Effect of aqueous extracts of *Grewia tenax* fruit (guddaim) on hematological, histological and ultrastructure changes in mice intoxicated with formalin

Aglal A. Alzergy

Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Omar Al Mukhtar University, AL Bayda Libya.
aglalalzergy@yahoo.com

Abstract: Eighty apparent healthy female albino Swiss mice 8-10 weeks old weighting 20-26 gm were divided into four groups. The first group was considered as a control and received distilled water only, the second group administered formalin (2.4ml/kg body weight) in drinking water for one week, the third group orally administered aqueous extract of *Grewia tenax* at dose level 800 mg/kg body weight once daily by oral gavage needle for one and two successive weeks. The fourth group Co- treated with formalin and aqueous extract of *G. tenax*. Blood samples were collected for hematological parameters; red blood corpuscles (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), total white blood cells count (WBCs), percentage of granulocytes, lymphocytes and monocytes as well as, platelets count were estimated. Specimens of spleen were processed for histological studies by light microscopy. Some spleen specimens were also processed to be studied by transmission electron microscopy (TEM). No abnormal signs in behavior and external features in control and *G. tenax* treated mice were noticed. Although obvious changes in behavior and external features included decrease in food intake, rough coat, fall hair around mount and hypoactivities were observed in few mice in formalin treated group, while aggressive fighting in few others was seen. The alterations in behavior and external features were less in mice administration *G. tenax* with formalin. No deaths were encountered in the control or treated mice during experimental period. Administration of G. *tenax* and formalin induced an improvement in the final body weight comparing to formalin only treated group. Treatment of mice with *G. tenax* for one week exhibited insignificant alterations in most hematological parameters. While, significant increase in RBCs and platelets counts were recorded. In contrast, administration of *G. tenax* for two weeks induced obvious disturbance in the hematological parameters. Also, disturbance in hematological parameters was recorded in formalin treated group. While, administration of *G. tenax* with formalin induced ameliorating changes and inhibited the decrease in Hb, RBCs and PCV compared to formalin only treated group. Also, an improvement in platelet count was demonstrated. No obvious histological alterations in spleen sections of mice treated with *G. tenax* for one week. While, administration of *G. tenax* fruit for two weeks induced some changes included hypocellularity in white pulp and red pulps, many necrotic cells in white pulp, edema and hemosedrine in red pulp. Spleen sections of mice treated with formalin showed many abnormal lesions included hyperplasia, disorganization of lymphoid follicles of white pulp, disappeared marginal zones, many dense nuclei, congestion and hemosedrine in red pulp. Mice Co-treated with formalin and *G.tenax* for one week revealed obvious alterations in the spleen including reduction of lymphoid follicles size with nearly normal cellularity, depletion of splenic cells in red pulp, some hemosidern granules and necrotic cells in both white and red pulps. TEM examination of the spleen sections of mice treated with *G. tenax* for one week did not reveal alterations. Ultrastructure of spleen of mice treated with formalin showed sever changes in splenocytes of white and red pulps included depletions of lymphocytes in white pulp, abnormal chromatin features, accumulation of dense materials in cytoplasm of some seplenocytes, marked congestion and increase granular leukocytes in red pulp. Also, some necrotic cells with pyknotic nuclei and few dense mitochondria with less distinct cristae, destructed organelles, destructed or less distinct cell membrane and vacuolated cytoplasm were seen. Spleen of mice Co-treated with formalin and *G. tenax* for one week revealed that *G. tenax* succeeded to lessened most abnormal ultrastructure alterations comparing to formalin only treated group. *G. tenax* succeeded to lessened most abnormalities on hematological, histological and ultrastructure levels in formalin intoxicated mice.

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Key words: Aqueous extract of *Grewia tenax*, Formalin, *Hematological*, Histological, Ultrastructure spleen and Female mice.

1. Introduction

Formalin is an aqueous solution (37%) of formaldehyde **(Khan *et al.*, 2006)**. Formaldehyde is commonly used in the production of various industrial and medical products **(Golalipour et *al*., 2008)**. It is colorless flammable gas with a pungent odor widely

