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Morphological and Histological Effect of Oral Intake of Rose Water in Mouse

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Abstract: *Rose water (Maa El-Ward) is one of the major products of rose, it is produced from the petals of Rosa damascene and contains about 0.08-0.12% of essential rose oil. Rose water is used widely between Saudi women (Saudi Arabia), in cooking and taken orally due to its calming effect. This study has been initiated to highlight the morphological and histological changes on mice and its kidney, using two types of rose water (RW1 & RW2), by oral uptake. Eighty four adult female albino mice were divided into one control group (12 mice) that was administered distilled water and two experimental groups. Each experimental group was subdivided into three subgroups 12 each and received oral doses twice/day for eight consecutive weeks under laboratory conditions. The first experimental subgroups were taken RW1 at doses 41, 82 & 174 µl /body weight. The second experimental subgroups were given RW2 at doses 42, 85, & 173 µl /body weight. Every two weeks, blood samples were collected and the sera were analyzed biochemically. Renal tissue samples were also taken and histopathological changes of the kidneys were examined using optical microscope. Biochemical analysis showed significant difference in the serum levels of both creatinine and uric acid between the experimental groups and the control. Varieties of histological changes were recorded in all experimental groups in terms of the intake dose and time interval. Results showed that slight degenerative changes in renal tubules elements of all treated groups, after two weeks of receiving the dose, in comparison with control. These changes increased with time and became more obvious in RW2 than RW1. These histological changes included wide spacing of tubules and atrophy of the lining epithelium. Proximal tubules showed large numbers of shrunken dark acidophilic cells with dark small nuclei. Renal medulla tubules appeared with degenerated cells and vascular congestion. In conclusion, the observed significant increases of serum parameters and histopathological changes in the kidneys of treated mice suggested that rose water may cause nephrotoxicity in mice. So, the results of this study send a public awareness for women who oral intake of rose water as it might cause histological damage to kidney for long term use.*

Keywords: *Rosa damascene, Rose water, kidney, microscopic changes.*

I. Introduction

Rosa damascene Mill well- known ornamental plant and have been referred to as the king of flowers (Cai *et al*., 2005 and Nikbakht *et al*., 2008). The name of *R. damascena* species is based on Damascus, Syria, where it originally existed as a wild plant (Shohayeb *et al*., 2014). However, it is now cultivated in different countries around the world (Das *et al*., 2012). Taif (Saudi Arabia) is known for the production of a high quality of rose essential oil from R. damascena. Apart from the use of *R. damascena* as ornamental plants in parks, gardens, and houses, they are principally cultivated for perfume, medicine and food industry (Jabbrazadeh and Khosh-Khui, 2005 and Baydar, 2006), and it is mainly known for its perfuming effects (Widrlechner, 1981). Because of the low oil content in R. damascena and the lack of natural and synthetic substitutes, essential rose oil of this plant is one of the most expensive ones in the world markets (Baydar and Baydar, 2005).

Rosa damascene has attracted considerable attention in biochemistry and in pharmacology because of the fragrance of the flowers and the high content of its biologically active substances (Zeinali *et al*., 2009) in addition it's well known as medicinal herbal in traditional medicine (Basim and Basim, 2003). Rose flower is very effective on emotions, anxiety, panic attacks, and nervous tension. It strengthens the heart, circulation, digestion and postnatal depression (Padecky, 2011). Rose oil has uses in aromatherapy for treatment of cardiac diseases (Loghmani-Khouzani *et al*., 2007). In addition aqueous extracts of rose petals showed anticonvulsant effect and therefore could be used as an adjunctive therapy for pediatric refractory seizer (Ashrafzadeh *et al*., 2007). The most important products obtained from oil rose are rose oil, rose water, rose concrete and rose absolute (Lawrence, 1991 and Loghmani-Khouzani *et al*., 2007). In Saudi Arabia, rose water (Maa El-Ward) used widely between Saudi women and preliminary data from questionnaire of 100 women showed that, large numbers of Saudi women (covering different sections of Saudi society) are taking rose water orally. Rose water obtained from petals of R. damascena is

