The Egyptian Journal of Hospital Medicine (2009) Vol., 34: 36– 56

Effect Of Geranium (*Pelargonium Graveolens)* Leaf Extract On Hepatic And Renal Functions In Mice.

Samia M. Abd El-Wahab, Eman G. E. Helal, Abdel-Mawgoud Asran*, Abdel-Sattar M. Metwally, Helmy A. Zedan* and Mona Abdel-Hameed Ali***

Zoology Department, Faculty of Science, Al-Azhar University for Girls, *Plant Protection Research Institute, Agricultural Research Center and **Agriculture Animal Department, Faculty of Agriculture, Al-Azhar University.

Abstract

Background: *Pelargonium graveolens L'Herit* is a perennial herb used for many medicinal treatment as staunch bleeding, heal wounds and antibacterial properties. Its common English name is geranium. In this study we follow up the effect of a high dose of this plant extract on two vital organs (liver and kidney) following treated and recovery periods.

Material and methods: Animals were divided into two major groups, a control and an experimental group. Animals of the experimental group received geranium extract orally day by day in a dose of 869.4mg / kg /day for 20 days. Animals were sacrificed after treated (20 days) and recovery periods (10 and 20 days). Blood samples were collected for hematological tests and biochemical analysis. Liver and kidney specimens were obtained and fixed in 10% formol saline for histological and histochemical study.

Results: AST, ALT, GGT, LDH, urea, creatinine, total lipid and cholesterol were significantly increased after treated and recovery period of 10 days. However, glucose level, total protein, albumin and globulin were significantly decreased in animals after treated period. Histological changes in treated sections of the liver showed evidence of degeneration, necrosis and mononuclear cellular infiltration and in kidney sections, tubular degeneration and glomerular atrophy were observed. Degenerative changes in both proximal (WBCs casts) and medullar tubules (hyaline casts) were also observed at recovery periods. Masson's trichrome stained sections in liver showed increased collagen fibers around congested central veins, blood sinusoids and portal areas. While in kidney sections, the extract could not induce any change in the collagen fibers in the connective tissue. Some parameters of the previous physiological and histological changes which were observed after treatment disappeared after a recovery period of 20 days. Histochemical studies revealed a significant decrease in PAS positive material in liver after the recovery periods. However DNA content showed non significant difference in all the experimental periods.

Conclusion: The over dose of geranium extract caused liver and kidney damage. So, it is well recommended to use this plant at a suitable dose for clinical therapy and if it's used for long time, kidney and liver functions must be tested periodically.

Keywords: *Pelargonium graveolens,* Geranium, Geraniaceae, Medicinal plants, Ethanolic extracts, liver, Kidney, Mice, Histology, Physiology.

Introduction

People usually prefer to use medicinal plants instead of drugs. They use medicinal plants for long time and may use it in over dose, thinking that these plants have no harmful effect. Many plants are used for treatment of diseases in human and animals (Botha and Penrith 2008 and Maikai *et al.*, 2008). A medicinal plant is any plant used for the extraction of its pure substances either for direct medicinal use or for hemi synthesis of medicinal compounds which can be used for the therapeutic purposes or as precursors for the synthesis of useful drugs (Sofowora, 1993).

Geranium *(Pelargonium graveolens L'Herit*) is a medicinal plant in the family

Geraniaceae, a perennial herb is native to South Africa and is produced in Egypt, France, China, Algeria, Morocco and Spain. Its leaf was containing an essential oil (Williams and Harborne, 2002). Geranium essential oil was traditionally used as a skin regenerator, applicable to fungal infections of the skin, or of the digestive system. It is believed to have an anti diabetic, antiseptic, astringent, diuretic, anti-infectious properties, anti-inflammatory, fungicidal, microbial and febrifuge (Mativandlela *et al.*, 2006). In South Africa, many *Pelargonium* species are used as traditional remedies for wounds, abscesses, fever, colic, nephritis and suppression of urine, colds and sore throats, haemorrhoids, gonorrhoea, and are also used for stimulating milk-production and for anti-helminthic infections (Lis-Balchin *et al.*, 1996).

Although *Pelargoniums* have a long tradition as ornamental and medicinal plants (Lis-Balchin, 2002), limited chemical sampling of members of this genus produced mainly common organic acids, derivatives of cinnamic acid, flavonoids, tannins, some coumarins and phytosterols (Williams and Harborne, 2002).

Tannins and triterpenoids (from *Geranium thubergii*) make up a large group of the major phytochemicals, found very widely throughout the plant kingdom have been reported to have anti-carcinogenic effects (Tanaka *et al.*, 2003 and Namura *et al.*, 2005) and affect the animal positively or negatively as a result of prolong usage (Maikai *et al.*, 2008).

Pelargonium graveolense, referred to as scented-geranium, has been studied extensively with respect to its conventional horticultural traits, although only a few reports have so far focused on its biochemical, histological and physiological studies (Lee, 2002).

Considering the medicinal value of geranium (*Pelargonium graveolens)*, physiology and histopathology of the over dose of ethanolic extract of the leaves was evaluated in the liver and kidney of mice to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings. In addition, clarify the reversible or irreversible action after two periods of recovery.

Material And Methods

Plant materials and extraction: Plant extraction was carried out using the method of Freedman *et al.* (1979) with minor modification. Fresh leaves of geranium were collected at El-Dakahlia government. The harvested fresh leaves were air dried at room temperature and ground into a fine powder. The dried material (500g) was macerated in 2.5 litters of ethanol (solvent). After 72 hrs, the extract was sieved and the juice was filtered using rotary evaporator under vacuum at temperature not exceeding 50 °C until total dryness. Then, the crude extract was put into small glass bottles and stored in a refrigerator until testing.

Phytochemical screening: The ethanolic leaf extract was screened as described by Karawya and Abd El-Wahab (1975).

Animals: Albino mice (23-25g) were used for this study. They were fed with standard pellets. Food and water were made available *ad-libitum* throughout the whole experimental period.

Determination of median lethal dose (LD_{50}): Albino mice of average weight 25g divided into five groups (4 animals each) were orally administered 2500, 3000, 3500, 4000 and 4500 mg/kg body weight, ethanolic leaf extract in water. Death was monitored over a period of 24 hr. LD50 was then determined using the method of Finny (1971).

Animals and Geranium administeration: Forty mice of both sexes were divided into two major groups, a control (n=10) and an experimental group (n=30). Animals of the experimental group received geranium extract orally day by day in a dose of 869.4mg ($^1/_4$ LD_{50}) / kg /day for 20 days. The animals were weighed at the beginning and end of the experiment. They were sacrificed 24 hours after the last administration and after recovery periods of 10 and 20 days respectively. Blood samples were collected for hematological tests and biochemical analysis.

Haematological tests: Total red blood cells (RBCs) count, total white blood cells (WBCs) count and haemoglobin (Hb) content were done according to the methods of Dacie and Lewis (1991), Mitruka *et al.* (1977) and Drabkin and Austin (1932) respectively.

Biochemical analysis: Levels of serum glucose was measured according to the method described by Tietz, (1986), activities of aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957), γ- glutamayil transfers (GGT) (Persijn *et al.*, 1976), lactate dehydrogenase (LDH) (Kachmar and Moss, 1976), urea (Patton and Crouh, 1977), creatinine (Jeffe, 1886), total protein (Doumas, 1975), albumin (Webster, 1977), globulin (Doumas, 1971), total lipid (Knight *et al.*, 1972) and cholesterol (Schettler and Nussel, 1975).

Histological and histochemical preparations: The livers and kidneys were dissected, fixed in formal saline for preparation of paraffin blocks and cut 5 um in thickness. For histological examinations, sections were stained with haematoxylin and eosin (Harris, 1900) and with Masson's trichrome for collagen fibers (Kiernan, 1999). In the histochemical study, sections were stained with periodic acid-Schiff's (PAS) method to demonstrate carbohydrates (Hotchkiss, 1948) and with Fuelgen technique to demonstrate DNA (Feulgen and Rosenback, 1942).

Histochemical analysis: Computerized image analyzer system (Leica Qwin 500 image) was used to evaluate the quantitative measurement of PAS and DNA contents of the liver and kidney stained sections in Image Analyzer Unit, Pathology Department, National Research Center.

Statistical analysis: The results were expressed as mean ±standard error (SE). The significance of differences between means was measured by student's t-test (Snedecor and Cochran, 1980). The P values below 0.05 were considered significant while those above 0.05 were considered insignificant. Degree of freedom = (n1+n2)-2.

Results

Phytochemical screening of the plant reveals the presence of sterols, triterpens, tannins, carbohydrates and/or glycosides, alkaloids, saponin and flavonoids (Table 1).

No death was observed throughout the period of experiment. The animals drenched the geranium extract showed insignificant change in percentage of the body weight gain as compared to the control (Table 2).

Hematological changes:

Hemoglobin content, RBCs and WBCs count were not significantly ($P< 0.05$) different from the control (Table 3).

