Evaluation of ameliorating role of avocado *Persea americana* fruit extract against monosodium glutamate-induced toxicity in pregnant female albino rats and their offspring

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Abstract

Background: Although monosodium glutamate (MSG) is commonly used as a food additive, the application of higher doses or prolonged uses significantly leads to accumulations in living cells and finally produces cellular toxicity. *Persea Americana* (avocado) has recently gained substantial popularity and is often marketed as a "superfood" because of its unique nutritional composition, antioxidant content, and biochemical profile**. Aim**: To evaluate the potential ameliorative role of avocado fruit extract against MSG-induced nephrotoxicity in pregnant rats and their offspring. Thirty-two (24 females and 8 males) albino rats were used in this study. After an acclimatization period of two weeks; the animals were mated, and the pregnant rats were randomly divided into four groups; control (G1), avocado (G2): they were supplemented with 50 mg/kg b.w. of avocado fruit extract, MSG (G3): they were given 3g / kg b.w. of MSG, every other day, and MSG &Avocado (G4): they were given an oral dose of MSG alternatively with avocado fruit extract. At the end of weaning, the female rats and their offspring were sacrificed and the blood was collected and the kidneys were excised to evaluate the renal biochemical and histopathological, and immunohistochemical investigations. **Results:** In MSG-treated mothers' rats, the renal cortical sections displayed severe histopathological lesions including little renal corpuscles, atrophied glomeruli, and relatively wide Bowman's space. However, the offspring displayed mild renal histopathological lesions compared with their mothers. The immunohistochemical results revealed strong PCNA and Bax expression in the renal tissues of MSG-exposed mother rats and their offspring if compared with the control. Furthermore, the mean percentage value of positively expressed cells for caspase-3 appeared significantly higher in the renal cells of MSG-induced mother's rats and their offspring if compared with the control. Additionally, the levels of serum antioxidants (SOD&CAT) and potassium ions appeared significantly lowered while the level of MDA, urea, and creatinine appeared significantly higher if compared with the control. Co-supplementation of avocado fruit extract to MSG-induced mothers rats and their pups successfully alleviated the histopathological, immune-histo-chemical, apoptotic as well as biochemical changes caused by MSG. Conclusion: Avocado fruit extract has a powerful ameliorative role against MSG-induced renal toxicity in mother rats and their offspring.

Introduction:

Preclinical studies have connected MSG consumption to hepatotoxicity. There have been claims that MSG usage has an impact on lymphocyte apoptosis [1]. MSG-fed rats have been proven to exhibit gonadal dysfunction. The kidneys are harmed by the overuse of MSG, as has been shown [2, 3]. In addition to humans, other animal species have also been reported to transmit flavors [4]. It has been demonstrated that a variety of types decrease when fruits are consumed. Numerous diseases are less common when fruits are consumed [5, 6]. For moms throughout the perinatal period, several of the important elements found in fruits are advised [7]. Fruits contain bioactive chemicals and are whole-food sources of magnesium [8]. All forms of food typically contain additives [9]. People were thought to be at risk from food additives [10]. The Acceptable Daily Intake (ADI) control method provides support for the evaluation of food additives [11]. The Food and Drug Administration (FDA) was established to safeguard consumers against any negative health impacts [12]. It is described as the additive's numerical value [13]. The highest concentration of an additive that is functionally beneficial in a food or food category is referred to as the maximum use dose [10]. To determine the possible harmful effects of a food additive these additives must be subjected to trials for an appropriate toxicity assessment [14].

Material and methods:

Monosodium glutamate (MSG) in the form of white crystals (99% purity) was purchased from the local market of Damanhur city, Egypt. Preparation of Avocado (*Persea Americana*) fruit Extracts. Fresh avocado pear fruits were purchased from the local market in Damanhour city, Egypt. The plant was identified as *Persea americana* (Lauraceae). Avocado fruit was extracted according to the method of Biglari et al. [15]. For this study, thirty- two albino rats (24 females and 8 males) weighing 180-200 g (8-9 weeks) were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Rats were mated in the special matting cages (1 male: 3 females) overnight. After 3-4 days and ensuring pregnancy via observation of a vaginal plug and using the vaginal smear method, pregnant females were separated from males. Mothers rats were weighed at the beginning of the experiment, at birth, and on postnatal day (PND) 21 (weaning). At the day of birth (PND0), the number of pups was recorded and weighed at birth and PNDs 21 days.

Experimental groups:

The pregnant rats were randomly divided into four groups as follows, six for each group (n=6). Group I (control): they received an oral dose of 1% carboxymethyl cellulose, every other day. Group II (Avocado): they were given a daily oral dose of Avocado fruit extract at a dose of 50 mg/kg b.w. Biglari et al. [15] dissolved in 1% carboxy-methyl cellulose (CMC), every other day from the 4th day of gestation till the end of weaning. Group III (MSG): they were given an oral dose of MSG at 3g / kg b.w. Contini et al. [16], every other day, from the 4th day of gestation till the end of weaning. Group IV (MSG &Avocado): they were given an oral dose of MSG alternatively with avocado fruit extract by the same previous doses.

Sample collection and tissue preparation: At the end of the experimental period (end of weaning = 21days postnatal), the mother's rats and their offspring were sacrificed to collect the blood and then dissected immediately to remove the kidneys. Histological techniques for Haematoxylin and Eosin stains were done according to Bancroft and Gamble [17].

Immunohistochemical staining technique: The demonstration of antigens by immunohistochemistry (IHC) is a two-step process involving first, the binding

of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary antibody may be used in IHC using manual techniques or using BioGenex Automated Staining System. BioGenex offers a variety of Super Sensitive detection systems including Link-Label and Polymer-based technologies to detect the chromogenic signal from stained tissues and cells. Immunohistochemical labeling of Proliferating cell nuclear antigen (PCNA): Five μm thick sections of kidneys from mother's rats and their offspring were cut, mounted onto positively charged slides, de-paraffinized, and rehydrated in descending grades of alcohol. Endogenous peroxidase activity was blocked by placing the sections in 1.4 % H_2O_2 in methanol for 10 min at room temp. The sections were retained at normal room temperature and processed for antigen retrieval by digestion in 0.05 % trypsin. After thorough washing in TRIS buffered saline (TBS), pH 7.6, the sections were then incubated in the following order: 10% normal horse serum (NHS, Vector Laboratories) in PBS for 1 h at room temperature, mouse monoclonal antibody against PCNA (Dako, Denmark) diluted 1:50 in PBS for 10–12 h at 48C, biotinylated horse anti-mouse IgG (Vector Laboratories) in PBS for 2 h at room temperature; ABC solution (Vector Laboratories) in PBS for 1 h at room temperature and finally immersed in DAB in 0.05M Tris-HCl buffer (TB, pH 7.4) for 1 min. The slides were washed in PBS, counter-stained with Mayer's hematoxylin then investigated under a bright field light microscope and photographed.

Immunohistochemical labeling of Bax: Kidney sections were fixed and sliced as aforementioned. Endogenous peroxidase was inactivated by incubating the sections in 3% H_2O_2 for 30 min at 37°C. The sections were incubated with 10% normal goat serum (catalog no. ZKP160724-1; Suzhou Zeke Biotech Co., Ltd., Suzhou, China) in 0.01 M PBS for 30 min at room temperature, and then incubated with rabbit anti-Bax antibody (catalog no. BA0315; 1:200; Wuhan Boster Biological Technology, Ltd) in PBS containing 0.3% Triton X-100 overnight at 4°C. The sections were washed three times for 5 min each with PBS and then incubated with peroxidase-conjugated goat anti-rat IgG (catalog no. ZDR-5118, 1:200; Zymed; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 1 h at room temperature. Finally, the sections were developed with DAB in 0.1 M TBS containing 0.001% H_2O_2 for 30 min at room temperature. The slides were washed in PBS, counter-stained with Mayer's hematoxylin then investigated under a bright field light microscope and photographed.