used in industrial and medical settings and it is a major source of occupational pollution compound **(Suh *et al*., 2000)**. Formaldehyde is also an important public health problem, because it is present in tobacco smoke, and is released from various household products such as plywood, particleboard, furniture, and carpeting. It is used in some cleaning products **(Cogliano et *al*., 2005 and IARC, 2006).** It had been also found in industrial exhaust, and used in some sterilizing and preserving solutions in medical and school settings **(World Health Organization, 1999).** A significantly greater number of people are exposed to lower levels of formaldehyde in the environment, as it is generated by automobile engines **(Turrio *et al*., 2004)**. Recently, there has been increased awareness of the possible toxic effects of formalin on the human health as well as, laboratory animals **(Aydin *et al*., 2013 and Louei Monfared *et al*., 2013)**. Epidemiological studies of industrial workers, embalmers and pathology anatomists have associated formaldehyde exposure with elevated risks for cancers at various sites, including the pancreas **(Stone *et al*., 2001),** brain**,** nasal cavities and lungs **(Coggon *et al*. 2003)**, and lympho-hematopoietic system **(Hauptmann *et al*., 2003 and Pinkerton *et al*., 2004)**. Both U.S. National Toxicology Program **(NTP, 2011)** and the International Agency for Research on Cancer **(IARC,2012)** classified formaldehyde as a human leukemogen based on epidemiological studies that suggest an increased risk of leukemia. Formalin exposure during the early postnatal period may lead to disorders and behavioral changes in adults **(Pryor, 1991 and Slomianka *et al*., 1992).** Moreover, acute and chronic inhaled formaldehyde had been associated with various toxic effects, including, neurotoxicity, in epidemiological and animal studies **(Tong *et al*., 2010).** Acute and chronic inhaled formaldehyde had been associated with various toxic effects, including hepatotoxicity and cancer, in epidemiological and animal studies **(Tong *et al*.,2010).** Acute and chronic inhaled formaldehyde have been associated with various toxic effects, including hepatotoxicity and cancer, in epidemiological and animal studies **(Tong *et al*.,2010)**.

G. tenax is known by utilization as a medicinal plant in different countries for a variety of medical purposes **(Khemiss *et al*., 2006).** It belongs to the *Tileacea* family and locally known as guddaim **(Gebauer *et al*., 2007).** *G. tenax* is a tree spread in African and Southeast Asiatic continents. It is an example of multipurpose plant species which is the source of food, fodder, fiber, fuel wood, timber and a range of traditional medicines that cure various perilous diseases and have mild antibiotic properties **(Sharma and Patni,2012).** *G.* fruits are a rich source of nutrients such as proteins, amino acids, vitamins, and minerals and contain various bioactive compounds, like anthocyanins, tannins, phenolics and flavonoids **(Zia-Ul-Haq *et al*., 2013).** It is widely used in traditional medicines to heal chronic wounds, gastric ulcers, burning sensation, itching and other allergic ailments **(Khadeer *et al*., 2009).** *G. tenax* also had been used in popular medicine to treat different diseases including hepatic disorders **(Khadeer *et al*., 2010).** The plant preparations are used for the treatment of bone fracture and for bone strengthening and tissue healing. The fruits are used for promoting fertility in females and are considered in special diets for pregnant women and anemic children **(Sharma and Patni, 2012).** In the experimental investigation some *G.* species were found to have antioxidant activities (**Kshirsagar and Upadhyay, 2009**). Different parts of this plant possess different pharmacological properties. Leaves have antimicrobial, anticancer, antiplatelet and antiemetic activities; fruit possess antioxidant and radioprotective properties while stem bark possesses analgesic and anti-inflammatory activities, also leaves and fruits possess anticancer, activity **(Zia-Ul-Haq *et al*., 2013)**.

Herbal medicine is a complementary therapy that uses plants to treat disorders. In various countries throughout the world, a large number of plants have been used as therapeutic agents in the traditional medicine **(Kumar *et al*., 2012),** but there are no enough documents in the literature about their probable toxic effects **(Monfared, 2013)**.

The alterations in hematological changes serve as the earliest indicator of toxic effects on tissue **(Paprika and Sharma, 2003).** Lymphoid tissues recently have received considerable attention as a target organ for the achieving the chemical materials toxicity **(Hsieh *et al*.,1992).** Spleen, as a part of hematopoietic and immune system, plays an important role in the life cycle of blood cells. **(Teske, 2000 and Dyce *et al*., 2002).** Spleen is the largest secondary lymphoid organ **(Cesta,2006).** Consequently, histopathological examination of spleen is highly recommended to evaluate the immune system **(Elmore, 2006).** Therefore, The present study was conducted to evaluate the effect of aqueous extracts of *G.tenax* fruit as used in traditional medicine in Libya against hematological, histological and ultrastructure alterations in spleen of female Swiss albino mice intoxicated with formalin.