Biochemical results:

Data showed significant decreased ($p<0.05$) in serum glucose level in animals at 24 hr. following the last administration of extract as compared to the control (Table 4). There were significant increase ($p<0.05$) in AST, ALT and GGT activities after treated period and after 10 days of recovery period. LDH activity, levels of urea and creatinine were significantly increased ($p<0.05$) after treated period only (Table 5 & 6) in comparison to the control.

Mice treated with geranium extract exhibited significant decrease ($p<0.05$) in serum total protein, albumin and globulin concentration after treated period, while albumin/globulin ratio (A/G) showed non significant change as compared to control group (Table 7).

Table (8) demonstrated that the levels of serum total lipids and cholesterol of mice after treated period of Geranium extract were significant increase ($p<0.05$) as compared to the control.

Histological changes in liver tissue:-

The control liver sections showed that the classic hepatic lobule was composed of a central vein and masses of liver cells (hepatocytes) arranged in the form of liver cords radiating from the central vein. The hepatocytes were polygonal or rounded in shape with central and vesicular nuclei. The liver cords were separated from each other by narrow blood sinusoids lined by endothelial cells and Von Kupffer cells (Plate 1A).

Examination of liver sections of mice 24 hr. following the last adminstration showed that the liver had lost its characteristic architecture (Plate 1 B) compared with the control group (Plate 1 A). Hydropic degeneration, mononuclear cellular infiltration, Kupffer cell hyperplasia and necrosis were also observed.

At recovery period of 10 days, mononuclear cellular infiltration was abundant around the congested blood vessel (Plates 1C). Necrosis and pyknotic nuclei were seen after recovery period of 20 days (Plate 1 D).

Few collagen fibers were seen around the central vein of a hepatic lobule in the control liver (Plate 2A), as well as scanty collagen fibers appeared in the portal area and surrounding the blood sinusoids (Plate 3A).

After treated period and 10 days recovery period, there was increase in the collagen fibers around the dilated central vein, around the blood sinusoids (Plate 2 B &C) and the portal area (Plate 3 B &C). In contrast, after recovery period of 20 days, there was a few of collagen fibers around the dilated central vein (Plate 2 D) and portal area (Plate 3 D). This decrease was nearly similar when compared to the control group (Plates 2 & 3 A).

Histological changes in renal tissue:-

Haematoxylin and eosin stained sections showed the normal histological structure of the renal cortex (Plate 4A). The cortex showed Malpighian renal corpuscles, proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs). Malpighian renal corpuscles consisted of normal glomerulus (G) with thin glomerular basement membrane and Bowman's capsules (BC), normal cellularity and patent capsular space, surrounding tubules (proximal and distal), interstitium and blood vessels were normal

Kidney sections of animals which received geranium extract for 20 days and sacrificed at different intervals (treated and two recovery periods) showed degenerative lesions in most convoluted tubules. Some glomeruli showed atrophy of the glomerular tuft of capillaries and the capillaries of most glomeruli appeared more or less congested, while the Bowman's capsules rupture was obvious (Plate 4B, C&D).

Inflammation cells were abundant between the tubules in groups of treated (Plate 4 B) and recovery period of 20 days (Plate 4 D) compared to the control group (Plate 4 A).

Masson stained sections of the control group showed minimal amount of collagen fibers around the glomerular capillaries, around the Bowman's capsule and between the tubules (Plate 5 A). Sections from animals which received geranium extract, showed mild decrease in the staining affinity of collagen fibers (Plate 5, B, C & D) as compared to the control (Plate 5 A). WBCs casts in kidney tubules and tubulointerstitium infiltrating inflammatory cells were seen at 10 days recovery group (Plate 5C). Hyaline casts are shown in the lumina of some tubules with flattened of the renal tubular epithelium in the medulla at recovery group of 20 days (Plate 5 D).

Histochemical changes in liver and kidney tissue:

Glycogen content: Control liver sections stained with PAS method are shown in Plate 6 A. Sections from animals after treated period had a slight decrease in glycogen in some hepatocytes (Plate 6 B). Sections examined at recovery periods of 10 and 20 days showed marked decrease in glycogen content (Plate 6 C & D respectively) as compared to the control (Plate 6 A).

Glycogen content 24 hr. following treated period, kidney sections appeared similar to liver sections. Tissue revealed a reduced amount of cytoplasmic glycogen, with diminished density of basement membranes, and brush borders of proximal convoluted tubules (Plate 6 F). Also, the glomeruli were less positive than those of the control group. However, glomeruli of the groups at recovery periods of 10 and 20 days revealed more positive PAS reaction, increased stainability of glomerular cells and intense brush border of the PCTs (Plate 6 G & H).

Table (9) illustrated that geranium extract induced non significant change after treated period, while significant decrease ($p<0.05$) in PAS positive material (increase in grey level) of liver cells after recovery

period of 10 and 20 days compared with the control group.

Optical density measurement showed significant decrease ($p< 0.05$) in PAS positive material of the kidney group after treated period (184.02), while the values at recovery periods of 10 & 20 days showed non significant difference compared to control group (176.67) (Table 9).

Deoxyribonucleic acid (DNA): After treated period, liver sections, stained by Fuelgen method, showed low content of coarse chromatin in the nuclei of the hepatocytes as well as in the nuclei of Kupffer cells (Plate 7 B).

At recovery period of 10 days, sections had densely stained nuclei of the Kupffer cells and darkly stained nuclei of the inflammatory cells (Plate 7C) than did the control group (Plate 7 A), but more than the group at treated period (Plate 7 B). At recovery period of 20 days, liver sections showed an increase in colored DNA material (Plate7D).

Kidney sections showed dense stained DNA material which appeared in the nuclei. In some cells, this DNA material was abundant, densely stained and scattered in the nucleoplasm, while in the other cells they appeared faintly stained. After treated period, renal sections showed low content of DNA material. At recovery periods of 10 and 20 days, renal sections have decreased DNA content in tubular cells, while increased stainablity of glomerular cells were obvious (Plate 7 G &H). The nuclei of the glomerular cells showed more positive reaction at recovery groups than those of the control group (Plate 7 E).

Image analyzer automatically express the DNA content in the liver and renal sections stained with Feulgen technique and showed non significant difference at all the experimental periods in relation to control (Table 10).

Table (1): Phytochemical screening of geranium (*Pelargonium graveolens).*

Extract	Sterols and triterpens	Tannins	Carbohydrates and / or glycosides	Alkaloids	Saponin	Flavonoids
Ethanolic extract	+	+	+	+	±	+

+ = Present. ± = Slightly present.

Table (2): Effect of geranium extract on percentage of the body weight gain (%) of male and female mice.

Parameter	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
Body weight (%)change	11.02 ±2.1	7.1 ± 0.5 NS	6.7 ± 0.7 NS	5.9 ± 0.9 NS	10.3 ±1.7	6.9 ±0.5 NS	6.3 ±1.4 NS	6.12 ±1.1 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
Percentage of change is in comparison with control.

Table (3): Effect of geranium extract on red blood cells (RBCs) Count, white blood cells (WBCs) count and hemoglobin (Hb) content in male and female mice.

Parameters	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
RBCs X 10^6	4.9 ±0.1	4.55 ±0.2 NS	4.7 ±0.1 NS	5.01 ±0.2 NS	4.5 ±0.2	4.11 ±0.2 NS	4.3 ±0.2 NS	4.3 ±0.1 NS
Hb g %	10.42 ± 0.1	10.3 ±0.1 NS	10.4 ±0.1 NS	10.36 ±0.2 NS	10.34 ±0.1	9.5 ±0.4 NS	10.4 ± 0.2 NS	10.4 ± 0.1 NS
WBCs X 10^3	6.7 ± 0.3	6.1 ± 0.3 NS	6.6 ± 0.4 NS	7.1 ± 0.3 NS	6.9 ± 0.3	6.5 ± 0.3 NS	7.04 ±0.3 NS	7.6 ± 0.3 NS

Each value is the mean of 5 animals ± SE.
NS: non significant

Table (4): Effect of geranium extract on serum glucose in male and female mice.

Parameter	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
Glucose(mg/dl)	92.2 ± 1.7	80.8 ± 3.5 *	90.6 ± 1.9 NS	92.6 ± 2.03 NS	92 ±1.4	79.8 ± 3.6 *	89.8 ± 1.9 NS	92.4 ± 2.7 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
* Significant value at $P< 0.05$

Table (5): Effect of geranium extract on aspartate transaminase (AST), alanine transaminase (ALT), γ- glutamayil transfers (GGT) and lactate dehydrogenase (LDH) activities in male and female mice.