Flow cytometry detection of caspase-3 activity in renal tissues: Flow cytometric detection of caspase-3 was done to check the number of apoptotic cells in the renal tissues of the different studied groups of mother rats and their offspring. This technique is applicable where the fluorochrome is directly linked to the primary antibody (PE and FITC conjugate). The cells were prepared appropriately. The cell suspension was adjusted to a concentration of 1×10^6 cells/ml with PBS/BSA buffer (phosphate-buffered saline and 1% BSA). An aliquot of 100μ L of cell suspension was put into test tubes as required. The antibody (FITC rabbit anti-active caspase-3, solid as, material No.559341, catalog No. 554714, from BD Pharmingen) was added at the recommended dilution (10μ L for each sample), mixed well, and incubated at room for 30 min. After that, the cells were washed with 2 ml of PBS/BSA then centrifuged at 1500 rpm for 5 min, and discard the resulting supernatant. The cells were re-suspended in 0.2 ml of PBS/BSA or with 0.2 ml of 0.5% Paraformaldehyde in PBS/BSA if required. The data were acquired by flow cytometry. This analysis was performed in the Mansoura University Hospital, Egypt, using FACS (flow activated cell sorter) Calibur Flow Cytometer (Becton Dickin-son, Sunnyvale, CA,

USA) equipped with a compact air-cooled low power 15 mW Argon ion laser beam (488 nm).

Serum urea content was measured according to Adekomi [18]. Creatinine Analyzer-2 (Beckman Coulter Inc., USA) in combination with a specific kit of reagents (Hichem Creatine Pak, Elan Diagnostics, USA) was applied to estimate serum creatinine content in blood samples [18]. Catalase (CAT) and superoxide dismutase (SOD) were assayed according to previous studies [19]. The determination of the Superoxide Dismutase (SOD) activity was done according to Bahrami et al. [20]. Malondialdehyde levels in samples were measured according to Placer et al. [21]. Serum magnesium was assayed by a spectrophotometer system (Beckman Instruments, CA) utilizing kits from BioMeriecux- France. The serum sodium and potassium concentrations were assayed by Beckman`s (659500) system E2A Analyzer, Ireland utilizing E2A sodium/potassium reagent kit-Ireland. Serum phosphorus and calcium levels were measured using a standard colorimetric method (Roche Diagnostics, Alameda, Calif). Serum phosphorus had an intra-assay coefficient of variation of 5.6%, and serum calcium had an intra-assay coefficient of variation of 2.5%. Statistical analysis of the data: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using mean, standard deviation, median and. The significance of the obtained results was judged at the 5% level. The used tests were: F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and the Post Hoc test (Tukey) for pairwise comparisons.

Results:

1. *Histopathological observations*

In the control as well as in the Avocado extract supplement mothers' rats and their offspring; the renal cortex revealed the normal histological structure of renal tubules and renal corpuscles. The renal tubules are represented by proximal tubules (PT), distal tubules (DT), and collecting tubules (CT). The PT is characterized by its narrow lumen that is lined with brush-bordered epithelium. The DT has a relatively wide lumen that is surrounded by cubical epithelium which lacks a brush border. The CT has a relatively wider lumen than the PT and DT and is lined with short cubical epithelium. The renal corpuscle consists of a glomerulus (tufts of blood capillaries) that is surrounded by Bowman's space and an intact Bowman's capsule that is lined with simple squamous epithelium (figures 1&2A-B1)

In MSG-supplemented mothers' rats, the renal cortical sections displayed little renal corpuscles, shrinkage or atrophied glomeruli and relatively wide Bowman's space, severe degeneration in their epithelial lining, and remarkable wide lumen and scattered hemorrhage spots among the cortical tubules (figure1C&C1). Post-treatment with avocado extract to MSG-induced mother's rats, the renal cortical sections restored to the normal histological architecture as control with little dilated cortical tubules still found in the sections (figure 1 D&D1).

In maternally induced MSG offspring, the renal cortical sections displayed mild histopathological lesions compared with their mothers. Such lesions included only mild atrophied glomeruli and wide inter-tubular spaces. However, the cortical tubules appeared intact without any hemorrhage spots among them (Figure 2C&C1). Supplementation of offspring with avocado fruit extract after exposure to MSG successfully restored the histological architecture of the renal cortex to the normal (Figures 2D&D1).

2. *Immunohistochemical observations*
Immunohistochemical expression of PCNA:
The renal cortical sections from control and avocado

extract-supplemented groups of mother rats and their offspring displayed weak to moderate PCNA expression. However, a strong positive expression of PCNA appeared in the cortical sections of MSG-induced mothers' rats. On treatment with avocado fruit extract in MSG-induced mother's rats, the immunoreactivity of PCNA appeared moderately expressed but still higher than control. Generally, the expression of PCNA appeared prominent in the cells of cortical tubules rather than the glomerulus (figures3&4).

Immunohistochemical expression of Bax: In control and Avocado fruit extract supplemented mothers' rats and their offspring, the cytoplasm of renal cortical cells appeared very weakly stained with Bax antibody. However, the renal cortical sections from MSG-induced mother rats and their offspring showed strong immunoreaction for Bax in the cytoplasm of nearly all the cells. In the MSG-induced group post-supplemented with Avocado fruit extract, the renal cortical sections appeared weakly stained with Bax antibody (figures5&6).

3. Changes in caspase-3 activity in the renal tissues

The obtained flow cytometric data revealed that the mean percentage value of caspase-3 activity was significantly higher in the renal cells of MSG-induced mother's rats and their offspring (73%, 60.1%) if compared with their control (39.7%, 36.2%) respectively. On treatment with avocado fruit extract after MSG induction, the mean percentage value of caspase-3 activity in the renal cells was significantly declined (mothers' rats =52.6%, Offspring =52.1%) if compared with MSG-induced group but still significantly higher than control (figure7).

4. Changes in serum creatinine and urea

A highly significant increase ($P<0.001$) in the level of serum creatinine was recorded in MSG-treated mothers and their offspring if compared with the control. However, on treatment with avocado fruit extract to MSG-induced mother's rats and their

offspring, the level of serum creatinine was restored to the normal value as in control (table1 and Figures 8&9)

The level of serum urea is not affected among the different studied groups of mother rats. However, the level of serum urea appeared significantly higher ($P<0.001$) in maternally MSG-treated offspring if compared with the control. Supplementation of avocado extract to MSG-induced offspring successfully decreased ($P<0.001$) the elevated level of urea caused by MSG but this level was still significantly higher ($P<0.001$) than the control (table1 and Figures 10&11).

5. Changes in serum antioxidants (CAT, SOD, and GPx) and MDA

In MSG-induced mothers' rats and their offspring, the serum levels of CAT and SOD enzymes appeared significantly lowered ($P <0.001$) however the levels of serum GPx appeared significantly higher ($P <0.001$) if compared with the control. Post-treatment with avocado fruit extract to MSG treated group, the serum levels of CAT and SOD appeared significantly higher if compared with the MSG-induced group but their levels still showed significant change if compared with control except for the level of SOD, which showed non-significant change with control mother. Correspondingly to the control, the levels of MDA appeared significantly higher ($P <0.001$) in MSG-induced mothers' rats and their offspring, however on treatment with avocado fruit extract, this level appeared significantly decreased ($P <0.001$) if compared with MSG-induced group but still significantly higher ($P <0.001$) than control (Table 2 and figures 12-17).

6. Changes in serum electrolytes:

The levels of serum ions appeared unchanged, in avocado supplemented group if compared with the control. In MSG-induced mothers' rats and their offspring, the levels of serum sodium ions appeared significantly higher ($P <0.001$) while the level of potassium ions appeared significantly lowered