2. Materials and Methods

Experimental animals:

Eighty apparent healthy adult female Swiss albino mice *(Mus-musculus*) 8 to 10 weeks old and weighing 20 -26 gm were obtained from the Animal Breeding House of faculty of veterinary medicine, Omar Al Mukhtar University, EL Beida, Libya. They

were housed in the laboratory animal room in clean plastic cages (10 mice/ cage) under controlled conditions of temperature (20 ± 2)°C and photoperiod (12h light: 12h dark) cycle. Animals were maintained on standard commercial pellet diet and clear drinking water *adlibitum*. Mice were acclimatized for 1 week prior to the start of experiments.

Materials used:

Fresh plant **(Fig. 1)** was purchased from a local herb grocery in Al-Jabel Alakhder, Libya. The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar Al Mukhtar University, EL Beida, Libya. All unwanted materials like stems, flowers, roots or stones were removed from the leaves. Plants were cleaned and used to prepare aqueous extract as used in traditional medicine.

Preparation of the aqueous extracts of *Grewia tenax* fruit:-

The dry fruit of *G. tenax* (50gm) were ground by domestic model electronic mixer and steeped with distilled water (250 ml) over night then mixed mechanically. Crude extracts were filtered by a piece of gauze. Filtrates were freshly prepared every day according to the prescriptions given by traditional healers. A dose was determined according to **Paget and Barnes (1964)**.

Formalin (formaldehyde) 37% was purchased from (Sigma Co, Germany). Formalin was chosen because it had been reported to induce hematopoietic toxicity, leukemia, liver necrosis and cancer **(IARC,1982; Takahashi *et al*., 1986; Soffritti *et al*.,1989; Tang *et al*.,2009 and Soni *et al*.,2013)**.

Fig. (1): *Grewia tenax*

Experimental Design

Eighty apparent healthy adult female mice were divided into 4 groups of 20 mice each and subjected to the following treatments:

Group I (control group): mice received distilled water at dose level 4 ml/kg by oral gavage for two weeks and served as negative control (untreated control group).

Group II (Formaline treated group): mice received formalin at dose level 2.4ml/kg body weight in drinking water for one week (Doses were estimated based on default drinking water intake values for mice).

Group III (Aqueous extract of *Grewia tenax* treated group): mice given orally by oral gavage 0.1 ml aqueous extract of *G. tenax* at dose level 800 mg/kg body weight once per day for one and two successive weeks.

Group IV (Aqueous extract of *Grewia tenax* and formalin Co- treated group): mice received formalin at dose level 2.4ml/kg body weight in drinking water for one week and treated orally by oral gavage with 0.1 ml aqueous extract of *G. tenax* at dose level 800 mg/kg body weight once per day for one week.

At the end of the experimental period, animals from both control and experimental groups were dissected without anesthesia. A minimum of 6 animals from each group were necropsied after sacrificed by cervical dislocation on days 8, 15 post-treatment intervals to evaluate hematological and histopathological changes.

I-Morphological studies:

Clinical signs and mortality:

Animals were observed daily to note and record any changes in the behavior, depression, food intake and signs of difficulty breathing, salivation, diarrhea, muscular weakness and any signs of toxicity or mortality.

Body weight:

Body weights of mice in all groups were measured at the beginning and the end of the experiment using electronic balance. Weight gains and body weight changes (%) were calculated according to **Tütüncü *et al*. (2010)**.

II- Haematological studies:

Twenty four hours after the end of experimental period, unanesthetized mice from both control and experimental groups were sacrificed by cervical dislocation. Peripheral blood samples were collected from the neck blood vessels. Blood samples were collected into clean sterile container containing (Ethylene diamine tetra acetic acid) EDTA; as anticoagulant for hematological studies.

Red blood corpuscles (RBCs), hemoglobin concentration (Hb), Total white blood cells (WBCs) count, percentage of lymphocytes and platelets count were counted and calculated according to **Lewis *et al*. (2001)** using an automatic hematocyte analyzer DLAGON LTd Auto Hematology Analyzer D. Cell 60**.**

III-Histopathological studies:

For the light microscopic examination tissues were fixed in 10% formalin solution and Bouin's fixative for 24 hours, washed in running tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, impregnated in paraffin wax, sectioned with rotary microtome (Leica RM 2125) at 5 µm thicknesses and stained with Harri's

hematoxylin and eosin (H & E) according to **Bancroft and Gamble (2008)**. Stained sections were examined under light microscope and histopathological changes were recognized and photographed using digital camera (Nikon Eclipse E400, Japan).