Parameters	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
AST (U/ml)	39.4 ±2.4	46.6 ±1.9 *	46.4 ±1.9 *	39.6 ±1.7 NS	40.4 ±2.01	47.8 ±1.6 *	46.8 ±1.8 *	39.4 ±0.9 NS
ALT (U/ml)	25.6 ± 1.9	36.4 ± 2.9 *	31.8 ± 1.6 *	26.4 ±1.1 NS	29.2 ± 1.7	37.8 ± 2.5 *	35.4 ±1.6 *	29.8 ± 1.1 NS
GGT (iu/L)	10.4 ± 0.8	15.06 ±1.3 *	14.32 ±1.5 *	10.76 ±1.2 NS	11.24 ±1.05	15.42 ± 1.3 *	15.48 ±1.2 *	10.43 ± 0.6 NS
LDH (U/L)	101.4 ±6.2	119.64 ± 2.4 *	103.92 ± 7.3 NS	97.6 ± 4.2 NS	96.22 ± 3.7	112.02 ± 3.2 *	98.62 ± 6.2 NS	95.1 ± 1.3 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
* significant value at $P< 0.05$

Table (6): Effect of geranium extract on serum levels urea and creatinine in male and female mice.

Parameters	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
Serum urea (mg/dl)	25.6 ±0.4	27.5 ± 0.5 *	26.72 ±0.3 NS	26.44 ±0.3 NS	26.1 ± 0.2	27.5 ± 0.5 *	26.74 ±0.4 NS	26.32 ± 0.4 NS
Serum creatinine (mg/dl)	0.63 ± 0.02	0.676 ± 0.01 *	0.65 ±0.02 NS	0.64 ±0.03 NS	0.64 ± 0.03	0.714 ± 0.02 *	0.67 ± 0.02 NS	0.65 ±0.03 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
* Significant value at P< 0.05.

Table (7): Effect of geranium extract on serum levels total protein, albumin, globulin and albumin / globulin (A/G ratio) in male and female mice.

Parameters	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
Total protein (g/dl)	7.8 ± 0.1	6.92 ± 0.3 *	7.3 ± 0.3 NS	7.7 ± 0.3 NS	7.5 ± 0.3	6.7 ± 0.2 *	7.1 ±0.4 NS	7.7 ±0.2 NS
Albumin (g/dl)	4.1 ± 0.1	3.6 ± 0.2 *	3.96 ± 0.2 NS	3.98 ± 0.2 NS	3.94 ± 0.2	3.42 ± 0.1 *	3.8 ±0.1 NS	3.94 ± 0.1 NS
Globulin (g/dl)	3.8 ± 0.2	3.34 ± 0.1 *	3.34 ±0.2 NS	3.72 ± 0.2 NS	3.6 ± 0.2	3.1 ± 0.1 *	3.26 ±0.3 NS	3.74.0. ± 2 NS
A / G ratio	1.084 ± 0.06	1.07 ± 0.1 NS	1.23 ± 0.1 NS	1.1 ± 0.1 NS	1.084 ± 0.03	1.04 ± 0.04 NS	1.2 ± 0.1 NS	1.1 ± 0.1 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
* Significant value at P< 0.05.

Table (8): Effect of geranium extract on serum levels total lipids and cholesterol in male and female mice.

Parameters	Male mice				Female mice			
	Control ♂	Treated period	Recovery periods		Control ♀	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
Total lipids (g/L)	9.06 ± 0.4	10.8 ± 0.5 *	10.2 ± 0.3 NS	8.8 ± 0.5 NS	8.81 ± 0.4	10.54 ± 0.5 *	9.94 ±0.4 NS	7.92 ±0.3 NS
Cholesterol(mg/dl)	66.8 ± 1.02	77. 6 ±3.7 *	73.4 ±3.6 NS	65.22 ± NS	62.6 ± 1.2	75.4 ± 4.1 *	68.8 ± 3.2 NS	60.4± 2.6 NS

Each value is the mean of 5 animals ± SE.
NS: non significant
* Significant value at P< 0.05.

Plate 1

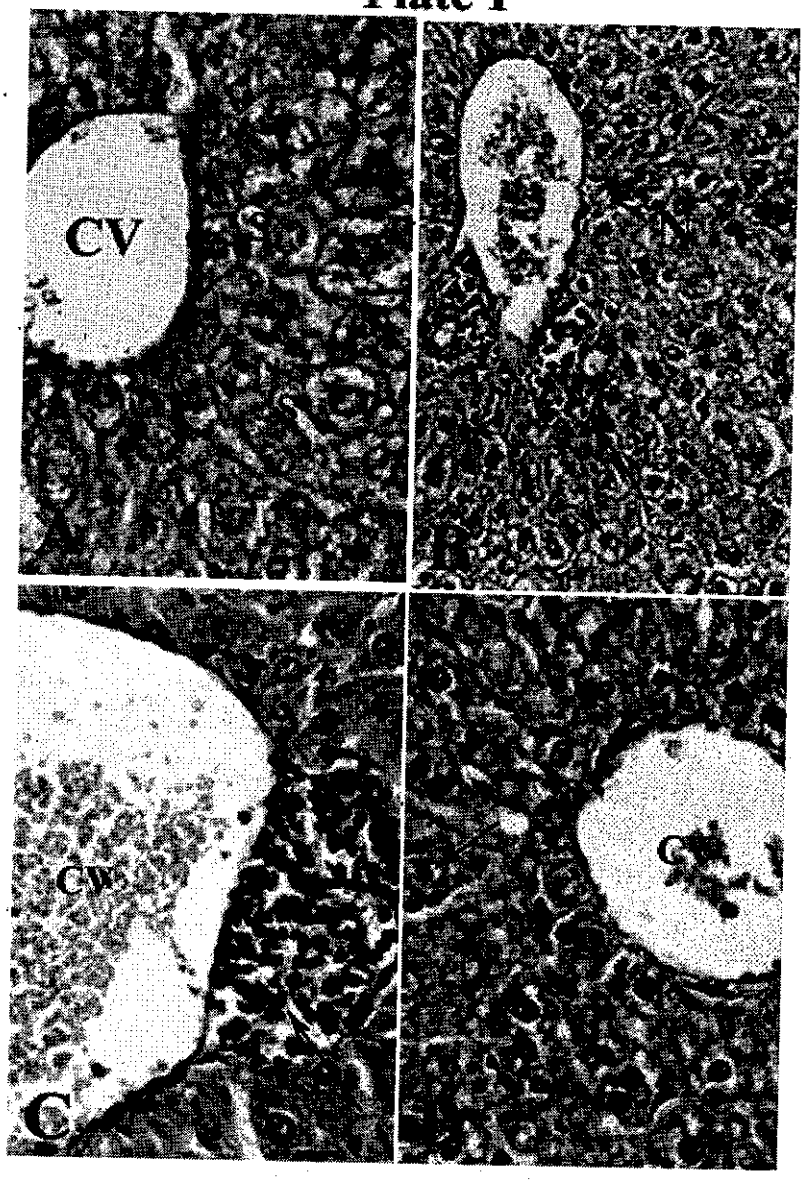

Plate (1): Photomicrograph of sections in livers of mice. (H & E X400).

A: Control, showing a central vein (CV), radiating cords of liver cells separated by blood sinusoids (S) and Kupffer cells (arrow). The liver cells show vesicular nuclei and granular cytoplasm. B: After treated period of geranium extract, displaying hydropic degeneration (arrow head) and necrosis (N) in some hepatic regions, cellular infiltration (arrow) at the periphery of the congested blood vessel and Kupffer cell hyperplasia. C: At a recovery period of 10 days, demonstrating cellular infiltration (arrow) at the peripherary of dilated central vein (CV) and karyorrhetic nuclei. D: At a recovery period of 20 days, revealed necrosis (N) in some hepatic regions and pyknotic nuclei (arrow).