(P<0.001) while the levels of phosphorus, calcium, and magnesium appeared unchanged if compared with control. Post-supplementation of avocado fruit to MSG-induced rats the levels of serum sodium and potassium showed a highly significant decrease and increase respectively if compared with MSG treated group but the level of sodium was still significantly higher in mothers and the level of serum potassium significantly lower in the offspring if compared with control (Table 3 &figures 18-25).
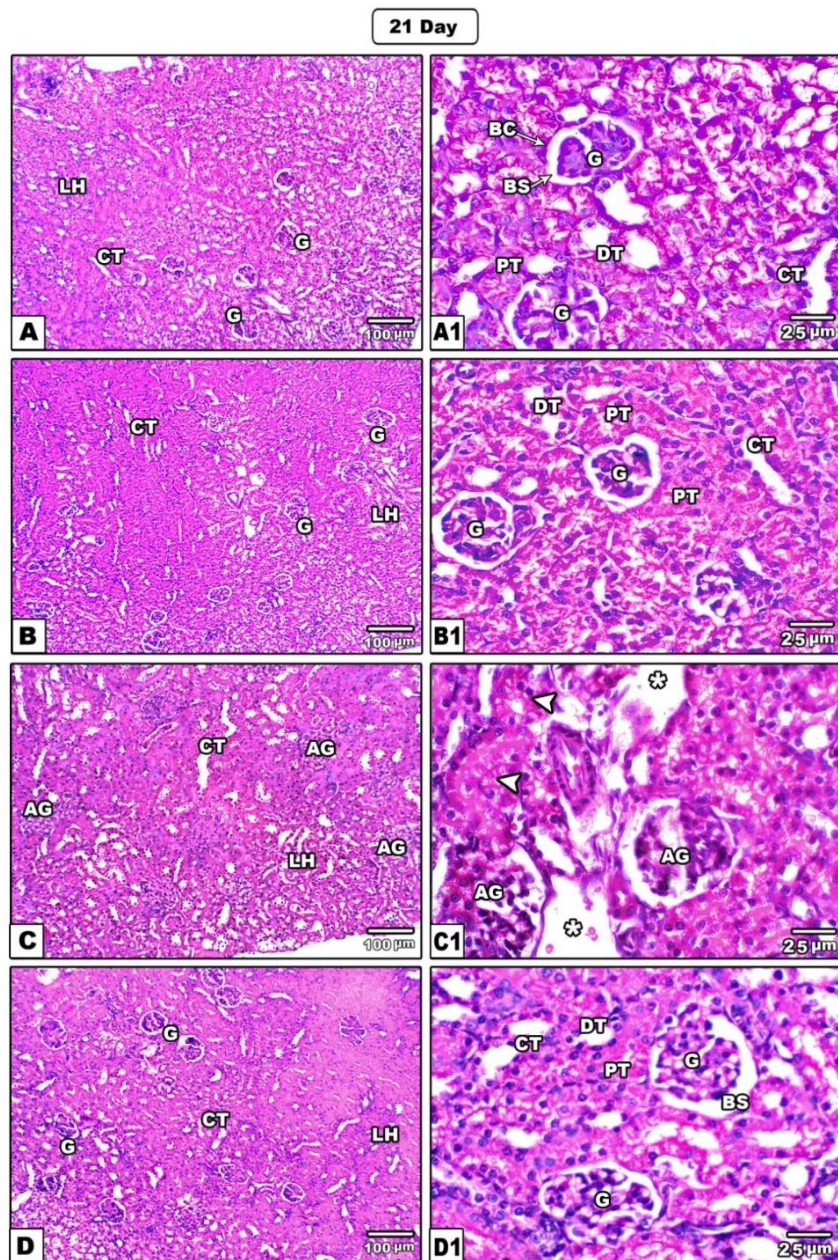

Fig.1 Photomicrograph of histological sections through the renal cortex of the different studied groups of mother's rats. (A and A1) control, (B and B1) Avocado, (C and C1) MSG, (D and D1) MSG &Avocado. Notes: Image A-B shows a normal histological architecture of the renal cortex. Images C&C1 show atrophied glomeruli (AG), dilated Bowman's space (curved arrow), and dilated tubules (star) with pronounced thickening of their epithelial lining (arrowhead). Multiple hemorrhage spots (H) appear among the renal cortical tubules. Images D&D1 show remarkable amelioration in the histological structure of the renal cortex. Abbreviations: BC: Bowman's capsule, BS: Bowman's space, G: glomerulus, AG: atrophied glomerulus, CT: collecting tubule, PT: proximal tubule, DT: distal tubule, LH: a loop of Henle, RC: renal capsule
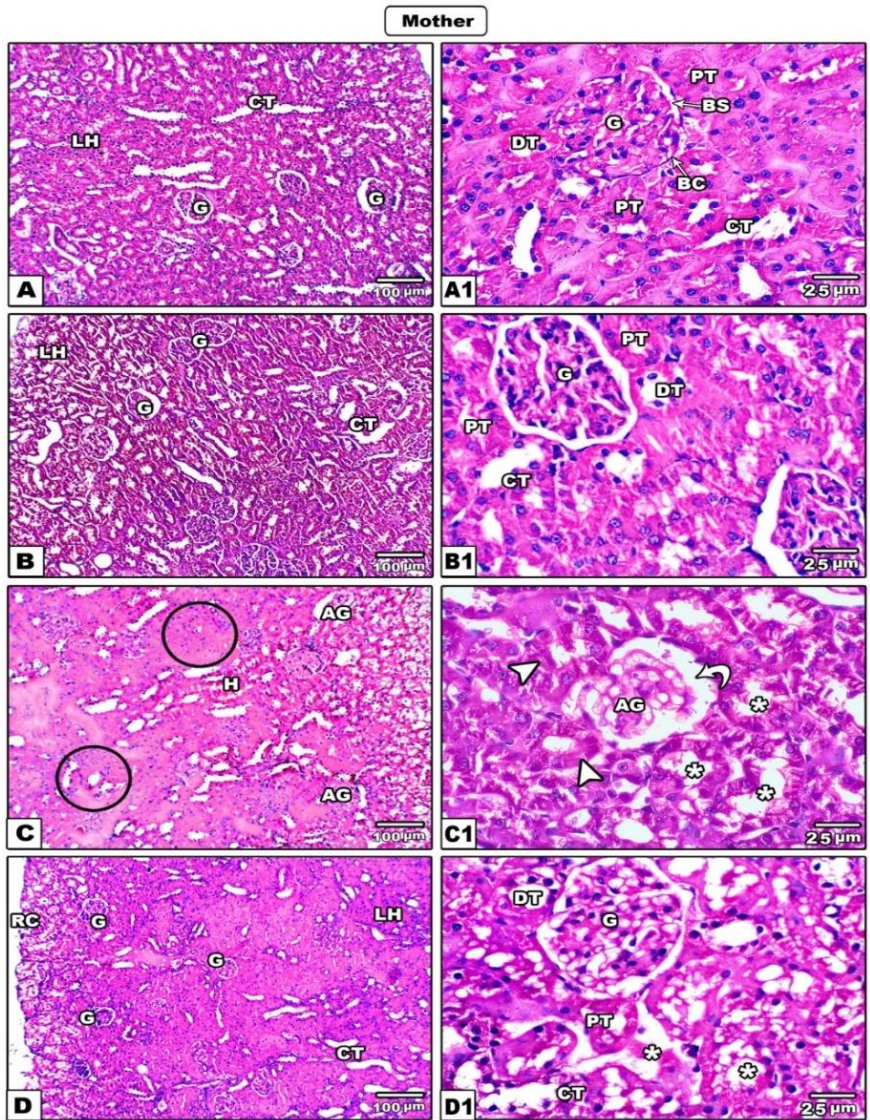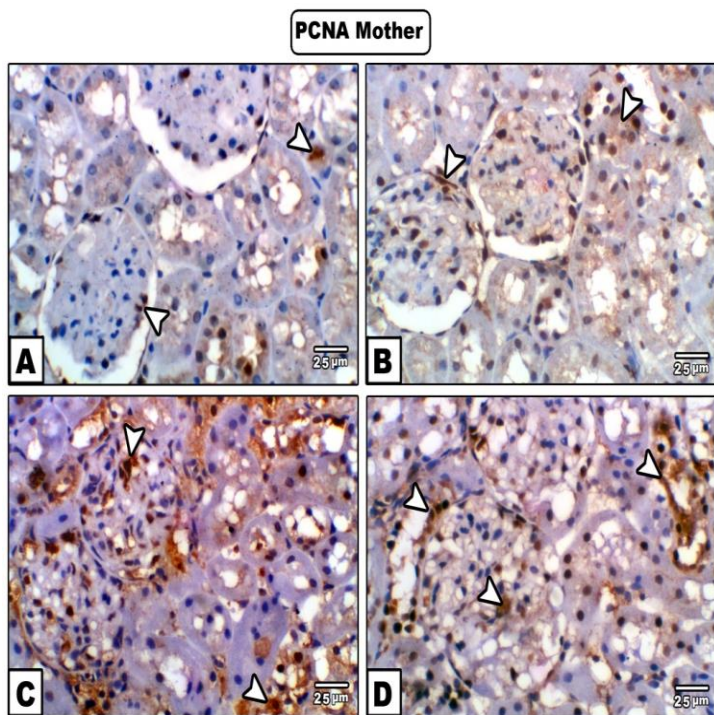
Fig. 2 . Photomicrograph of histological sections through the renal cortex of the different studied groups of 21 days old rats. Images A & A1: control, B & B1: Avocado, C & C1: MSG, and D & D1: MSG &Avocado. Notes: images A-B show the normal histological architecture of the renal cortex. Images C&C1 show atrophied glomeruli (AG) and dilated tubules (star) with pronounced thickening degeneration of their epithelial lining (arrowhead). In mages D&D1, the renal cortical sections appeared with normal architecture to be more or less similar to the control. Abbreviations: BC: Bowman's capsule, BS: Bowman's space, G: glomerulus, AG: atrophied glomerulus, CT: collecting tubule, PT: proximal tubule, DT: distal tubule, LH: the loop of Henle, RC: renal capsule
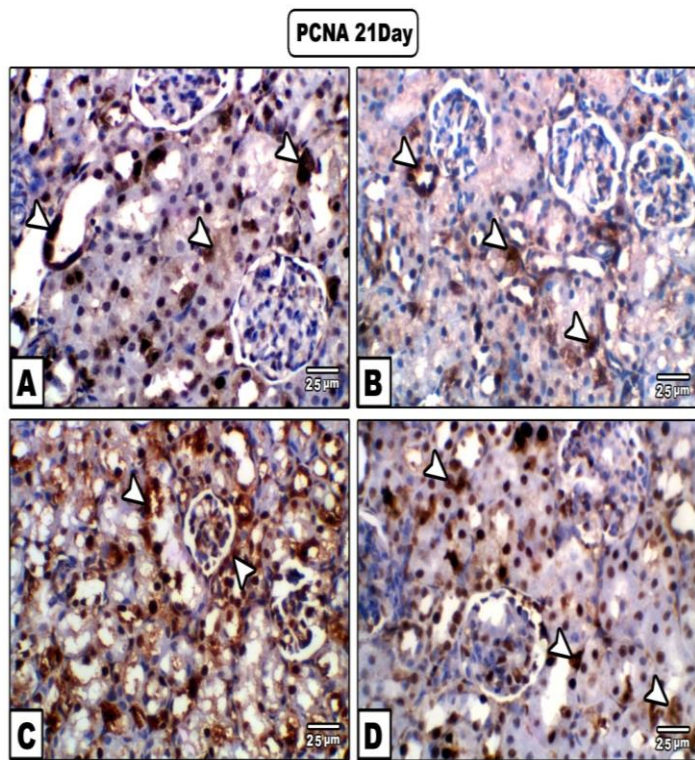
Fig.3. A photomicrograph of paraffin-embedded sections through the renal cortex of among the different studied groups of mothers rats stained with PCNA antibody. A: control, B: Avocado, C: MSG, D: MSG &Avocado Note: In images A&B, the renal cortex displays weak to moderate immunoreaction stain for PCNA protein. In image C, the renal cortex reveals strong expression for PCNA protein. In image D, the renal cortical section displays moderate immune expression for PCNA. (PCNA antibody, X=250). *The arrowhead refers to the localization of PCNA immunoreactivity*