For transmission electron microscopy (TEM) study small pieces of fresh specimens of spleen were removed and fixed by immersing them immediately in about 2 ml of 4F1G buffered with 0.1 M phosphate buffer (pH7.3) for 24 hours. Specimens were then postfixed in 2% OsO4 at 4°C for 2 hours, dehydrated in graded series of ethanol and embedded in Epon-araldite mixture in labeled beam capsules. LKB ultramicrotome was used to obtain ultrathin sections (50 nm thick) which were picked upon 200 mesh naked copper grids. Grids were double stained with uranyl acetate for ½ h and lead citrate for 20-30 min **(Reynolds,1963).** Scoping the grids was achieved by using Jeol 100 CX TEM.

Statistical analysis:

Data were expressed as means ± Standard Error of Mean (SEM), analyzed through one way analysis of variance (ANOVA), followed by the post hoc Duncan's test for comparison of various treatments using the SPSS software version 19.0. A p-value of less than 0.05 ($P<0.05$) was considered statistically significant. Excel programs was also used for analysis and drawing the figures.

3. Results

Morphological studies:

No abnormal signs in behavior and external features were noticed in mice administrated *G. tenax* for one week. Also, no obvious changes in behavior and external features were observed in mice treated with formalin for one week. Although, few animals showed decrease in food intake, rough coat and fall hair around mount. In addition, hypoactivities in some individuals and aggressive fighting in few others were observed. The alterations in behavior and external features were less in mice administrated *G.tenax* with formalin. No deaths were encountered in the control or treated mice during experimental period.

Our data of body weight illustrated in (Table 1) and (Fig. 2). The final body weight of mice treated with formalin for a week decreased by -1.8% comparing to initial body weight. Also, the final body weight of mice treated with formalin decreased significantly comparing to control animals. Mice treated with *G. tenax* showed slight insignificant increase in the final body weight comparing to initial body weight and it was found to be significantly decreased compared to final body weight of control group. Administration of *G. tenax* with formalin induced improvement in the final body weight comparing to formalin only treated group. While the statistical analysis revealed that the final body weight of mice treated with *G.tenax* with formalin decreased significantly comparing to control group.

Table (1): Effect of aqueous extract of *G.tenax* fruit with and without formalin on body weight (gm) gain of mice.

Time Groups	Mean of Initial body weight (gm)	Mean body weight after one week	Mean body weight after two weeks	The mean of change in body weight (%)
Control	20.7± 0.3[a]	21.6 ± 0.45[a]	22.6± 0.6[b]	9.1 %
Formalin only	21.2 ± 0.4[a]	20.2 ± 0.4[a]	20.8 ± 0.4[a]	-1. 8 %
Aqueous extract of *Grewia tenax*	20.9 ± 0.6[a]	20.9 ± 0.5[a]	21.3 ± 0.4[a]	1. 9 %
Formalin & *Grewia tenax*	21.4 ± 0.4[a]	21 ± 0.8[a]	21.6 ± 0.4[a]	.9 %

Each value represent the mean ±S.E. of body weight of survival animals in each group.
Values, within raw and colum with no common superscripts are statistically significant at P≤ 0.05

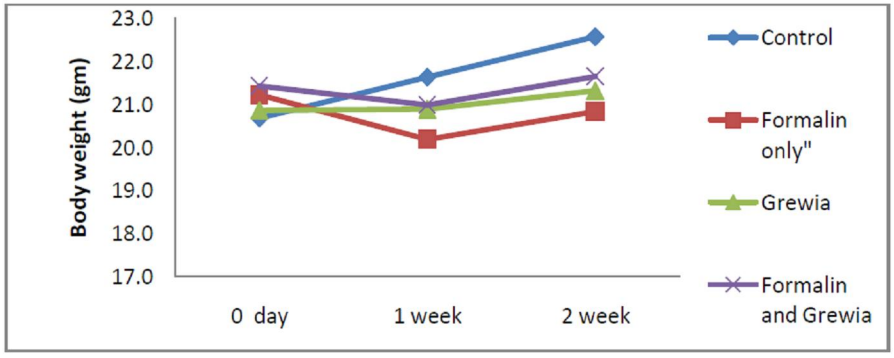

Fig. (2): Effect of aqueous extract of *G.tenax* fruit with and without formalin on body weight gain of mice.