Plate 2

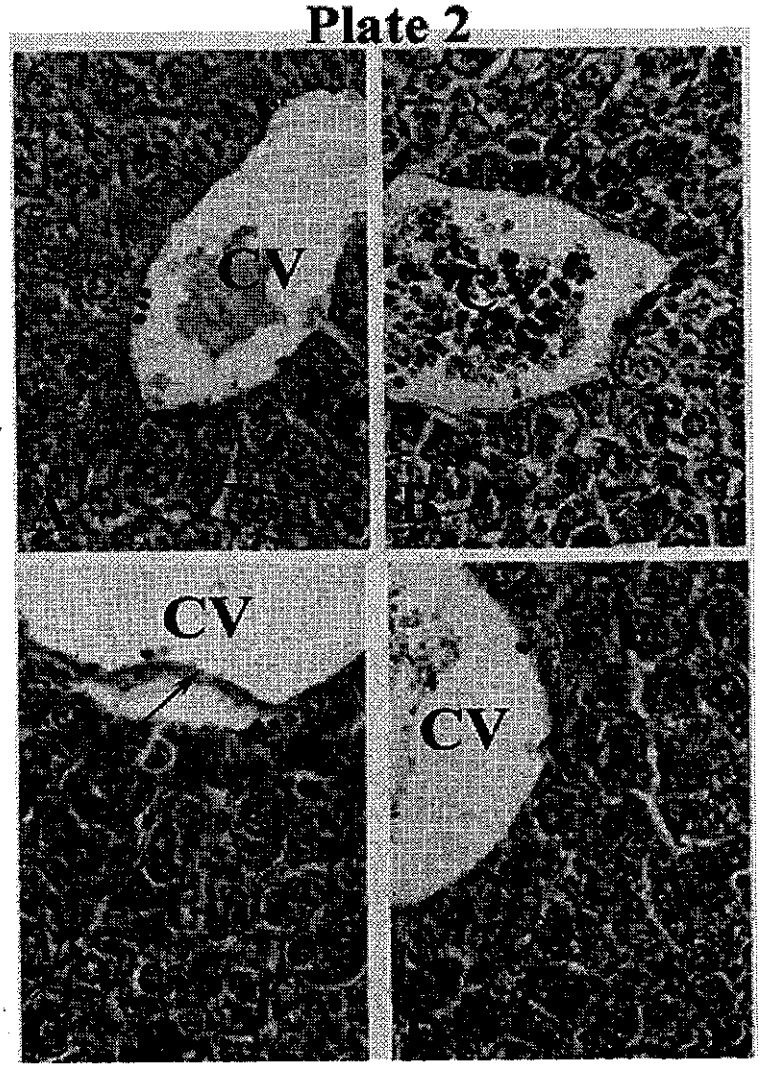

Plate (2): Photomicrograph of sections in livers of mice. (Masson's trichrome X400).
A: Control, showing very minimal faintly stained collagen fibers around the central vein (CV) and blood sinusoids (arrows). **B**: After treated period of geranium extract, showing increase in the collagen fibers around dilated and congested central vein (CV) and surrounding the blood sinusoids (arrows). **C:** At a recovery period of 10 days, demonstrating slight increase in collagen fibers around central vein (CV) and detachment of the wall of the central vein from blood sinusoid (arrow). **D:** At a recovery period of 20 days, revealed that the collagen fibers (arrow) around the central vein (CV) appeared nearly similar to the control group.

Plate 3

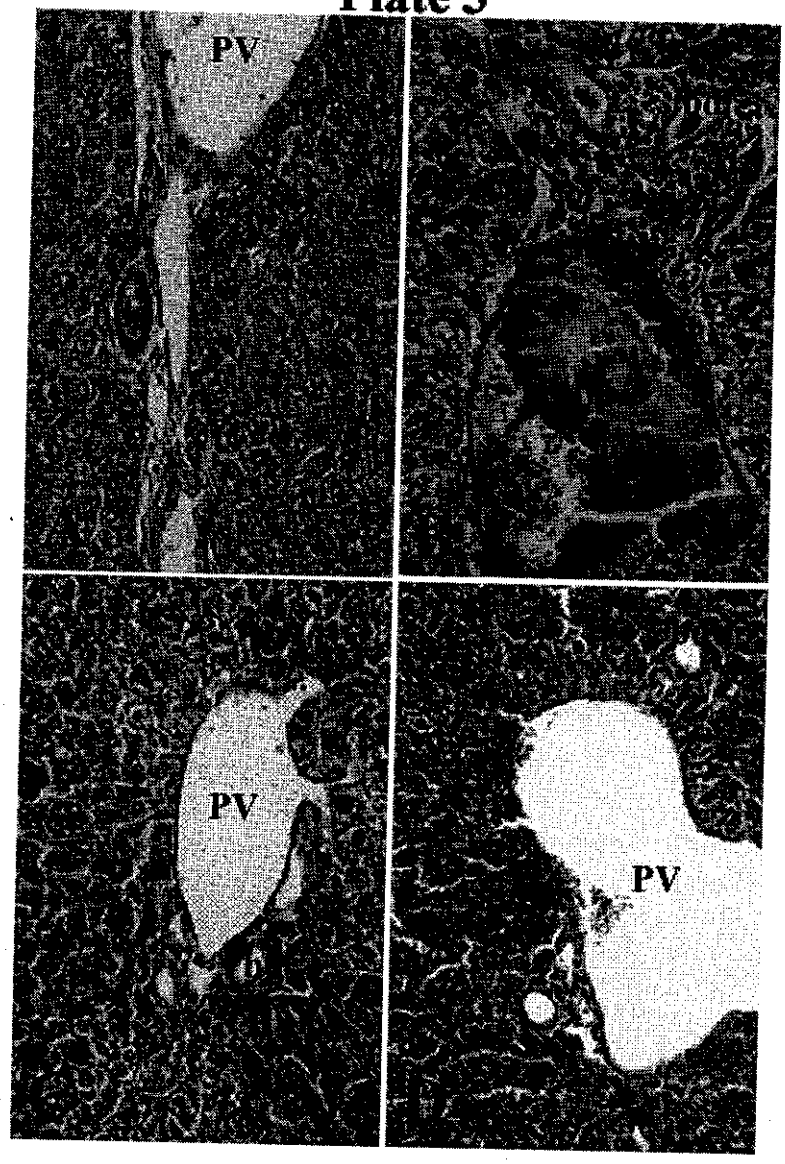

Plate (3): Photomicrograph of sections in livers of mice. (Masson's trichrome X 200).
A: Control, showing scanty collagen fibers in the portal area, portal vein (PV) and bile duct (bd). **B:** After treated period of geranium extract, showing increased in the collagen fibers around the portal lobule, dilated portal vein (PV) and bile duct (bd). **C:** At a recovery period of 10 days, demonstrating slight increase in collagen fibers around portal area, portal vein (PV) and bile duct (bd). **D:** At a recovery period of 20 days, showing that the collagen fibers around the portal areas, portal vein (PV), bile duct (bd) appeared nearly similar to the control group.

Plate 4

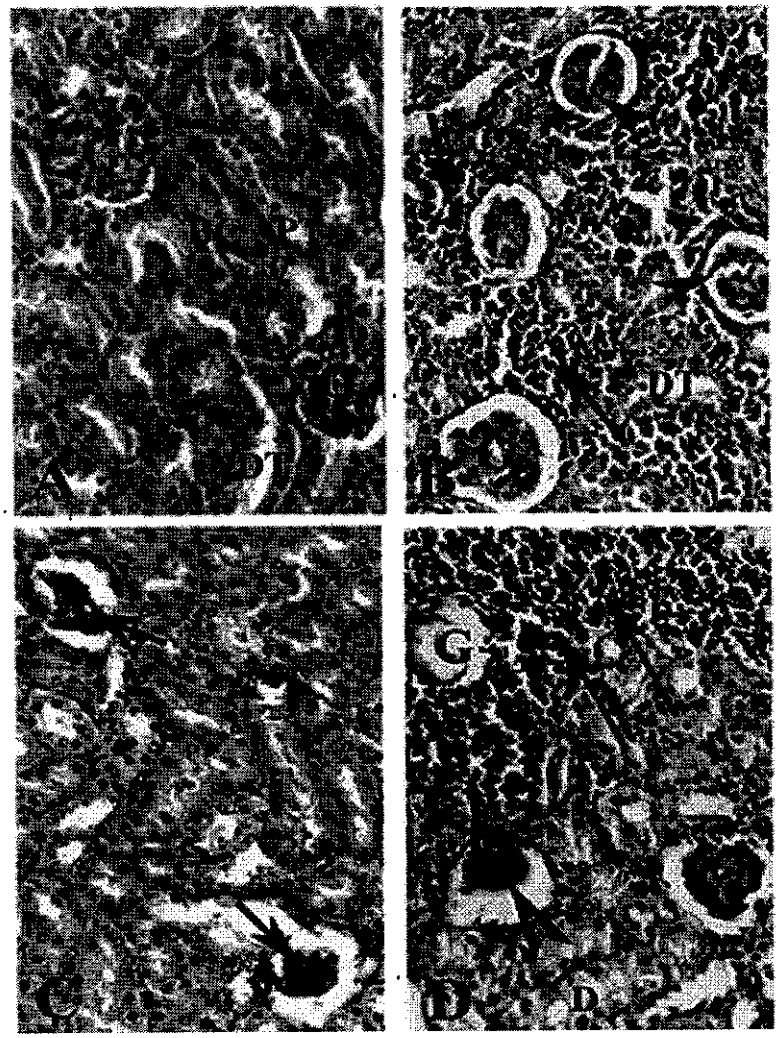

Plate (4): Photomicrographs of sections of renal cortex of mice. (H&E X 200).

A: Control, showing Malpighian renal corpuscles with normal glomerulus (G) and Bowman's capsules (BC), normal proximal convoluted tubules (PCTs) with brush border and distal convoluted tubules (DCTs). **B**: After treated period of geranium extract, displaying inflammatory cellular infiltration (arrows) and degenerative lesions (D) in convoluted tubular epithelium. Most of the glomeruli are lobulated and congested (arrow head). **C:** At a recovery period of 10 days, displaying the congested atrophied glomerular tuft of capillaries and rupture of BC (arrows). **D:** At a recovery period of 20 days, showing inflammatory cellular infiltration (arrows) around the glomerular capsule and in between the renal tubules. Vacuolar degeneration (D) of some convoluted tubules and disappearance of some glomeruli (G), others became atrophied (arrow head).