Fig.4. A photomicrograph of paraffin-embedded sections through the renal cortex of among the different studied groups of 21 days old rats stained with PCNA antibody. A: control, B: Avocado, C: MSG, D: MSG &Avocado. In images A&B, the renal cortical tubule cells display moderate immunoreaction stains for PCNA protein. In image C, the cortical tubules and glomeruli show strong expression for PCNA protein. In image D, the renal cortical section displays moderate immune expression for PCNA. (PCNA antibody, X=250). *The arrowhead refers to the localization of PCNA immunoreactivity*.
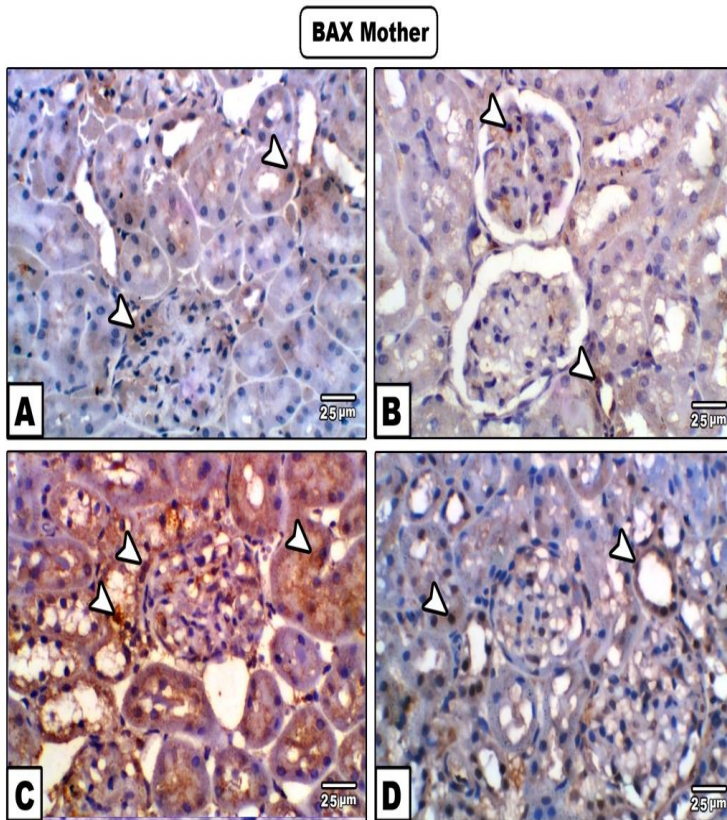
Fig.5. A photomicrograph of paraffin-embedded sections through the renal cortex of among the different studied groups of mothers rats stained with Bax antibody. A: control, B: Avocado, C: MSG, D: MSG &Avocado. In images A&B, the glomeruli and tubules display weak immunoreaction stains for Bax protein. In image C, the renal cortical tissues display strong expression for Bax protein. In image D, the renal cortical section shows weak to moderate immune expression for Bax.   (Bax antibody, X=250). *The arrowhead refers to the localization of Bax immunoreactivity.*
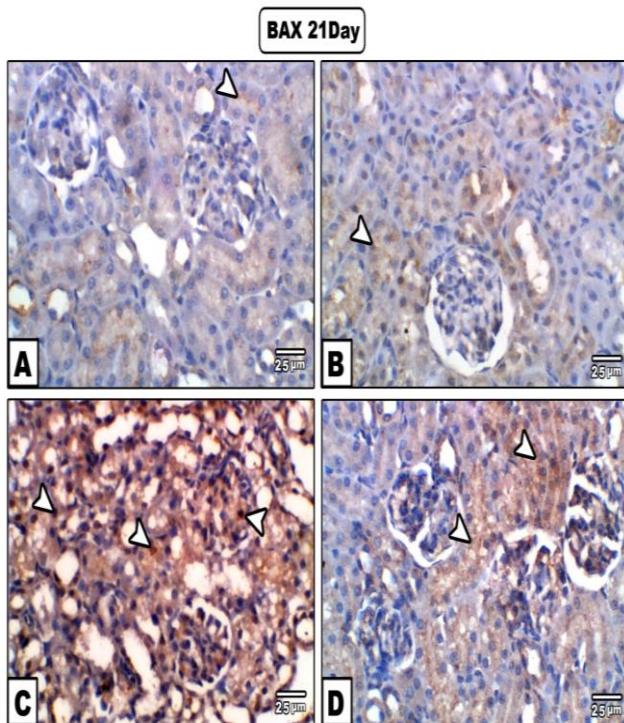

