given by intraperitoneal route was 455.19±23 mg/kg. [11,12, 13]. The difference in the lethal dose 50 may be due to different solvent, extraction method and rout of administration.

Acute toxicity study of R. canina ethanol extract

Acute toxicity of *R. canina* was done by using 20 rats (male and female) divided to two groups. The first group was the controlled group that includes 5 male and 5 female. While the 2nd group includes treated group that contains 5 male and 5 female. After given single dose of 5 g/kg of ethanolic extract of *R.canina* to animals, there were no any toxicity signs or death cases during fourteen days of experiment. The weight of animal was taken at day 0, 7 and 14. The body weight is acceptable if did not exceed ± 20% depending on organization for economic cooperation and development guideline 423. [14,13] So, there is no significant change between control and treated one. P> 0.05.

Relative organ weight in acute toxicity study

At day fourteen of acute oral toxicity study, the relative organ weight of heart, liver, spleen, kidney, lung, stomach, testes and ovaries were taken. [15]. The result was there is on significant difference between controlled and treated groups. P >0.05. Ethanolic extract did not interact with regular metabolism of tested animals

Measurement the Serum biochemical tests for rats in acute toxicity study

At day fourteen of experiment, serum biochemical tests were taken. Serum glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin were done. The lower increased levels of ALT in both controlled and treated group in male rats only may be physiologically alteration. However, ALT levels in both controlled and treated female are normal. The levels of ALT and AST may be increased by raised strenuous exercise. The result was there is no significant difference between controlled and treated groups P > 0.05[16]

Histopathology examination for certain organs in the acute toxicity study

Histopathological examination was done at day fourteen of experiment on heart, liver, spleen, kidney, lung, stomach, testes and ovaries. The result sowed the ethanolic extract of *R. canina* did not change the function of liver or renal, according to histopathology examination, there is no toxic conditions appeared. Therefore, there is no significant change between controlled and treated groups. P >0.05. This study agrees with previous studies about ethanolic extract of *Rosa canina* have hepatoprotective effect against hepatotoxicity induced by CCL_4

Induced hepatic damage. [17]. Previous study about genotoxicity and sub chronic toxicity of ethanol Rose hip extract, the result observed there were no genotoxicity signs and adverse effects found in the dose more than 1000 mg /kg in rats. In addition to the absolute and relative organ weight were no significant difference. Finally hematological tests after 90 days of toxicity dose were the same as to controlled group and there is no significant alteration in biochemistry between control and treated groups. (18) The rats that were used as experimental animals are 2-3% of body weight (4-5 gram) that is mean the weight of the liver was considered as 2.51 % of body weight in rabbits. [19]. The animals that are used in laboratory must be healthy young adult. Female must be nulliparous and not pregnant. At dosing of each animal must be between 8 and 12 weeks. The weight of tested animals should be within the range ± 20 % of the average weight of each formerly dosed animals. [20]

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