known for its soothing effect and also found to be beneficial in ophthalmopathy. In addition, it is mainly used in cosmetics industry in various fairness creams and for cleansing of face (Kamran *et al*., 2014). Rose water is also of high value in the food industry and some special foods are prepared using this product (Nikbakht *et al*., 2008).
Herbal medicines may be associated with some risk depending on the toxicity of ingredients, proposed dosage and appropriateness of the indications. The adverse health effects can occur as a result of misuse of botanical ingredients (Rietjens *et al*., 2008). The potential danger of an essential oil is generally relative to its level or grade of purity. Many essential oils are designed exclusively for their aroma-therapeutic quality; they generally should not be used in their undiluted form. Some can cause severe irritation, provoke an allergic reaction and, over time, prove hepatotoxic (Liu *et al*., 2010). Non-therapeutic grade essential oils are never recommended for topical or internal use. Essential oils contain a rich blend of highly functional molecules some of which are beneficial and others, which are not (Anthony *et al*., 2009). With the growing awareness of these issues, efforts to ensure safety of botanicals and botanical ingredients are also increasing. Rose water is one of the major products of rose worldwide which contains essential rose oil (0.08-0.12%), and it is widely used in cooking, drinking and can be taken orally by some women. *Rosa damascena* is traditionally used as herbal medicines. Based on traditional uses of the plant, this study aimed to investigate the morphological and histological effects of two types of rose water collected from Saudi markets on the body and kidney of the female mouse. It will evaluate the effects depending on the time and proposed dosage of rose water taken orally. As parameters of the kidney function, uric acid, creatinine, and urea concentrations in serum will be determined.

II. Material & Method

Rose Water

Two types of rose water commonly used by Saudi society were chosen for this study. Rose water type 1 (RW1) is known as virgin rose water and it is the first water collected after distillation process of rose petals. Rose water type 2 (RW2), is the refined water after distillation for several times. Rose water were manufactured in AL-Gadhi factory based on Taif city (Saudi Arabia) and purchased from the local markets.

Animals

Eighty-four albino Swiss mice (SWR) ranging from 30 to 35g weight were used. Female mice were purchased from animal house of King Fahad Research Center under the rules of the Canadian ethical approval from the local biomedical ethical committee of King Abdul-Aziz University. They were maintained at the animal house of King Abdul-Aziz University, Jeddah. All mice were kept for one week to acclimatize conditions before being subjected to any treatment. Then mice groups were placed in cages at 20 ± 2^0C and relative humidity (50±5%) with alternating light and dark cycle of 12 hours. All experimental mice were fed on a standard meal and drinking water was available throughout the day.

Experimental design

Mice were divided into three main groups: the first group (12 mice) is the control was taken distilled water. The second group was taken RW1 and subdivided into three subgroups (LRW1, MRW1, & HRW1). They administered (41, 82 & 174 µl /body weight) respectively. The third group was taken RW2 and subdivided into three subgroups (LRW2, MRW2, & HRW2). They administered (42, 85 & 143 µl /body weight) respectively. The use of equivalent rose water dose to mice was calculated according to Paget and Barnes (1964). The human dose was determined depending on questioner of a group of Saudi women (10ml twice /day/average body weight). All groups were given doses twice a day for 8 weeks by using a gastric feeding. Body weights of mice were taken at the beginning of the experiment and then recorded at the end of each week. Every two weeks, mice were observed for signs of abnormalities, then euthanized by ether (Dimmock and Kennedy, 1978), dissected and kidney specimens were collected and fixed into 10% buffered formalin for 48 hours for light microscope study (Lamberg and Rothstein, 1978). All sections were examined at magnification of 4X, 10X and 40X by light microscope (Olympus dp72 microscope digital camera) at King Abdul-Aziz University, Central lab, King Fahd Center for Research.

Collection of Blood

For kidneys function test (uric acid, creatinine, & urea concentrations), blood was collected by cardiac puncture and peri-orbital sinus every two weeks using 1ml syringe. Biochemical analysis was performed without using EDTA according to Mohan (2007).

Statistical Analysis

Data were analyzed using the statistical software package SPSS version 14. All values were expressed in mean ± SEM. Treatment effects over time were compared between control and treated groups by analysis of covariance. The results were analyzed statistically using analysis of variance one-way ANOVA to identify possible difference of body and kidney weights, and biochemical values. P values were considered statistically significant at $P \leq 0.05$.

III. Results

Effects of Rose Water on Body & Kidney Weight

Control group showed no physical changes in their appearances, while mice treated with RW1 & RW2 showed change in the color of body hair, eyes became pinker and urinary smell became less in comparison to control group. In addition to gradual increase in the breast size and nipples started after two week to the end of the experiment. The recorded body weights for control and all experimental groups were analyzed and summarized in Table (1). The statistical analysis showed that there was positive correlation between groups treated with RW1 & RW2 in terms of dose and time intervals. No significance difference in values of kidney weights between all treated mice compared to control group.