Plate 5

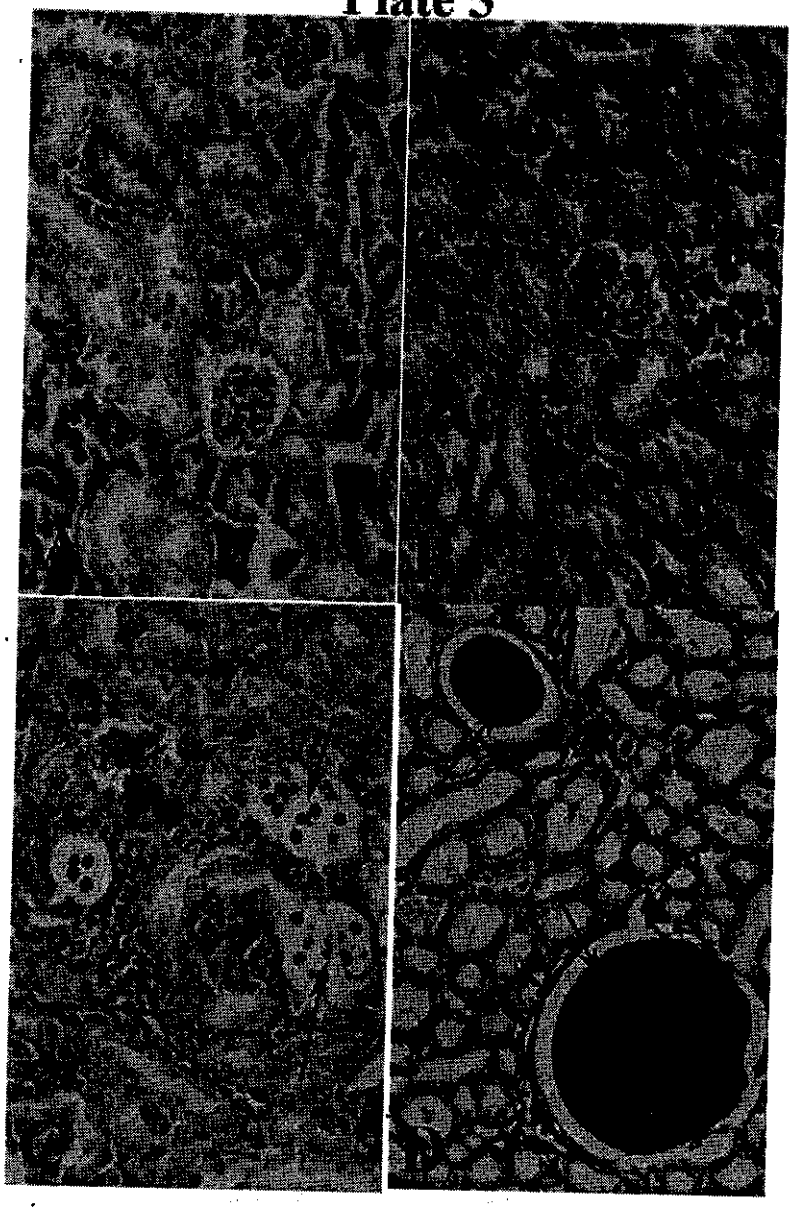

Plate (5): Photomicrographs of Kidney sections of mice.

(Masson's trichrome X 200).

A: Control, exhibiting collagen fibers supporting the tubules. **B**: After treated period of geranium extract, demonstrating a mild effect of accumulation of collagen fibers in the interstitium of kidney and venous congestion (arrow). **C:** At a recovery period of 10 days, exhibiting WBCs casts (arrows) inside kidney tubules with degeneration of the renal tubular epithelium (arrow head). Notice cellular infiltration (CI) between the tubules. **D:** At a recovery period of 20 days, showing hyaline casts (arrows) in lumina of some tubules with flattened renal tubular epithelium (arrow head) in the medulla.

Plate 6

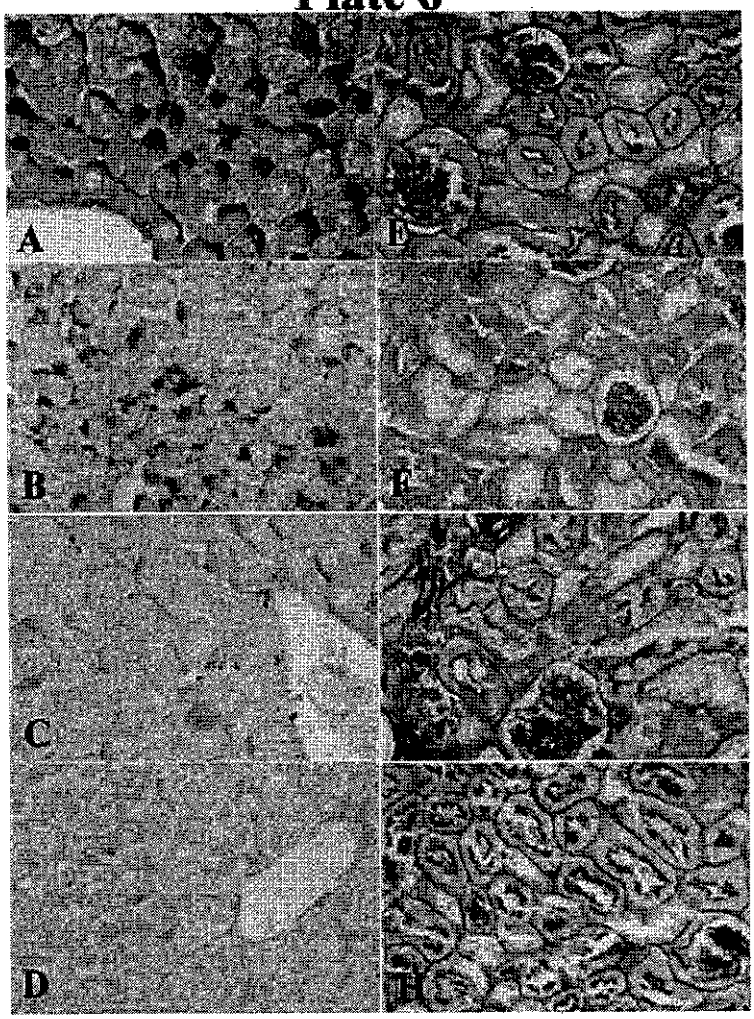

Plate (6): Photomicrograph of the liver (A, B, C & D) and kidney (E, F, G & H) sections showing distribution of glycogen. **A.** Control liver, glycogen in hepatocytes with intense red color and nuclei had no stains. **B.** After treated period of geranium extract, liver section with a slight decrease in glycogen. **C.** At a recovery period of 10 days with apparent decrease of glycogen. **D.** At a recovery period of 20 days with a severe decrease in glycogen. **E.** Control kidney section with moderate PAS positive material in the cytoplasm and brush borders of the PCTs. Glomeruli were intensely positive to PAS reaction. **F.** After treated period of geranium extract, with marked decreased amount of PAS positive material in tubules and glomeruli. **G&H.** At recovery periods of 10 and 20 days respectively with decreased amount of glycogen in tubular epithelium while increased stainability of brush borders of PCTs and glomerular cells. Sections were stained with periodic Schiff's method. Magnifications, X 400 (A, B, C &D) and X 200 (E, F, G & H).

Table (9): Histochemical changes in the PAS content in the liver and kidney sections from control and geranium extract –treated mice.

Parameters	Liver				Kidney			
	Control	Treated period	Recovery periods		Control	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
PAS +ve material	174.92 ± 1.03	178.25 ± 1.2 NS	180.11 ± 2.2 *	181.83 ± 2.44 *	176.67 ± 2.61	184.02 ± 2.12 *	180.26 ± 1.28 NS	175.76 ± 1.2 NS

Each value is the mean of 10 frams ± SE.
NS: non significant
* Significant value at $P< 0.05$.