Hematological studies:

Results of hematological parameters are shown in (Table2) and (Fig.3–10). Our study revealed that the hematological parameters of control group were within normal values. Administration of *G.tenax* fruit for one week induced significant increase in RBCs and insignificant increase in the Hb and PCV were recorded. Insignificant decrease in total WBCs count accompanied by insignificant increase in granulocytes, insignificant decrease in the percentage of lymphocyte and slight insignificant elevation in the percentage of monocytes were demonstrated. Whereas, platelet count was found to be increased significantly. In contrast to this result, administration of *G. tenax* for two weeks induced obvious disturbance in the hematological parameters. Whereas, animals treated with *G. tenax* for two weeks showed significant decrease in Hb, RBCs and PCV values comparing to control and aqueous extract of *G. tenax* for one week treated group. In addition marked but insignificant increase in total WBCs count accompanied by insignificant increase in the percentage of granulocytes and lymphocyte. While, insignificant decrease in the percentage of monocytes were recorded. Furthermore, administration *G.tenax* for two weeks caused disturbance in platelets count which was found to be significantly decreased compared to corresponding value in the control group.

Table (2): Influence of aqueous extract of *G. tenax* fruit administration after one and two weeks on the peripheral blood parameters of mice.

Group Item	**control**	Aqueous extract of ***G. tenax*** for one week	Aqueous extract of ***G. tenax*** for two weeks
Hb (g/dl)	16.9 ± 0.5^{a}	17.2 ± 0^{a}	13.4 ± 0.3^{b}
RBCs (10^{12}/L)	12 ± 0.3^{a}	12.9 ± 0.1^{b}	9.5 ± 0.3^{c}
PCV (%)	48.2 ± 0.8^{a}	50.2 ± 0.4^{a}	40.5 ± 1.2^{b}
WBCs (10^{9}/L)	11.6 ± 0.6^{a}	8.4 ± 1.6^{a}	18.5 ± 11.1^{a}
Granulocytes (%)	16.6 ± 2.6^{a}	25.8 ± 8.3^{a}	20.3 ± 0.4^{a}
Lymphocytes (%)	79 ± 2.9^{a}	70.9 ± 6.8^{a}	76.9 ± 0.8^{a}
Monocytes (%)	3.6 ± 0.4^{a}	3.6 ± 1.6^{a}	2.6 ± 1.2^{a}
Platelets (10^{9}/L)	410 ± 49.3^{a}	878 ± 8.1^{b}	130.3 ± 22.4^{c}

Each value represent the mean ±S.E. of 6 animals in each group.
Values, within row with no common superscripts are statistically significant at $P \leq 0.05$
Hemoglobin concentration (Hb). Red blood corpuscles (RBCs). Packed cell volume (PCV). White blood cells count (WBCs).

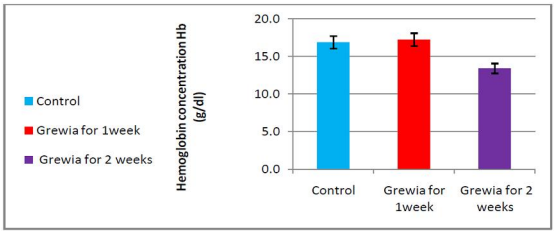

Fig. (3): Effect of aqueous extract of *G. tenax* fruit for one and two weeks on Hb (g/dl).

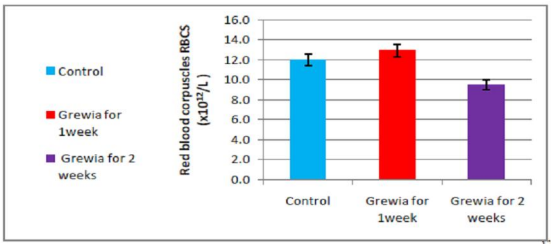

Fig. (4): Effect of aqueous extract of *G. tenax* fruit for one and two weeks on RBCS (x10^{12}/L)

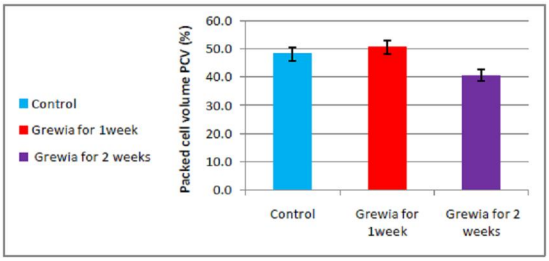

Fig. (5): Effect of aqueous extract of *G.tenax* fruit for one and two weeks on PCV (%).

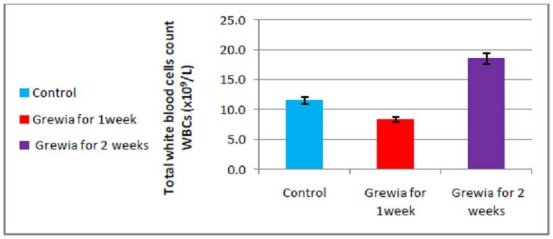

Fig. (6): Effect of aqueous extract of *G.tenax* fruit for one and two weeks on total WBCs count (x10^{9}/L).