Fig.6. A photomicrograph of paraffin-embedded sections through the renal cortex of among the different studied groups of 21 days old rats stained with Bax antibody. A: control, B: Avocado, C: MSG, D: MSG &Avocado. In images A&B, the glomeruli and tubules display negative to weak immunoreaction stains for Bax protein. In image C, the renal cortical tissues display strong expression for Bax protein. In image D, the renal cortical tubules show weak to moderate immune expression for Bax. (Bax antibody, X=250). *The arrowhead refers to the localization of Bax immunoreactivity.*
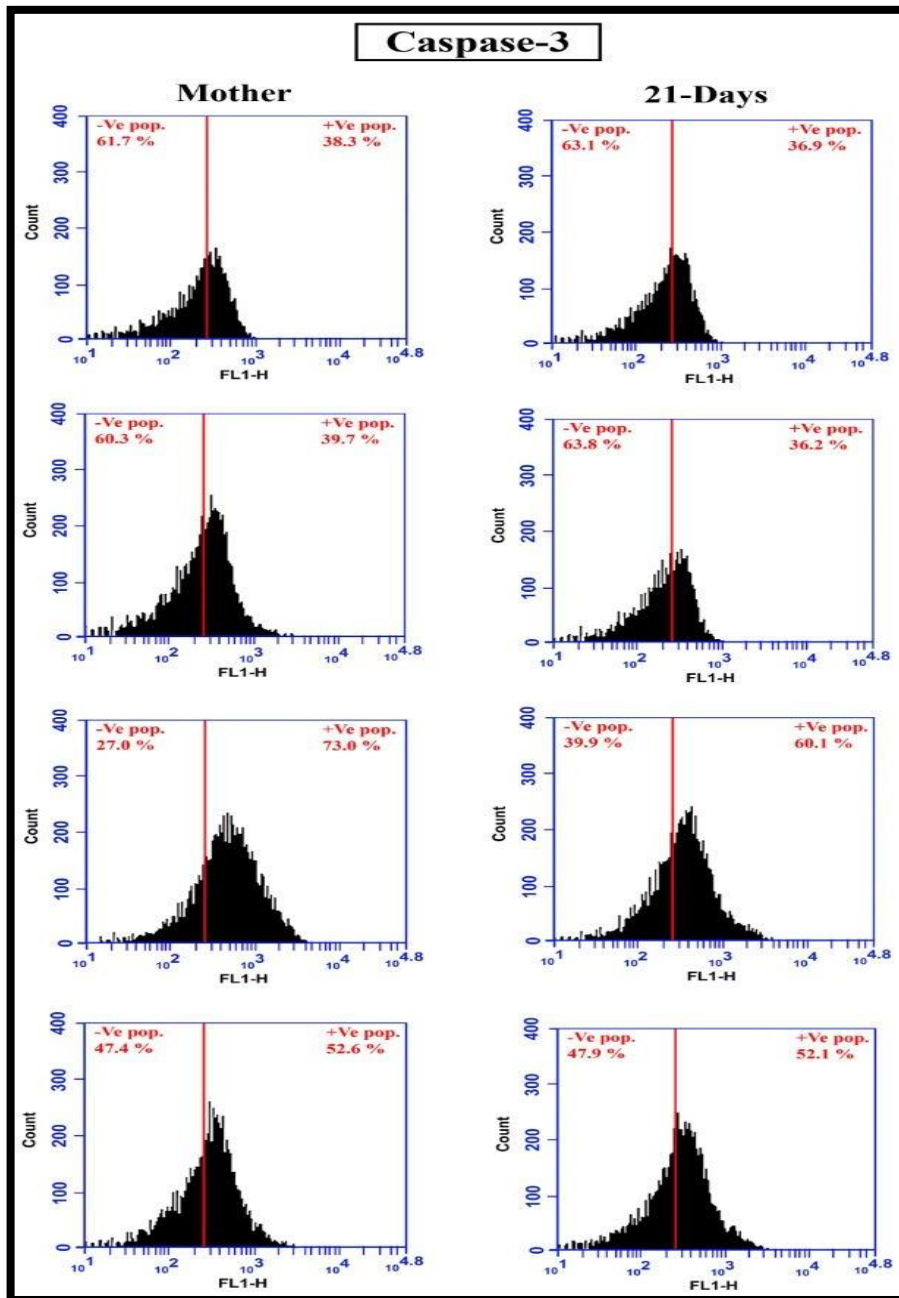
Figure 7: A flow cytometric chart showing the mean % value of caspase-3 activity in the renal tissues of mother's rats and their offspring. Note a high % value of capase3 activity in MSG-induced mothers' rats and their offspring (73%, 60.1%) if compared with their control (38.3%, 36.9%) respectively. On treatment with avocado fruit extract after MSG induction, the mean %value of caspase-3 activity in the renal cells significantly lowered (mothers' rats =52.6%, Offspring =52.1%) if compared with MSG-induced group but still significantly higher than control.
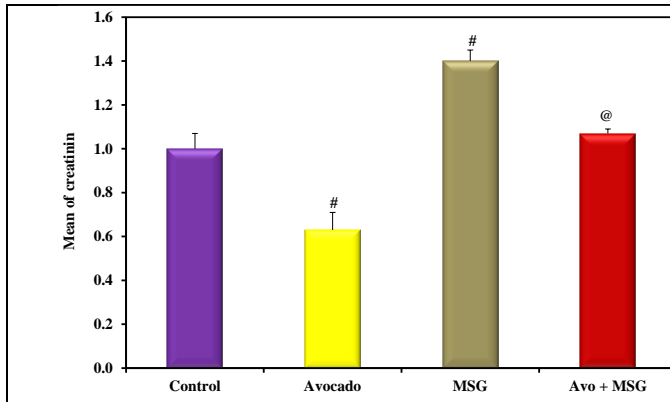
Figure (8): The levels of serum creatinine (mg/dl) among the different studied groups of mother rats.
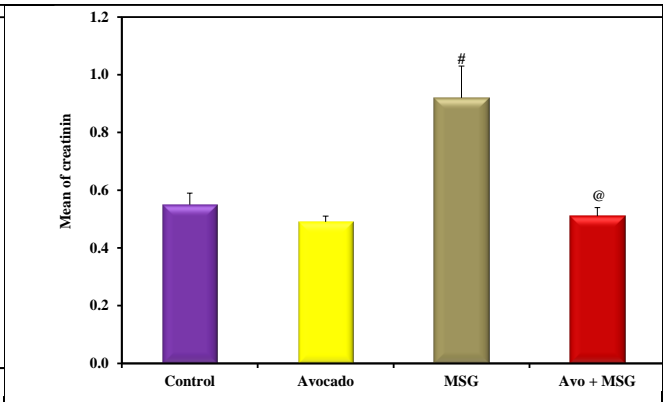

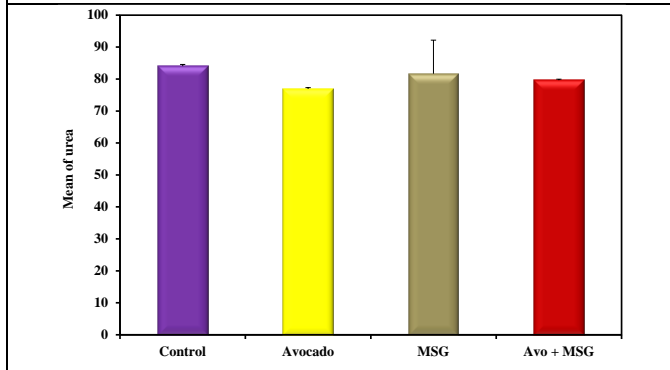gure (9): The levels of serum creatinine (mg/dl) among the different studied groups of 21 days old rats.

gure (10): The levels of serum urea (mg/dl) among the fferent studied groups of mother rats.
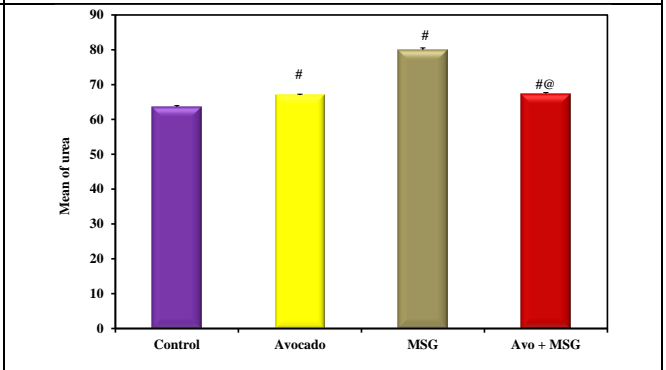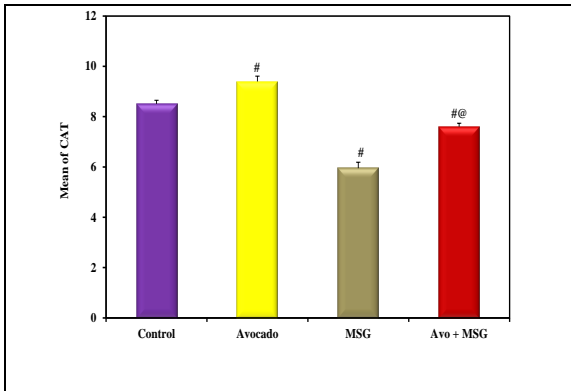

gure (11): The levels of serum urea (mg/dl) among the different studied groups of 21 days old rats.