Table (1) Changes in body weight (grams) of all treated groups and control of female mice: H for high dose; M for medium dose, and L for low dose for both rose water (RW1) & (RW2).

Time	Mean ± Std. Error (g)						
	HRW1	MRW1	LRW1	HRW2	MRW2	LRW2	Control
two weeks	0.650 ± 0.429	0.767 ± 0.407	0.867 ± 0.178*	0.575 ± 0.323	1.075 ± 0.486*	0.933 ± 0.278*	-.1833±.7732
four weeks	0.811 ± 0.459	1.533 ± 0.436*	1.922 ± 0.468*	0.600 ± 0.442	2.162 ± 1.115	1.444 ± 0.601*	-.5583±1.4324
six weeks	0.933 ± 0.358*	-0.167 ±0.618	0.467 ± 0.497*	0.383 ± 0.449	0.400 ± 0.251	-0.250 ± 0.368	-1.2333±1.1810
eight weeks	1.933 ± 0.348*	-0.767 ±0.606	1.133 ± 0.088*	0.200 ± 0.404	0.667 ± 1.036	0.233 ± 0.233	-.7333±1.0786

Biochemical analysis

The results of blood analysis of urea showed that values have increased after two weeks for both RW1 and RW2 subgroups at high dose, then decreased again till the end of experiment (Fig. 1a & b). Serum levels of uric acid recorded different values for RW1 & RW2 treated group's at different doses after two weeks. For serum creatinine, the lowest values have been recorded after two weeks, while the highest values were recorded after four, six and eight weeks for all experimental groups and control.

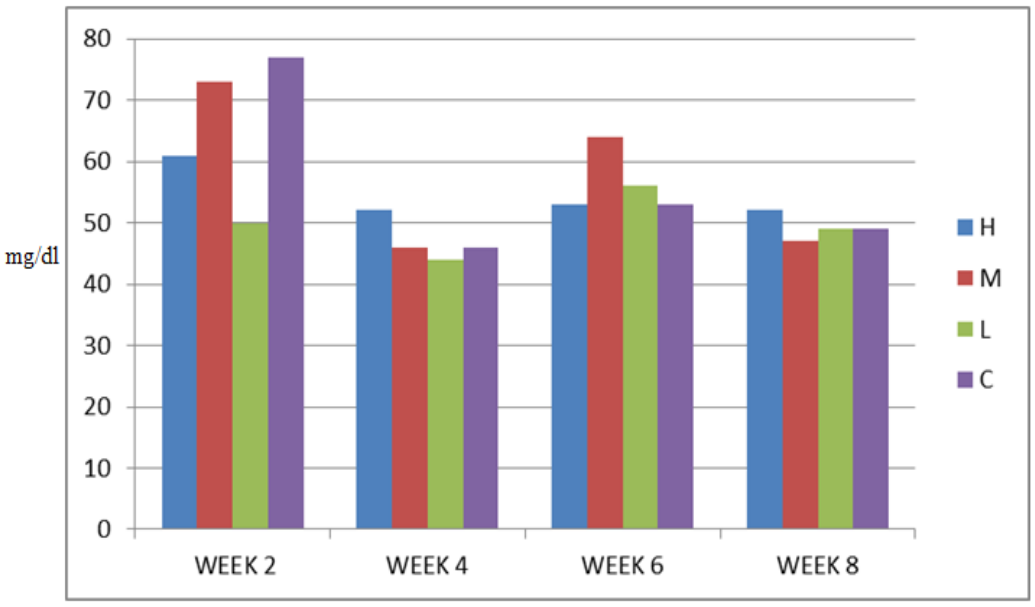

Fig. (1a) Changes In The Mean Values Of Urea With Time For Both Control (C) And Different Subgroups Treated With RW1