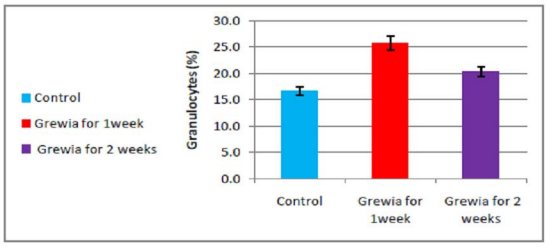

Fig. (7): Effect of aqueous extract of *G.tenax* fruit for one and two weeks on granulocytes (%).

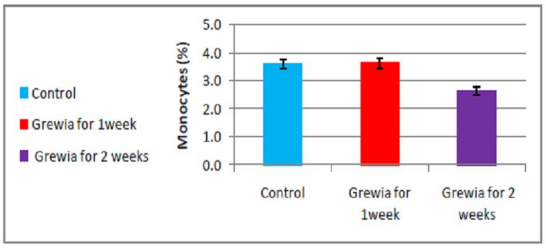

Fig. (9): Effect of aqueous extract of *G. tenax* fruit for one and two weeks on monocytes (%).

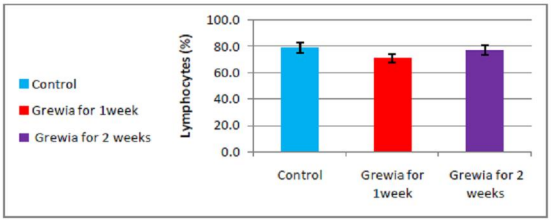

Fig. (8): Effect of aqueous extract of *G. tenax* fruit for one and two weeks on lymphocytes (%)

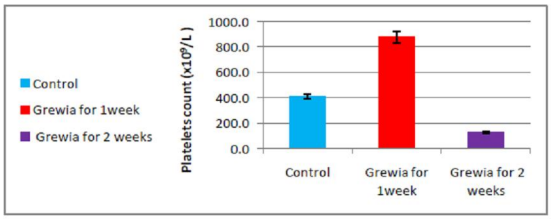

Fig. (10): Effect of aqueous extract of *G. tenax* fruit for one and two weeks on platelets count (x10^9/L).

Our data of hematological parameters are illustrated in (Table 3) and (Figs.11-18). A Statistical analysis clearly demonstrated that repeated administration of formalin for one week induced obvious disturbance in hematological parameters whereas Hb, RBCs and PCV decreased significantly. In addition, administration of formalin caused significant decrease in total WBCs count accompanied by insignificant increase in the percentage of monocytes and granulocytes while, insignificant decrease in the percentage of lymphocyte was recorded. Furthermore, significant elevation in platelets count was detected. Treatment of mice with *G. tenax* for one week exhibited insignificant alterations in most hematological parameters. However, animals treated with *G.tenax* showed significant increase in RBCs and platelets count. Administration of *G. tenax* with formalin induced ameliorating changes and inhibited the decrease in Hb, RBCs and PCV compared to formalin only treated group. Also an improvement in platelets count was demonstrated but, the value of platelets count was lower than value of (insignificant decrease) normal control group. Although, significant decrease in total WBCs count accompanied by insignificant increase in the percentage of granulocytes and monocytes and insignificant decrease in the percentage of lymphocyte were recorded.

Table (3): Enfluence of aqueous extract of *G. tenax* fruit for one week with and without formalin on peripheral blood of mice.

Group Item	Control	Formalin only	Aqueous extract of *G. tenax* only	Formalin & aqueous extract of *G. tenax*
Hb (g/dl)	16.9 ± 0.5^{ac}	15.3 ± 0.3^{b}	17.2 ± 0^{c}	15.6 ± 0.7^{ab}
RBCs (10^{12}/L)	12 ± 0.3^{a}	11.4 ± 0^{b}	12.9 ± 0.1^{c}	12.8 ± 0.2^{c}
PCV (%)	48.2 ± 0.8^{ac}	44.8 ± 0.2^{a}	50.2 ± 0.4^{c}	45.8 ± 2.6^{a}
WBCs (10^{9}/L)	11.6 ± 0.6^{a}	6.1 ± 1.4^{b}	8.4 ± 1.6^{ab}	5.4 ± 2.1^{b}
Granulocytes (%)	16.6 ± 2.6^{a}	22.7 ± 4.4^{a}	25.8 ± 8.3^{a}	20.3 ± 1.5^{a}
Lymphocytes (%)	79 ± 2.9^{a}	72.7 ± 5.8^{a}	70.9 ± 6.8^{a}	74.4 ± 2.2^{a}
Monocytes (%)	3.6 ± 0.4^{a}	4.5 ± 1.4^{a}	3.6 ± 1.6^{a}	5.3 ± 0.8^{a}
Platelets (10^{9}/L)	410 ± 49.3^{ac}	541 ± 94.1^{c}	878 ± 8.1^{b}	310 ± 46.1^{a}