Fig(12): The levels of serum CAT among the different studied groups of mother rats

Fig (13): The levels of serum CAT among the different studied groups of 21-day-old rats

Fig. (14): The levels of serum SOD among the different studied groups of mother rats
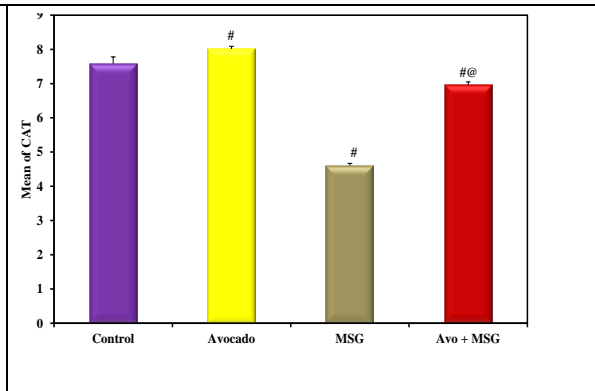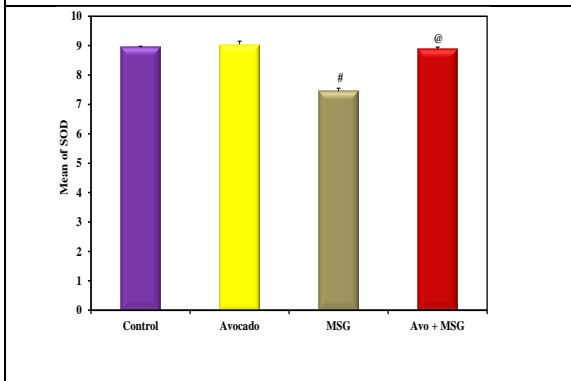

Fig (15): The levels of serum SOD among the different studied groups of 21-day-old rats

Fig. (16): The levels of serum MDA among the different studied groups of mother's rats.

Fig(17): The levels of serum MDA among the different studied groups of 21 days old rats.

Figure (18): The levels of serum sodium among the different studied groups of mother rats.

Figure (19): The levels of serum potassium among the different studied groups of mother rats.
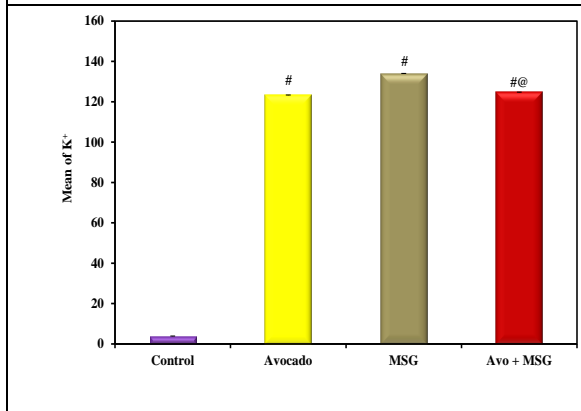
Figure (20): The levels of serum potassium among the different studied groups of 21-day-old rats.
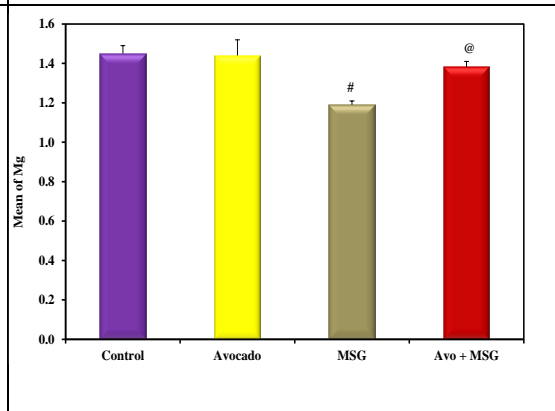
Figure (21): The levels of serum magnesium among the different studied groups of mother rats.
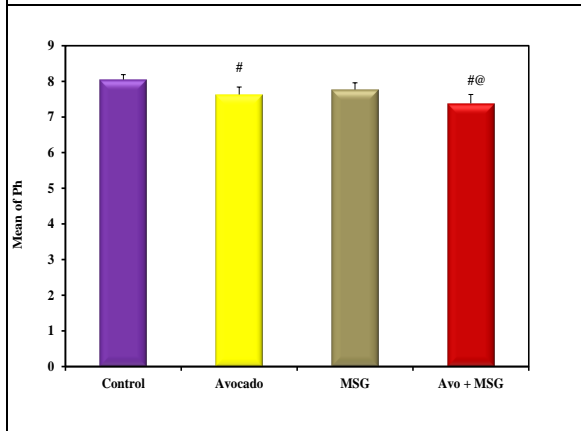
Figure (22): The levels of serum phosphorus among the different studied groups of mother's rats.
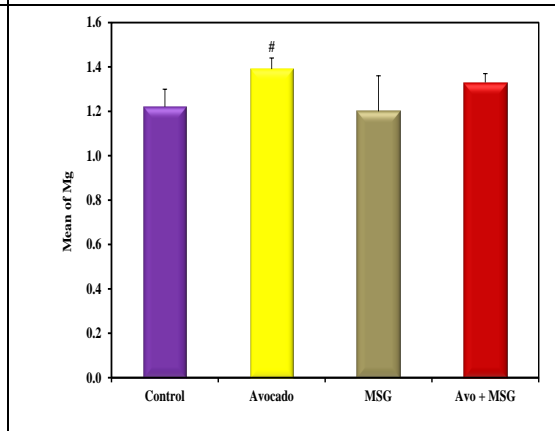
Figure (23): The levels of serum magnesium among the different studied groups of 21 day's old rats.

Figure (24): The levels of serum phosphorus among the different studied groups of21 day's old rats.

Figure (25): The levels of serum sodium among the different studied groups of 21 days old rats.

Figure (26): The levels of serum calcium among the different studied groups of mother rats.
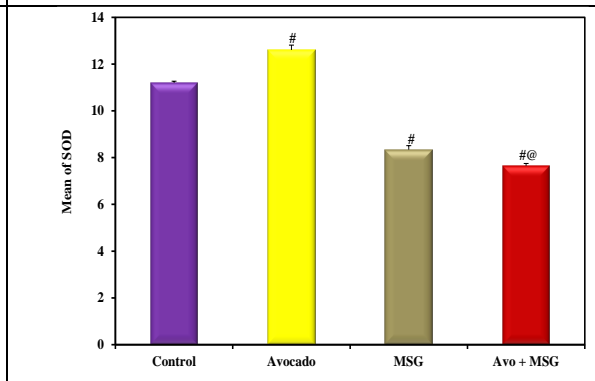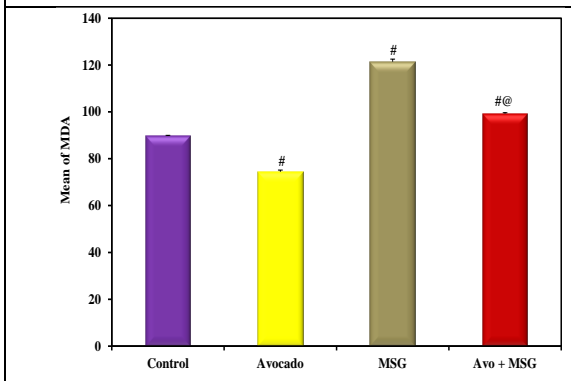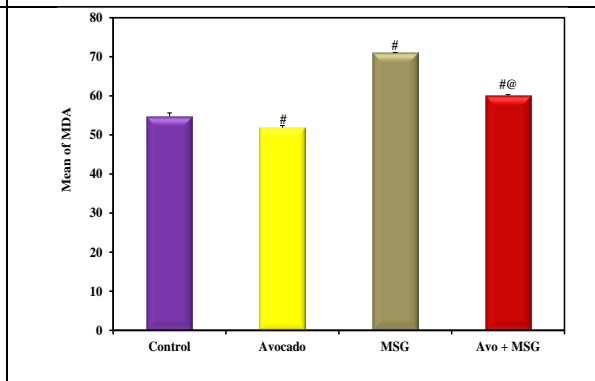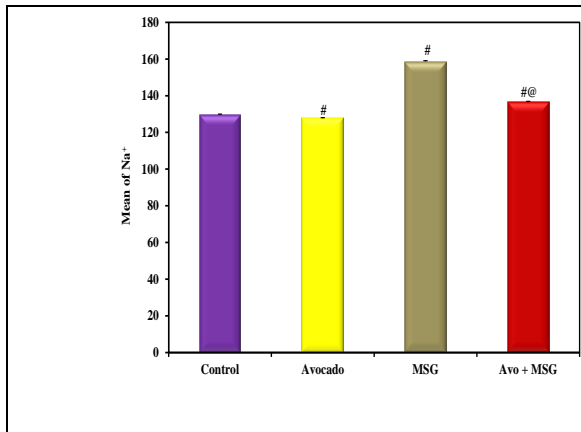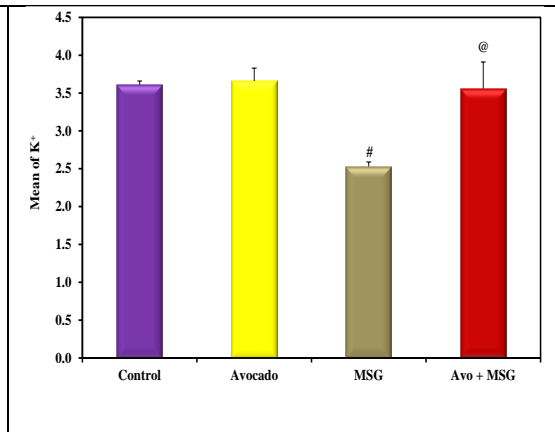