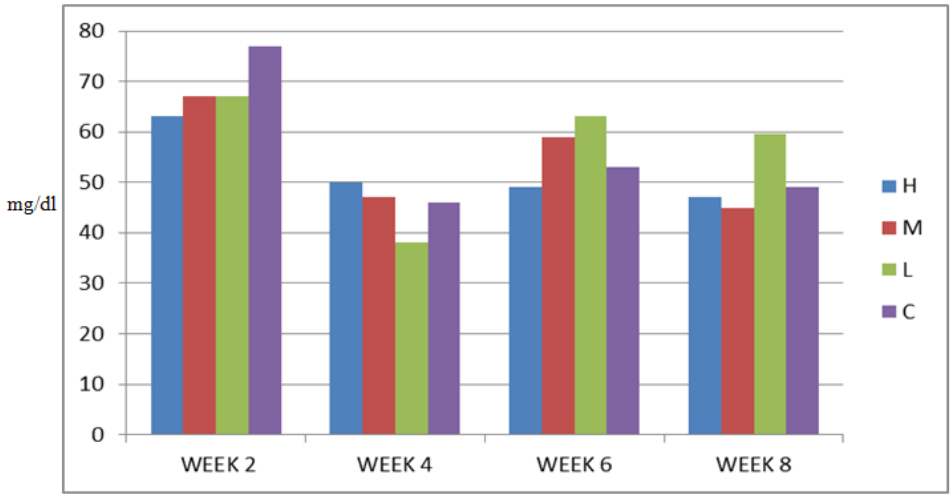

Fig. (1b) Changes in the mean values of urea with time for both control (C) and different subgroups treated with RW2

Histopathological findings

In control group, the renal corpuscles appeared with normal glomular capillaries and the renal tubules characterized by thin walled tubular capillaries as shown in Figure (2).

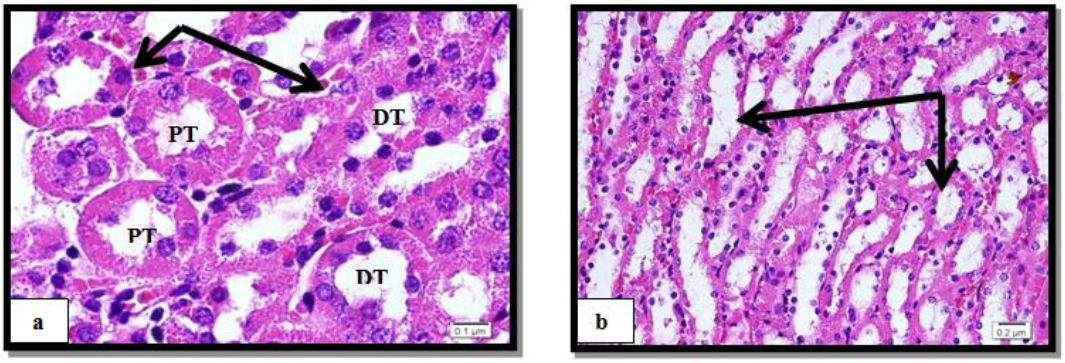

Fig. (2): Hematoxylin and Eosin stained section in mice kidney of control group: (a) Proximal tubules (PT) and distal tubules (DT). Notice the thin walled per tubular capillaries (black arrows). (b) Magnified section of (a) showing longitudinally cut collecting tubules.

The histopathological changes observed in experimental groups received RW1 and RW2 at low doses were summarized in Figure (3-6). After two weeks, at a dose of 41 µl /body weight of RW1, the examined tissues showed dilated congested vessel, renal corpuscle with degenerated glomeruli. Renal tubules are more or less normal while renal medulla showing slight dilated tubules. After four weeks, atrophy and shrinkage with lobulation of glomeruli and dark degenerated macula densa was observed. Most tubules showed dilated lumina and degenerating lining epithelium. At the end of week six, disorganization of renal corpuscles and glomeruli were clearly visible and the nuclei of most tubule cells appeared small and dark with granular cytoplasm. By week eighth, the renal corpuscle appeared with atrophy of glomerular capillaries and widen Bowman's space. Beside, inflammatory cells around blood vessels were visible.

In experimental group received a dose of 82 µl /body weight of RW1. The results of two weeks of treatment showed slight changes in renal corpuscles. Some collecting tubules showed early sloughing of lining cells. Also section of renal tubules showed marked increase in apoptotic cells. Renal medulla appeared with slight dilated tubules and slight congested vessels. After four weeks of treatment, glomeruli atrophy and some degenerated cells in tubules were appeared. Congestion in blood vessels and dilated tubules with degenerated epithelium were also seen.