Each value represent the mean ±S.E. of 5 animals in each group.
Values, within row with no common superscripts are statistically significant at $P \leq 0.05$.
Hemoglobin concentration (Hb). Red blood corpuscles (RBCs). Packed cell volume (PCV). White blood cells count (WBCs).

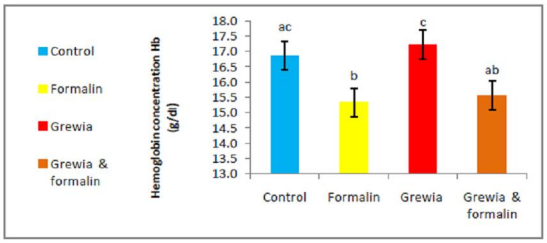

Fig. (11): Effect of aqueous extract of *G.tenax* fruit with and without formalin on Hb (g/dl).

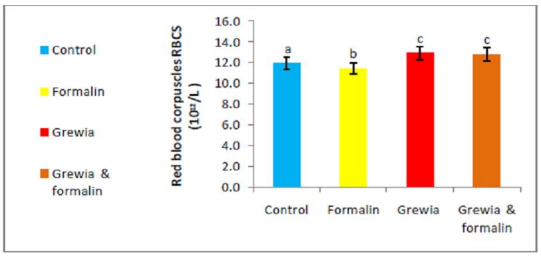

Fig. (12): Effect of aqueous extract of *G.tenax* fruit with and without formalin on RBCS (x10^{12}/L).

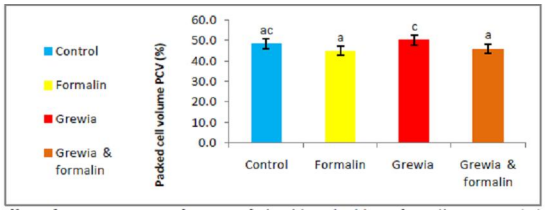

Fig. (13): Effect of aqueous extract of *G.tenax* fruit with and without formalin on PCV (%).

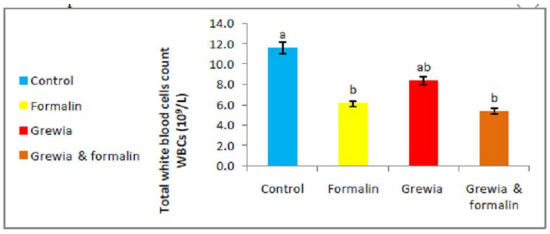

Fig. (14): Effect of aqueous extract of *G.tenax* fruit with and without formalin on total WBCs count (x10^{9}/L).

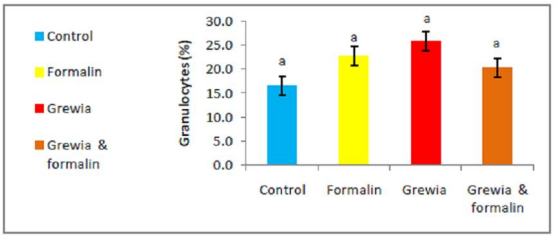

Fig. (15): Effect of aqueous extract of *G.tenax* fruit with and without formalin on granulocytes (%).

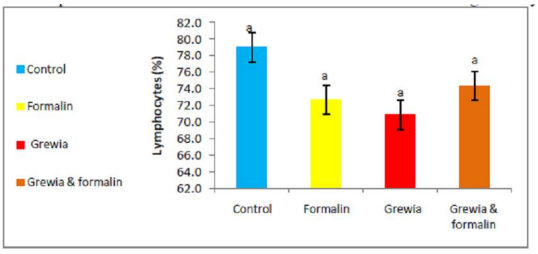

Fig. (16): Effect of aqueous extract of *G.tenax* fruit with and without formalin on Lymphocytes (%).