Figure (27): The levels of serum calcium among the different studied groups of21 day's old rats.
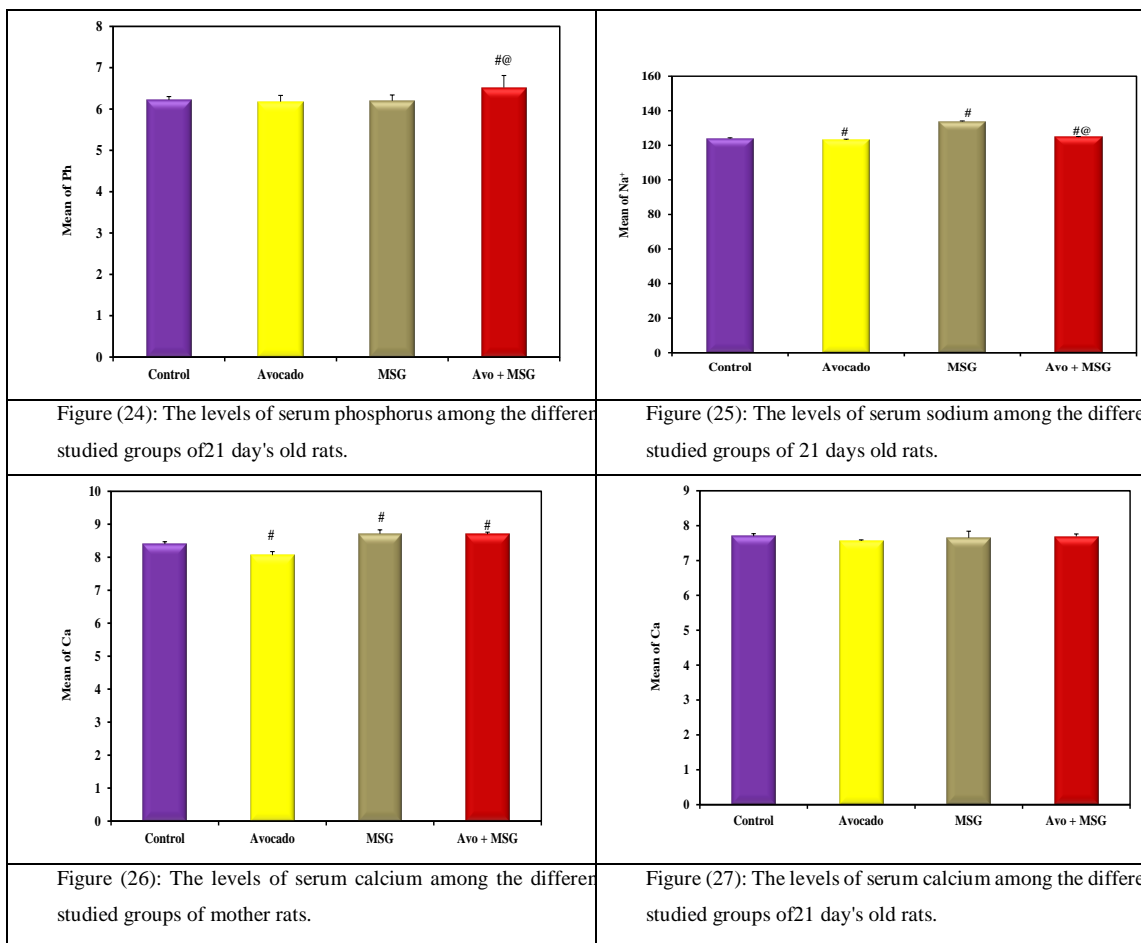
Table 1. The levels of serum creatinine and urea (mg/dl) among the different studied groups of mother's rats and their offspring

		Control (n=6)	Avocado (n=6)	MSG (n=6)	Avo + MSG (n=6)	p
Creatinine	Mother	1.0± 0.07	0.63#± 0.0	1.40#± 0.05	1.07± 0.02	<0.001*
	21 day	0.55± 0.0	0.49± 0.02	0.92#± 0.11	0.51± 0.03	<0.001*
Urea	Mother	84.02± 0.55	76.92± 0.4	81.65± 10.53	79.63± 0.33	0.153
	21 day	63.58± 0.40	67.03#± 0.22	79.89#± 0.66	67.37#± 0.36	<0.001*

F for ANOVA test, Pairwise comparison bet. every 2 groups were done using Post Hoc Test (Tukey) .p: p-value for comparing between the four studied groups. p_1: p-value for comparing between MSG and Avo + MSG.*: Statistically significant at $p \leq 0.05$. #: Significant with Control. @: Significant MSG with Avo + MSG group in graph.

Table (2). The levels of serum CAT, SOD, and MDA among the different studied groups of mother's arts and their offspring

		Control(n=6)	Avocado(n=6)	MSG(n=6)	Avo + MSG (n=6)	p
CAT (U/L)	Mother	7.58 ± 0.20	$8.01^{\#} \pm 0.08$	$4.60^{\#} \pm 0.07$	$6.95^{\#} \pm 0.10$	$<0.001^{*}$
	21 day	8.51 ± 0.14	$9.40^{\#} \pm 0.21$	$5.95^{\#} \pm 0.24$	$7.60^{\#} \pm 0.14$	$<0.001^{*}$
SOD (U/L)	Mother	8.95 ± 0.03	9.04 ± 0.11	$7.45^{\#} \pm 0.10$	8.88 ± 0.07	$<0.001^{*}$
	21 day	11.20 ± 0.07	$12.60^{\#} \pm 0.21$	$8.34^{\#} \pm 0.17$	$7.65^{\#} \pm 0.10$	$<0.001^{*}$
MDA (nmol/L)	Mother	89.81 ± 0.14	$74.42^{\#} \pm 0.74$	$121.4^{\#} \pm 1.14$	$99.33^{\#} \pm 0.33$	$<0.001^{*}$
	21 day	54.73 ± 0.89	$51.82^{\#} \pm 0.58$	$70.94^{\#} \pm 0.16$	$60.03^{\#} \pm 0.31$	$<0.001^{*}$

Data were expressed in mean ± SD. F: F for ANOVA test, Pairwise comparison bet. Every 2 groups were done using Post Hoc Test (Tukey). p: p-value for comparing between the four studied groups p_1: p-value for comparing between MSG and Avo + MSG. *: Statistically significant at $p \leq 0.05$. #: Significant with Control. @: Significant MSG with Avo + MSG group in graph.