Plate 7

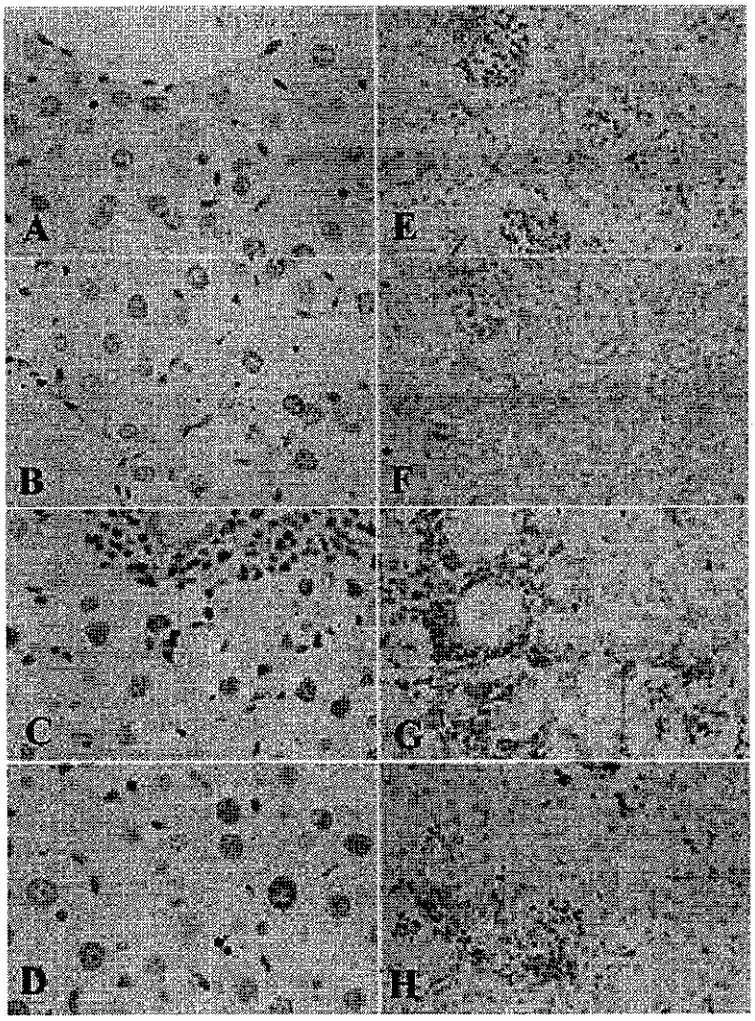

Plate (7): Photomicrograph of the liver (A, B, C & D) and kidney (E, F, G & H) sections showing the DNA content. **A.** Control liver with faint red purple colored materials in the nucleoplasm of hepatocytes and Von Kupffer cells. **B.** After treated period of geranium extract, liver section with low amount of DNA in hepatocytes increased coarse chromatin. **C.** At recovery period of 10 days with densely stained nuclei of the Kupffer cells and inflammatory cells. **D.** At recovery period of 20 days with a slight increase amount of DNA content. **E.** Control kidney section with DNA material appeared as faint red purple color. **F.** After treated period of geranium extract with low amount of DNA. **G&H.**, Groups at recovery period of 10 and 20 days respectively with decreased DNA content in tubules while increased stainablity of glomerular cells. Sections were stained with Feulgen technique. Magnifications, X 400 (A, B, C &D) and X 200 (E, F, G & H).

Table (10): Histochemical changes in the DNA content in the liver and kidney sections from control and geranium extract-treated mice.

Parameters	Liver				Kidney			
	Control	Treated period	Recovery periods		Control	Treated period	Recovery periods	
			10 days	20 days			10 days	20 days
DNA content	189.01 ± 0.69	190.68 ± 0.75 NS	190.33 ± 1.31 NS	189.44 ± 0.73 NS	196.01 ± 0.74	195.93 ± 0.81 NS	195.84 ± 0.83 NS	194.31 ± 0.73 NS

Each value is the mean of 10 frams ± SE.
NS: non significant
* Significant value at P< 0.05.

Discussion

Increasing interest in medicinal plants has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decision on their usage.

The present results showed that the body weights of treated animals were not significantly different from the control. This may suggests that the ethanolic extract did not interfere with the absorption of nutrients such as proteins and minerals.

It appears that the ethanolic extract did not affect hematological parameters of the animals. This is very surprising because the extract contained saponnins which has been reported (Sofowora, 1993) to have deleterious hemolysing effect on circulating erythrocytes.

Because of the most vital function of the liver which neutralizes and eliminates toxic substances from the body (Boorman *et al.*, 1990 and Effendy *et al.*, 2006) the present study showed that the dose of *Pelargonium graveolens* extract signify-cantly increased the levels of serum AST, ALT, GGT and LDH. This is the direct consequences of organ damage, especially of the liver and kidney (Jubb *et al.*, 1995 and Wannang *et al.*, 2005). Hepatocellular damage caused the death of numerous liver cells (extensive hydropic degeneration and necrosis) in the present study. Hydropic degeneration means that disturbance of the mitochondria function with the result of lack of energy and may be due to disturbance in the metabolism of the cell (Abdin, 1981). Changes in the cells due to necrosis and vacuolation may be due to acute injury causing inflammatory response (Alberts *et al.*, 2001). This may be attributed to the excessive release of such enzymes from the damaged liver cells into the blood circulation. Where, there is an inverse relationship between the liver activity and the level of enzymes in serum (Boorman *et al.*, 1990).

There was also significant increase in serum urea and creatinine after treatment with geranium extract. This may be resulted from failure of the body to excrete the metabolic end products of proteins (Guyton and Hall, 2000). On the other hand, the significant increase in the level of creatinine may be regarded to glomerular damage and excessive muscular catabolism (Al-Sultan *et al.*, 2003). This conclusion is confirmed by the present histopathological investi-gation which indicated glomerular damage and corticotubular degeneration mainly in the PCTs. Serum total proteins, albumin and globulin concentrations showed a significant decrease after the treated period in mice. The hypo albuminaemia may be related to the hepatic damage (Jubb *et al.*, 1995). The decrease in total serum protein may be due to increased conversion rate of glycogenic amino acids to CO_2 and H_2O (Mortimore and Mandon, 1970), reduction in protein synthesis which in turn may be due to a decrease in the amount and availability of mRNA (Peavy *et al.*, 1985). In general, the pathological changes may lead to impaired liver function which interferes with the secretion of plasma

proteins (Lapeyre-Mestre *et al.* 2006). This leads to decreased blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the congestion observed in the tissue (Ebaid *et al.*, 2007).

The increase of serum total lipids and cholesterol in the present work indicates liver damage (Bush, 1991). The lipid content changes in pathological liver were recorded by Glaser and Magner (1972) to be due to imbalance between the normal rates of lipid synthesis, utilization and secretion. In addition, Zhang and Wang (1984) suggested that the cytoplasmic vacuolation is mainly a consequence of considerable disturbance in lipid inclusions and fat metabolism occurring during pathological changes. Vacuolar degeneration also has been regarded by Durham *et al.* (1990) to be an alteration produced to collect the injurious substances in the cells. Lalli *et al.* (2008) suggested that the antimicrobial activity of the geranium extracts may be ascribed to general cytotoxic effects.

The present results also showed a remarkable cellular infiltration in the hepatic and renal tissues. This supports El-Banhawy *et al.* (1993) whose studies suggested that abundance of leucocytes, in general, and lymphocytes, in particular, are a prominent response of body tissues facing any injurious impacts. Leukocyte elevations and adherence to the vascular endothelium have been suggested by McCafferty *et al.* (1995) to play an important role in the pathogenesis of non steroidal anti-inflammatory drug (NSAID)-associated injury.

Masson's stained sections revealed a slight increase in collagen fibers around the central vein, blood sinusoids and the portal areas after geranium extract administration. Horn *et al.* (1985) declared that the presence of collagen in perisinusoidal space would reduce the exchange of metabolite which might cause hepatocellular dysfunction and necrosis.

Histochemical analysis of the hepatic tissue evidenced a decrease in glycogen content, probably correlated with the pathological status within the liver tissue. The glycogenolytic effect study may be due to its direct action on the cells, stimulating glycogenolysis, or due to its effect on the other cytoplasmic membranous organelles and the associated enzymes necessary for glycogen synthesis (Saleh *et al.*, 1986).

Kidney is the second target in the body after the liver in this study. The main functions of the kidney are the excretion of the by-products of the metabolism, foreign substances such as body pigments (Effendy *et al.*, 2006) and maintaining homeostasis. Exposure to circulating toxins will lead to pathological changes and disruption of both glomerular and renal tubular functions (Wannang *et al.*, 2005).

It was observed in the present study that the kidney was associated with a wide variety of histological alterations, such as degeneration in some convoluted tubules, atrophied glomeruli and tubular casts after extract treatment. Hathaway *et al.* (1991) stated that the tubular lesions started with signs of degeneration which gradually progressed to massive tubular necrosis. The observed necrosis may be due to either a severe degeneration (Dukes and Belly 1991) or to metabolic disturbances (Casarett and Doull 1975). In addition, Stevens and Lowe (1997) reported that the drug concentration in the blood is affected by capillary constriction leading to a decrease in glomerular filtration of that drug which minimizes its effect and protects the tubular cells. This may cause the shrinkage and atrophy of the glomeruli.