Six weeks later of treatment, vascular congestion decrease in cellularity or atrophy of glomeruli with wide capsular space or urinary space had appeared. Marked aggregation of inflammatory cells in the interstitial tissue, enlarged renal corpuscle with wide capsular space, inflammatory cells and shrunken degenerated tubules were also seen. Renal medulla showed dilated tubules and vascular congestion. By week eighth, inflammatory cells around blood vessels and vascular congestion of peri tubular capillaries were obvious. Apoptosis of tubular epithelium which appear dark acidophilic cytoplasm with small dark nuclei were also

appeared.

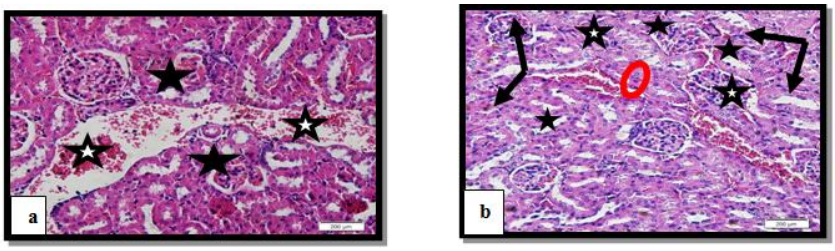

Fig.(3) H & E stained section in mice kidney after two weeks of treatment at low dose, (a) RW1 and (b) RW2: renal corpuscle with degenerated glomeruli (black stars), vascular congestion (whit stars), slight tubular dilation (black arrows), Macula densa (red circle).

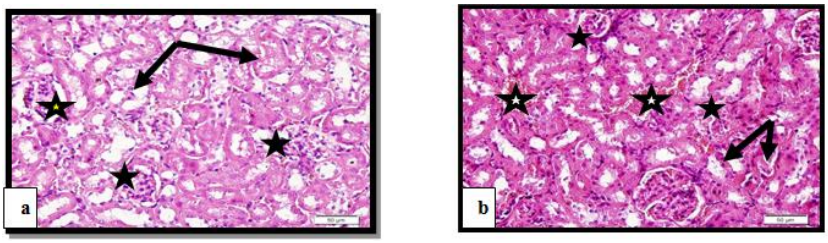

Fig.(4) H & E stained section in mice kidney after four weeks of treatment at low dose, (a) RW1 and (b) RW2: degeneration of renal glomeruli (black stars), tubules showed dilated Lumina and degenerated lining epithelium (black arrows), congestion of cortical vessel (whit

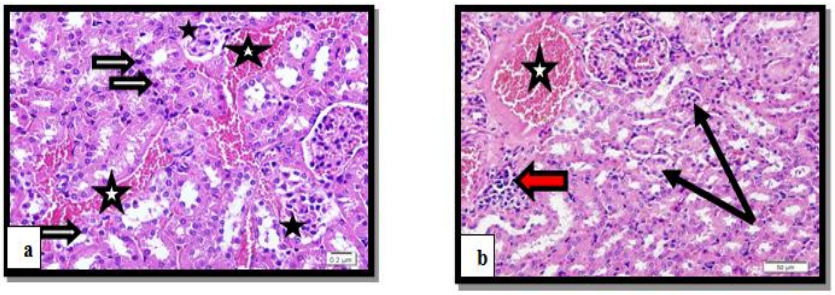

Fig.(5) H & E stained section in mice kidney after six weeks of treatment at low dose, (a) RW1 and (b) RW2: congestion of blood vessels (whit stars), disorganization of renal corpuscles (black stars). The cytoplasm became granular (whit arrows), vascular congestion (whit star),

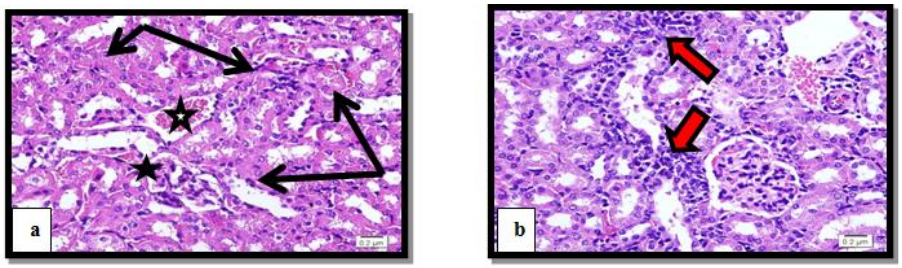

Fig.(6) H & E stained section in mice kidney after eight weeks of treatment at low dose, (a) RW1 and (b) RW2: disorganization of renal corpuscle with atrophy of glomerular capillaries (black stars), tubules showed slight deformity (black arrows), some vessels showed congestion