Table (3). The mean levels of serum electrolytes among the different studied groups of mother's rats and their offspring

		Control (n=6)	Avocado (n=6)	MSG (n=6)	Avo + MSG (n=6)	p
Na$^+$ (Meq/ l)	Mother	129.7 ± 0.33	127.7 ± 0.29	$158.7^{\#} \pm 0.41$	$136.6^{\#} \pm 0.32$	$<0.001^{*}$
	21 day	124.0 ± 0.36	123.3 ± 0.27	$133.9^{\#} \pm 0.24$	124.7 ± 0.44	$<0.001^{*}$
K$^+$ (Meq/l)	Mother	3.61 ± 0.05	3.66 ± 0.17	$2.53^{\#} \pm 0.06$	3.56 ± 0.35	$<0.001^{*}$
	21 day	3.86 ± 0.09	3.67 ± 0.03	$2.22^{\#} \pm 0.12$	$3.29^{\#} \pm 0.04$	$<0.001^{*}$
Mg (mg/dl)	Mother	1.45 ± 0.04	1.44 ± 0.08	$1.19^{\#} \pm 0.02$	1.38 ± 0.03	$<0.001^{*}$
	21 day	1.22 ± 0.08	$1.39^{\#} \pm 0.05$	1.20 ± 0.16	1.3 ± 0.04	0.091
Ph (mg/dl)	Mother	8.05 ± 0.14	7.63 ± 0.21	7.77 ± 0.19	7.3 ± 0.25	0.016^{*}
	21 day	6.22 ± 0.08	6.18 ± 0.15	6.20 ± 0.14	6.52 ± 0.29	0.032^{*}
Ca (mg/dl)	Mother	8.40 ± 0.07	$8.06^{\#} \pm 0.11$	8.71 ± 0.12	8.7 ± 0.06	$<0.001^{*}$
	21 day	7.70 ± 0.07	7.57 ± 0.02	7.65 ± 0.19	7.69 ± 0.07	0.939

Data were expressed in mean ± SD.F: F for the ANOVA test, Pairwise comparison bet. every 2 groups were done using Post Hoc Test (Tukey).p: p-value for comparing between the four studied groups. p_1: p-value for comparing between MSG and Avo + MSG.*: Statistically significant at $p \leq 0.05$.#: Significant with Control. @: Significant MSG with Avo + MSG group in graph

Discussion:

Metabolites of the drugs that are excreted by the kidney may also cause cellular damage leading to kidney dysfunction [22].   Several studies in animals have shown that MSG is toxic to various organs such as the liver [23]. Other studies revealed that if female rats are exposed to MSG during the gestation or lactation period this leads to deleterious developmental changes in their offspring [24]. *Persea americana* has gained substantial popularity [25]. The current work is mainly designed to evaluate the potential therapeutic role of avocado fruit extract against MSG-induced nephrotoxicity in pregnant rats and their neonates. The results of the present work revealed that exposure of pregnant rats to MSG during the lactation period is followed by pronounced histopathological lesions in the renal cortical sections of both mother rats and their offspring.

In mother rats, these renal lesions included atrophied glomeruli. In the offspring, the histopathological lesions were represented by atrophied glomeruli.

The obtained results go parallel with the findings of previous reports on adult rats [26]. Another report revealed that MSG can induce changes in the renal cytoarchitecture, and increase glomerular hypercellularity [27]. The histopathological signs that appeared in the renal tissues of offspring maternally induced with MSG go parallel with the findings of Bhattacharya & Ghosh [28] who found dilated urinary space in mice neonates maternally induced with MSG. Previous research confirmed that exposure to an overdose of MSG is implicated in the liberation of free reactive oxygen species [29]. Furthermore, MSG supplementation by oral intake has been shown to alter lipid peroxidation byproducts [30]. Paul et al. [30] found reduced activities of SOD and CAT in the kidney after MSG administration. Supplementation of avocado fruit extract to MSG-treated pregnant rats restored the renal histopathological lesions induced by MSG. The obtained results are in agreement with the

findings of Al-Okbi et al. [31] who found a pronounced ameliorative effect of avocado against cisplatin-induced nephrotoxicity in rats.

Previous reports declared that avocado is a good source of unsaturated fatty acids [32]. Avocado is rich in other natural antioxidants as phenolic compounds [33].   The present study showed that the administration of MSG to pregnant rats is implicated in the induction of renal dysfunction as evidenced by the increased levels of serum creatinine in both mother rats and their offspring. These findings are in agreement with the studies of [34] who reported that prolonged administration of MSG can induce alteration in the glomerular. Adejuwon and Adokiye [35] added that serum creatinine level relates to glomerular function. The significant increase in creatinine content of the serum following the administration of MSG may be attributed to the compromise of the renal functional capacity [36]. Tawfik and Al-Badr [37] added that exposure to MSG can harm renal function which might be due to oxidative stress induced by MSG on the renal tissue. In MSG-administered groups and co-supplemented with avocado fruit extract revealed an improvement in renal functions. These findings go parallel with the study of Mahadeva et al. [38] who reported that avocado fruit acts as a nephroprotective agent. An avocado oil-rich diet has been shown to modify renal functions [39]. The data of the present study revealed that in MSG-exposed mother rats and their offspring the levels of serum sodium ions appeared significantly higher while the level of potassium ions appeared significantly lowered if compared with the control. The data concerned with significant elevation of serum sodium in MSG-exposed rats go parallel with the finding of Ilegbedion et al. [40] who reported that the glutamate ingredient of MSG is implicated in the elevation of sodium ions as a result of damaged renal tubular cells. In contrast to our obtained result concerned with declined potassium levels, Peterson

and Levi [41] found that MSG can induce hyperkalemia. Edible plants containing high potassium content appear to be healthier when compared with meals containing high potassium content [42]. The proliferating cell nuclear antigen (PCNA) has a central role during DNA replication, acting to recruit enzymes to the DNA replication fork. PCNA is also required for DNA repair and is involved in the processes of nucleotide excision repair (NER), long-patch base excision repair (BER), and mismatch repair (MMR) [43]. In the current work, the renal cortical sections from MSG-induced female rats and their offspring revealed higher immune expression of PCNA if compared with the control. Similar observations were reported in the liver tissues of MSG-treated rats [44]. Increased activity of PCNA indicates intensive DNA replication and enhancement of abnormal cell proliferation. The apoptosis phenomenon involves the activation of BAX and protease caspase-3. This in turn initiates a cascade of signal transduction pathways leading to altered cellular responses including cell-cycle arrest and apoptosis [45]. Caspase-3 is a pro-apoptotic marker that accelerates programmed cell death. This study showed that the administration of MSG led to significant increases in Bax and caspase-3. This was in the same line with Sarhan et al. [46] who explained that glutamate induced the Ca^{2+} influx and destruction of the internal mitochondrial membrane potential [47]. Avocado fruit extract was found to decrease the higher levels of caspase 3, and Bax caused by MSG. Roset et al. [48] revealed an increase in caspase-3 activity in the rats treated with MSG in the testicular tissues. Schelman et al. [49] indicated that glutamate receptors may be the result of apoptosis depending on the severity of stimulation induced by MSG. This is explained by Kanki et al. [50] as glutamate-induced Ca^{2+} influx and disruption of the inner transmembrane potential of the mitochondria. According to Walker and Lupien [51], the circulating of MSG was dissociated in sodium (Na^+) and L-glutamate which crosses the peritoneal cells and the bloodstream and then transformed into glutamine which causes damage to cells. Diabetic rats' kidney tissue exhibited enlarged basement membranes of the proximal and distal convoluted tubules, increased glycogen deposition, and increased mesangial cell and matrix of glomeruli. The glomeruli are reduced as a result of these modifications [52]. The diabetic rats displayed glomerular shrinkage, tubular casts, and inflammatory cellular infiltration [52]. Rats on a high-fat diet for two weeks displayed more inflammatory cells, cardiac muscle fibers in decline, and round and clogged blood arteries [53].

Conclusion: Based on our findings, avocado fruit extract has a powerful ameliorative role against MSG-induced nephrotoxicity in pregnant rats and their neonates. Such amelioration was represented by the alleviation of histopathological, biochemical, and apoptotic changes induced by MSG. As per previous reports and our obtained result, the ameliorative role of avocado fruit extract is mainly attributed to its vital antioxidant, anti-inflammatory, and anti-apoptotic constituents.

Contribution:

E H Radwan and A Elbeltagy, N Nazeh wrote the draft of the article, R Ibrahim did the practical work, Prof G Tabl revised the draft all authors read and approved the manuscript. The data are available with the corresponding author if needed. The article has the approval of Ethical approval of Damanhour University, Egypt. The article has no funds either in the practical work or the publication.
All authors read and approved the article. There is no conflict of interest.

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