Renal casts (hyaline and WBCs casts) with flattened of renal tubular epithelium have been observed in the kidneys at recovery periods of 10 and 20 days respectively. The presence of tubular casts might cause obstruction of the tubular lumen, together with the disruption of tubular basement membrane might produce interstitial inflammatory reaction (Ozen *et al.*, 2001). Increased plasma proteins into the glomerular filterate resulted in proteinuria and the hyaline casts which were found may be due to this proteinuria deposits around the lumen walls of renal tubules (Anderson, 1985). Cast formation is augmented if plasma proteins are present, solutes are increased, the pH is acidic, and filtrate flow through the lumen is slow. In addition, the leukocyte casts indicate the presence of a renal inflammation or infection (http://cancerweb.ncl.ac.uk/cgi-bin/omd?hyaline+cast. 2000).

Regarding the histochemical changes observed in this study under geranium extract administration, results clearly indicated reduction in the polysaccharides in the liver and kidney tissues. These changes were consistent with those induced histopathologically for the group after treated period. This may indicate a decrease in the absorptive function of the tubules that induce catabolism of glycogen in animal cells (Grodsky, 1977). Also, the decrease in carbohydrate content was attributed by some investigators to be due to increased stress on organs, leading to high energy consumption which allowed an equalized pressure to be exerted upon them (Ibrahim, 1999). The significant decrease in DNA in the present results is confirmed with the significant decrease in serum total protein in treated mice which could be attributed to the disruption of lysosomal membranes under the effect of various toxicants leading to the liberation of their hydrolytic enzymes in the cytoplasm resulting in marked lysis and dissolution of the target material. This result confirmed that of Awasthi *et al.* (1984) who found elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acids content in respose to organophosphate insecticide.

At recovery period of 20 days, it was observed that hepatocytes seemed approximately normal. Other investigators have reported tissue adaptation to the injury produced by NSAID (Ibrahim, 1999). However, no histochemical changes improvement, particularly PAS, was noted in the liver tissue after recovery periods. Actually, hepatocytes DNA content was low. Depleted and disturbed DNA in the hepatocytes indicated that these cells remained impaired by the action of the extract.

Biochemical, histological and quantitative histochemical studies showed some toxic effects on the liver and kidney of treated mice with statistically significant changes in some enzymatic content. Although signs of recovery were noticed after 20 days of last administration of the extract, they were not complete in some parameters and a longer time might be needed for full recovery.

It has been concluded that *Pelargonium graveolens* is safety used as medicinal plant. Its overdose has a reversible action, so, its damage can be ameliorate after recovery period (equivalent to the time of treatment).

References

1. **Abdin F (1981):** Cell and tissue damage in: Abdin's General Pathology, 4th ed., p.7.
2. **Alberts B, Johnson A, Lewis J, Raff M, Roberts K, and Walter P (2001):** Molecular Biology of The Cell. 4th ed. Chapter 17, P. 1011. Published by Garland Science, New York.
3. **Al-Sultan SI, Hussein YA and Hegazy A (2003):** Toxicity of *Anagallis arvensis* plant. Pakistan Journal of Nutrition, 2 (3): 116-122.
4. **Anderson JR (1985):** Urinary system. In: Muir's Textbook of pathology, 12th ed., English Language Book Society, Ed word Arnold, Pp.22-24.
5. **Awasthi M, Shah P, Dubale M, Gadhia P (1984):** Metabolic changes induced by organophosphates in the piscine organs. Environm Res; 35:320-325.
6. **Boorman GA, El-well MR, Eustis SL and Leiniger JR (1990):** Pathology of the fisher rat. Reference and Atlas, Academic press. Inc. Harcourt Brace Jovanoich. Publishers: San Diego, New York, Boston, London, Sydney, Tokyo and Toronto, P. 150.
7. **Botha CJ and Penrith ML (2008):** Poisonous plants of veterinary and human importance in southern Africa. Journal of Ethnopharmacology, 119 (3): 549-558.
8. **Bush BM (1991):** Part 2. Plasma biochemistry. In: interpretation of laboratory results for small animal clinicians. Blackwell Scientific Publications London. Pp. 221-229.
9. **Casarett L and Doull J (1975):** Toxicology: The basic science of poisons. New York, Toronto, London, Pp. 468-470.
10. **Dacie JV and Lewis SM (1991):** Hematology. 7th ed., the English language book society and Churchill Iirringstone, Pp. 37-58.
11. **Doumas BT (1971):** Clin. Chem. Acta., 31: 87-96.
12. **Doumas BT (1975):** Colourimetric determination of total protein in serum or plasma. Clin. Chem., 21 (8): 1159-1166.
13. **Drabkin DL and Austin JH (1932):** Spectrophotometric studies: Spectrophotometer constants for common hemoglobin

derivatives in human, dog and rabbit. J. Biol. Chem., 98: 719-752.

14. **Dukes M and Belly L (1991):** Side effects of drugs. Elsevier, Amesterdam, New York, Oxford.
15. **Durham SK, Brouwer A and Barelds RJ (1990):** Comparative endotoxin-induced hepatic injury in young and aged rats. J Pathol; 162: 341-349.
16. **Ebaid H, Dkhil MA, Danfour MA, Tohamy A and Gabry MS (2007):** Piroxicam-induced hepatic and renal histopathological changes in mice. Libyan J Med., 13:51-56.
17. **Effendy AWM, Siti-Nurtahirah J, Zamri-Saad M and Hussin ZM (2006):** The side effects of Kacip Fatimah extract on liver and kidney of white rats. Journal of Sustain. Sci & Mngt., 1(1): 40-46.
18. **El-Banhawy MA, Sanad SM, Sakr SA, El-Elaimy IA and Mahran HA (1993):** Histopathological studies on the effect of the anticoagulant rodenticide "Brodifacoum" on the liver of rat. J Egypt Ger Soc Zool; 12(C):185-227.
19. **Feulgen R and Rosenback HC (1942):** Manual of histological demonstration technique. Butterworth & Co. (Publishers) Ltd. London, The ford, Harverhill.
20. **Finney DJ (1971):** Probit Analysis 3rd Ed., Cambridge Univ., Press, London U.K. Pp. 318.
21. **Freedman B, Kopek WF and Nowak J (1979):** A bioassay for plant derived pest control agent using the European comborer. J. Econ. Entomal., 72: 45-54.
22. **Glaser G and Magner J (1972):** Biochemical studies on the mechanism of action of liver poisons. II. Induction of fatty liver. Biochem. Biophys. Acta., 269: 500.
23. **Grodsky GM (1977):** The chemistry and functions of hormones in: review of physiological chemistry. 16th ed., P. 484, Editors: Harper, H.A., Rodwell, V.W. and Mayes, P.A. Lange Medical publications: California.
24. **Guyton AC and Hall JE (2000):** Text book of medical physiology. Endocrinology and reproduction. Insulin, Glucagons and Diabetes Mellitus. 10th ed. W. B. Sounders Company in U. S. A.
25. **Harris (1900):** After Bruse, Casselman. W.G. (1959): Histochemical technique by Nethien and Co. L T D.
26. **Hathaway GJ, Fischman ML, Hughes JP and Proctor NH (1991):** Proctor and Hughe's chemical hazards of the workplace. 3rd ed. New york, NY: Van Nostrand Reinhold.
27. **Horn T, Christoffersen P and Jung J (1985):** Alcoholic liver injury: early changes of the Disse space in acinar zone 3. Liver, 6: 301-310.
28. **Hotchkiss RD (1948):** A micro chemical reaction resulting in the staining of polysaccharide structure in fixed tissue preparation. Arch. Biochem., 16: 131-136.
29. **http://cancerweb.ncl.ac.uk/cgi-bin/omd?hyaline+cast.** 2000.
30. **Ibrahim MA (1999):** A study of the histochemical changes in some mammalian tissues induced by a NSAID (Diclofenac). M. Sc. Thesis. Fac Sci Helwan Univ.
31. **Jeffe MZ (1886):** Determination of creatinine in serum. Phys. Chem., 10: 391.
32. **Jubb KVF, Kennedy PC and Palmer N (1995):** Pathology of Domestic Animals. 3rd edition, Academic Press Inc., New York.
33. **Kachmar JF and Moss DW (1976):** In Fundamentals of Clinical chemistry, 2nd ed. Nw Tietz, Editor. WB Sounders, Philadelphia, P. 652.
34. **Karawya MS and Abd El-Wahab SM (1975):** Practical applied pharmacognosy note, for fourth year pharmacy students. Cairo Univ., P. 103.
35. **Kiernan jA (1999):** Methods for connective tissue: Collagen: Masson's trichrome. In: Histological and histochemical methods. Third edition, Pp. 154-155. In Great Britain by the bath. Press, Somerset.
36. **Knight JA, Anderson S and Rawie JM (1972):** Chemical basis of the sulfo-phospho-vanilin reaction for estimation total serum lipid. Clin. Chem., 18: 199-202.
37. **Lalli JYY, Van Zyl RL, Van Vuuren SF and Viljoen AM** (2008): In vitro biological activities of South African *Pelargonium* (Geraniaceae) species. South African Journal of Botany, 74 (1): 153-157
38. **Lapeyre-Mestre M, de Castro AM, Bareille MP, Del Pozo JG, Requejo AA, Arias LM, Montastruc JL, Carvajal A (2006):** Non-steroidal anti-inflammatory drug-related hepatic damage in France and Spain: analysis from national spontaneous reporting systems. Fundam Clin Pharmacol; 20(4):391-395.
39. **Lee MY (2002):** Effects of Na_2SO_3 on the activities of antioxidant enzymes in geranium seedlings. Phytochemistry, 59 (5):493-499

Lis-Balchin M (2002): Geranium and *Pelargonium*, Taylor & Francis, London.