The result of experimental group received a dose of 174 µl /body weight of RW1 after two weeks of treatment include slight dilation in the tubules; the collecting duct appeared with desquamation of lining cells. In addition a glomular atrophy is evident, the renal corpuscles is characterized by the presence of inflammatory cells and wide Bowman's space. The proximal tubules appeared with large number of acidophilic cells. After four weeks of treatment slight shrinkage of renal glomerulus and widen capsular space, marked atrophy and shrinkage of proximal tubules that their lining cells appeared separated from the basement membrane .Apoptotic dark cells, renal medulla tubules with degenerated cells and vascular congestion were also seen. While in weeks six, results showed magnified congestion of blood vessel and Glomular capillaries. Also shrunken cells with deep acidophilic cytoplasm and dark small nuclei were seen. Blood vessel congestion, renal corpuscle with their glomeruli appeared deformed and disorganized. Some sections showed aggregation of inflammatory cells. Proximal tubules are shrunken and their lining cells showed separation from basement membrane. At the end of experiment (eight weeks) a vascular congestion of per tubular capillaries, apoptosis of tubular epithelium appeared with dark acidophilic cytoplasm and small dark nuclei. Degeneration and maculation of tubular epithelium of collecting ducts were seen. Also kidney tubules showed dark stained lining with dark small nuclei indicated apoptosis.

The result of experimental group received a dose of 42 µl /body weight of RW2 after two weeks of treatment, recorded vascular congestion, slight tubular dilation, and numerous apoptotic cells were shown. While in four weeks period, congestion of cortical vessel and glomerular atrophy were visible. Some tubules showed hyaline degeneration, while others lost their nuclei and appeared irregular and shrunken. The sixth week sections showed tubules cells with unstained cytoplasm and small dark nuclei. In addition to enlargement in kidney glomeruli and marked tubules disorganization were also seen. By week eighth, marked tubular granular degeneration with capillary congestion has been observed, beside the appearance of few inflammatory cells.The result of experimental group received a dose of 85 µl /body weight of RW2, after two weeks, of treatment showed lobulation in renal glomeruli, vascular congestion and atrophy of renal glomeruli. Renal medulla showed dilated tubules and congested vessels. While after four weeks, slight granular appearance in tubules, and some shrinking with degenerated cells were visible in. After six weeks, many tubules showed complete hyaline degeneration and by week eighth, numerous proximal tubules with granular degeneration and unstained shrunken glomerular has appeared. In addition, distal tubules showed degenerated cells and few cells appeared apoptotic.

The result of experimental group received a dose of 173 µl /body weight of RW2, after two weeks of receiving the dose, showed renal corpuscle with congestion within Bowman capsule. Macula densa are prominent. Some sections showed vascular congestion and peri vascular inflammatory cells. By week four, marked atrophy of renal glomerular was visible and the proximal tubules had granular appearance, beside many apoptotic cells were observed. At six weeks, tubular dilation and glomerulus atrophy and degeneration change of renal tubules with marked inflammatory cell infiltrates were shown. After eight weeks, marked decreasing in glomeruli cellularity with congestion and degenerated cells were seen. Moreover, there was granular degeneration and numerous scattered apoptotic cells.

IV. Discussion

Rose water (Maa El-Ward) is one of the major products that produced from the petals of *Rosa damascene* and contains essential rose oil (0.08-0.12%). Rose water is used widely between Saudi women in cooking and taken orally due to its calming effect. This study aimed to investigate the morphological and histological effects of two types of rose water on female mice body and kidney subjected to different doses of rose water by oral intake. Up to date there is no study showed the effect of rose water on mice kidney taken orally. The results of this study showed that, oral intake of rose water caused a significant increase in the whole body weight of all experimental groups in comparison to control group as shown by statistical analysis. While the values of kidney weight showed no significant difference between the control and the experimental groups

As a measure of kidney function, serum uric acid, urea and creatinine are often regarded as reliable markers (Henry *et al.,* 1982, Bonsnes and Taussky, 1982). Biochemical analysis of blood serum for uric acid, creatinine and urea recorded variable changes in their concentration from week two up to the end of week eight. Urea concentration increased by week two in both group's received high dose of two types of rose water and these increase is significantly difference in comparison to control. This might indicate to failure in kidney function as a previous study concluded that serum urea level is used as an index of kidney function (Lesley *et al.,* 2005). For creatinine levels, the results of all experimental groups recorded an increase throughout the experiment. The level of creatinine

in the blood rises if the kidney does not function properly. Uric acid has also recorded an increase in the serum of all experimental groups receiving different doses of rose water.