40. **Lis-Balchin M, Hart SL, Deans SG and Eaglesham E (1996):** Potential

agrochemical and medicinal usage of essential oils of *Pelargonium* species, Journal of Herbs, Spices & Medicinal Plants 3: 11–22.

41. **Maikai VA, Kobo PI and Adaudi AO (2008):** Acute toxicity studies of aqueous stem bark extract of *Ximenia Americana.* African Journal of Biotechnology, 7 (10): 1600-1603.
42. **Mativandlela SPN, Lall N and Meyer JJM (2006):** Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South African Journal of Botany 72: 232–237.
43. **McCafferty D, Granger DN, and Wallace JL (1995):** Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. Gastroenterol; 109:1173-1180.
44. **Mitruka BM, Rawnsley HM and Vadehra BV (1977):** Clinical, biochemical and hematological reference values in normal experimental animals. Masson publishing USA, INC. New York, Paris, Barcelon, A. Milan.
45. **Mortimore GE and Mandon CE (1970):** Inhibition of insulin of valine turnover in liver. J. Biol. Chem., 245: 2375-2383.
46. **Namura M, Tsukada H, chimatsu D, Ito H, Yoshida T and Miyamoto KI (2005):** Inhibition of epidermal growth factor-induced cell transformation by tannins. **Phytochemistry**, 66 (17): 2038-2046
47. **Ozen S, Usta Y, Sahin-Erdemli I, Orhan D, Gumsel B, Yang B, Gursoy Y, Tulumoy O, Dalkara T, Bakkaloglu A and El-Nahas M (2001):** Association of nitric oxide production and apoptosis in a model of experimental nephropathy. Nephrol. Dial. Transplant., Jan.,16 (1): 32-40.
48. **Patton CJ and Crouh SR (1977):** A colorimetric method for the determination of blood urea concentration. J. Anal. Chem., 49: 464-469.
49. **Peavy DE, Jefferson LS and Taylor JM (1985):** Time course of changes in albumin synthesis and mRNA in diabetic rats. Am. J. Physiol., 248: 656.
50. **Persijn JP, Slik WJ and Van D (1976):** Clin. Chem. Clin. Biochem., 14: 421-427.
51. **Reitman S and Frankel S (1957):** A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28: 57-63.
52. **Saleh AM, Beshir SR, Mohamed AA and Mousa S (1986):** Histological and histochemical studies on the effect of chloramphenicol on the liver of albino rat. Egypt. J. Histol., 9 (1): 3-10.
53. **Schettler G and Nussel E (1975):** Arbeits med. Sozialmed. Preventive med; 10: 25.
54. **Snedecor Gw and Cochran WG (1980):** Statistical methods. Oxford and J. B. H. Publishing Co., 7th ed.
55. **Sofowora EA (1993):** Medical plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, P. 289.
56. **Stevens A and Lowe J (1997):** Histology Gower Medical Publishing. London. New York.
57. **Tanaka R, Minami T, Ishikawa Y, Matsunaga S, Tokuda H and Nishino H (2003):** Cancer chemopreventive activity of serratane-type triterpenoids on two-stage mouse skin carcinogenesis, Cancer Lett. 196:121–126.
58. **Tietz NW (1986):** Text book of clinical chemistry. W. B. Saunders Co., London, Philadelphia, Pp.: 1389-1390.
59. **Wannang NN, Wudil AM, Dapar LMP and Bich LA (2005):** Evaluation of *Securidaca longepedunculata* in rats. J. Pharm. Bio. Res., 2 (2): 80-83.
60. **Webster D (1977):** Albumin standards and measurements of serum albumin with bromocreasol green. Clin. Chem., 23: 663.
61. **Williams CA and Harborne JB (2002):** Phytochemistry of the genus *Pelargonium.* In: M. Lis-Balchin, Editor, *Geranium and Pelargonium*, Taylor and Francis, London ISBN 0415284872.
62. **Zhang LY and Wang CX (1984):** Histopathological and histochemical studies on toxic effect of brodifacoum in mouse liver. Acta Acad Med Sci; 6 (5):386-388.

تأثير مستخلص أوراق الجيرانيم على وظائف الكبد والكلى في الفئران البيضاء.

سامية محمد عبد الوهاب، إيمان جمال الدين هلال ، عبد الموجود عسران* ،
عبد الستار متولي** ، حلمي زيدان* و منى عبد الحميد علي*
قسم علم الحيوان– كلية العلوم– جامعة الأزهر– فرع البنات ،*معهد بحوث وقاية النبات — مركز البحوث الزراعية و** قسم الحيوان الزراعي – كلية الزراعة– جامعة الأزهر.

يهدف هذا البحث إلى إبراز تأثير مستخلص أوراق الجيرانيم على وظائف الكبد والكلى في الفئران والتغيرات الهستولوجية والهستوكيميائية التي تطرأ عليهما خلال فترتي المعاملة والإستشفاء. و قد قسمت الفئران إلى مجموعتين رئيسيتين. إعتبرت المجموعة الأولى مجموعة ضابطة وأعطيت المجموعة الثانية مستخلص إيثانولى لنبات الجرانيم عن طريق الفم يوم بعد يوم بجرعة مقدارها 869.4 مجم/كجم/يوم ولمدة عشرون يوماً. تم ذبح الفئران بعد فترة المعاملة و فترتي الإستشفاء على التوالي وأخذت عينات من الدم لعد كريات الدم الحمراء والبيضاء و قياس محتوى الهيموجلوبين و بعض وظائف الكبد والكلى ، وأخذت عينات الكبد والكلى وأعدت للفحص الهستولوجي والهستوكيميائي . وقد أوضحت النتائج حدوث زيادة إحصائية في نسبة إنزيمات LDH ,GGT, ALT, AST وفى نسبة البولينا والكرياتينين في الدم وبعض محتويات مصل الدم الأخرى مثل الدهون الكلية والكوليستيرول. بينما أظهرت النتائج إنخفاضا ذو دلالة إحصائية في نسبة الجلوكوز وبعض محتويات مصل الدم مثل البروتين والزلال والجلوبيولين.

أوضح الفحص النسيجي ظهور ملامح مرضية في أنسجة الكبد والكلى على هيئة أتساع و إحتقان في الأوردة المركزية في الكبد مع وجود إنتشار خلوي أحادى النواة حولها، وإشتملت الأضرار على إضمحلال في السيتوبلازم وإنكماش للأنوية وظهور بعض المناطق الكبدية ميتة و حدوث بعض تغيرات هستولوجية بالكلى حيث إعترى الكبات التقصص والإنكماش والضمور وكذلك فقد لبعض الكبات ولوحظ أيضا إضمحلال في خلايا الأنابيب الملتفة مع وجود إرتشاح خلوي بين الأنيبيبات ، وكذلك وجود قوالب شفافة في الأنيبيبات الكلوية . كما لوحظ زيادة نسبية في ألياف الكولاجين حول الأوردة المركزية و المنطقة البابية في الكبد وفى الكلى ظهر تليف بين الشعيرات الدموية للكبيبات الكلوية وبين الأنابيب المعوجة . وقد كان ترك الحيوانات المعالجة لفترة إستشفاء مدتها 20 يوم كافية لإعطاء صورة شبيهة إلى حد ما لتلك المأخوذة من الحيوانات غير المعاملة بالمستخلص.

وبالنسبة للدراسة الهستوكيميائية الكمية للكبد والكلى فقد وجد أن المستخلص النباتي تسبب في إنخفاض ذو دلالة إحصائية في كمية الجليكوجين. بينما لم يحدث أي تغير ملحوظ في كمية الحامض النووي (د.ن.أ) في كل من الكبد والكلى.

وتدل نتائج هذا البحث على أن المعاملة بالمستخلص الإيثانولى لنبات ألجيرانيم قد سببت إضطرابا في مختلف القياسات الفسيولوجية في الفئران سواء كانت ذكورا أو إناثا. كما ثبت أن تأثيره الضار يختفي بعد التوقف من إستخدامه (لفترة مكافئة لفترة الاستخدام). كما ثبت أن جرعة نصف الميتة عالية جدا مما يؤكد أنه آمن للإستخدامات الطبية لذا فلا مانع من إستخدامه. كما يلزم إستخدامه لفترات أطول وإجراء تجارب للسمية لكل النباتات المستخدمة طبيا قبل إستخدامها.