The histological examination of kidney thin section by light microscopy showed different histological changes based on type of rose water, dose and time intervals. In all experimental groups, it was clear that the most affected parts of the kidney were the renal tubules. The proximal and distal tubules showed degenerative changes in the form of slight shrinking and separation from basement membrane which were more evident after two and four weeks of receiving different doses of both RW1 and RW2. Apoptosis was observed in the form of dark acidophilic shrunken cells with deeply stained nuclei. After sixth and eighth weeks, in general, it was observed that some compensatory improvement was present.After two weeks, renal corpuscles with wide Bowman space and inflammatory cells were observed at low dose for RW1 subgroup, while vascular congestion, atrophy of renal glomeruli, numerous apoptosis shrunken dark acidophilic and dilation of tubules were marked for RW2 subgroup. At medium dose, RW1 subgroup showed slight decrease in glomerular cellularity, while RW2 subgroup appeared with many cells possessing granular cytoplasm and small degenerated nuclei and apoptotic cells are also numerous. At high dose, RW1 subgroup showed slightly dilated tubules of collecting duct and desquamation of lining cells while the RW2 subgroup showed vascular congestion tubules with degenerated cells, and bleeding within Bowman capsule. After four weeks, at low dose, RW1 subgroup had a marked degeneration of proximal tubule cells, while RW2 subgroup observed with some apoptosis, marked congestion, hemorrhage and slightly dilated tubules. Apoptosis on renal tubule cells were described by many authors worked on nephrotoxicity such as (Kubin *et al.,* 2012; Elias *et al.,* 2010 and Pastewski *et al.,* 2008).At medium dose, RW1 subgroup showed the tubules with dilated lamina and few desquamated cells. Moreover, a prominent macula densa and many tubular apoptotic cells were observed. RW2 subgroup appeared with many apoptotic cells. At high dose, RW1 subgroup showed a slight shrinkage of renal glomerulus and widening in capsular space. For RW1 subgroup, some marked atrophy of renal glomerular, congestion of proximal tubules and granular appearance were observed.

After six weeks, at low dose, there were a lot of inflammatory cells with dark nuclei for RW1 subgroup, while RW2 subgroup showed decreases in cellularity of renal glomeruli and capillary congestion. Some tubules cells appeared with unstained cytoplasm and small dark nuclei. At medium dose, RW1 subgroup showed renal tubules with marked disorganization that had separated from the basement membrane. While many tubules were showed complete hyaline degeneration for RW2 subgroup. At high dose, RW1 subgroup had damage in renal corpuscles and tubules, congestion of blood vessel and glomular capillaries. Some tubules showed cells with dark nucleus. While in RW2 subgroup, atrophy of glomeruli and degeneration tubular epithelial were observed. After eight weeks, at low dose, RW1 subgroup showed dilated tubules due to atrophy of lining epithelium and most tubules looked disorganized with unstained basal parts due to degeneration. For RW2 subgroup, inflammatory cells around degenerated tubules were observed. There were inflammatory cells around blood vessels for RW1 subgroup at medium dose, while RW2 subgroup showed granular degeneration, numerous scattered apoptotic cells. Beside, the renal corpuscle appeared with wide Bowman space due to atrophy of glomeruli. At high dose, RW1 subgroup showed a decrease in width of cortex, widening of medulla in addition vascular congestion of peri tubular capillaries, apoptosis of tubular epithelium which appeared dark with acidophilic cytoplasm and small dark nuclei. RW2 subgroup showed glomeruli cellularity with congestion and marked degeneration. A previous study showed that the destruction of glomeruli caused significant decrease in the glomerular filtration rate and increasing in the blood urea lead to chronic renal failure (Ramakrishnan *et al.,* 1995). In this study, low dose of RW2 was appeared to have a greater effect on kidney function and histological changes than RW1, Followed by the medium and then high dose. From this study, it was also clear that kidney damage increased as doses decreased in particular at glomerulus. This could be due to that glomerular cortex is considerably more sensitive to oxidative injuries than other nephron parts as indicated by Yi *et al.,* (2011).In conclusion, our results clearly demonstrated that both types of rose water used in this study had some histological changes on female mice kidney.

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