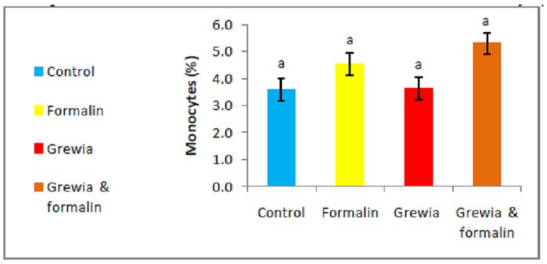

Fig. (17): Effect of aqueous extract of *G.tenax* fruit with and without formalin on monocytes (%).

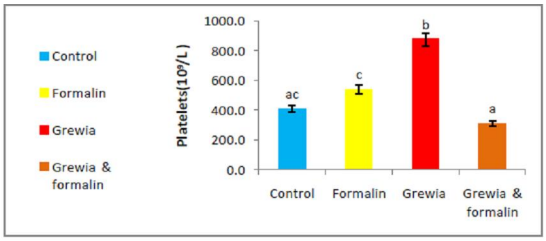

Fig. (18): Effect of aqueous extract of *G.tenax* fruit with and without formalin on platelets count (x10^{9}/L).

Histological studies:

Histological examination of the spleen sections of control mice showed normal architecture. The spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue. White pulp consisted of lymphoid follicles with central artery located eccentrically. Lymphoid follicles of white pulp separated from red pulp with well visible marginal zone. Red pulp composed of splenic cords and sinusoids. Megakaryocytes with an irregularly lobulated nuclei were visible among the cells of red pulp (Figs.19 and 20). Spleen sections of mice treated with *G. tenax* for one week showed lymphoid follicles of white pulp with normal features. Red pulp with mild hypocellularity, marginal zones were noticed. Also, few hemosedrine granules in red pulp were frequently noticed (Fig. 21). Administration of *G.tenax* for two weeks induced more severe changes include hypocellularity in white and red pulps, many necrotic cells in white pulp as manifested by pyknotic or dense stained nuclei, edema and hemosedrine in red pulp were also observed (Fig. 22).

Examination of the spleen of mice treated with formalin for one week showed many abnormal lesions included hyperplasia and disorganization of lymphoid follicles of white pulp, disappeared marginal zones, congestion and hemosedrine in red pulp. Also, many cells with dense nuclei in red and white pulps were detected (Fig. 23). Spleen sections of mice treated with formalin and *G. tenax* for one week revealed obvious alterations including reduction of lymphoid follicles size which appeared with nearly normal cellularity and depletion of splenic cells in red pulp. Some haemosidern granules and necrotic cells in red and white pulps were noticed (Figs.24 and 25).

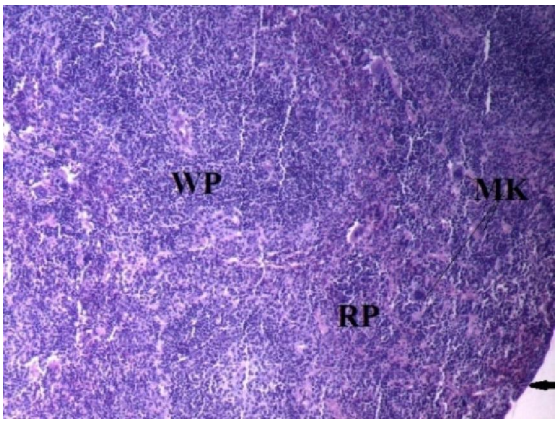

Fig. (19): A section of spleen of female mouse from control group showing normal architecture of spleen, white pulp (WP), Red pulp (RP), Capsule (Arrow), Megakaryocyte (MK) (H & E stain, X100).

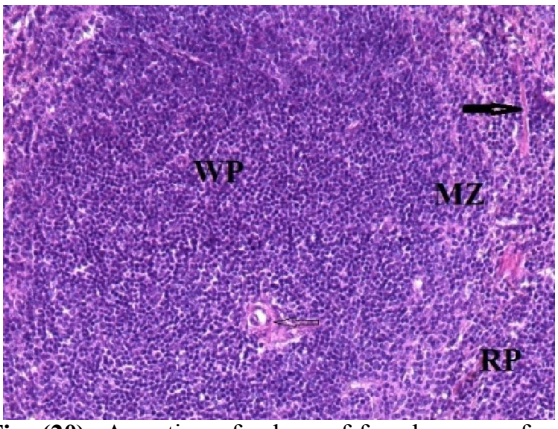

Fig. (20): A section of spleen of female mouse from control group showing normal architecture of spleen, white pulp (WP), eccentric artery (Thin Arrow), Red pulp (RP), marginal zones (MZ), Trabeculae (Arrow) (H & E stain, X200).

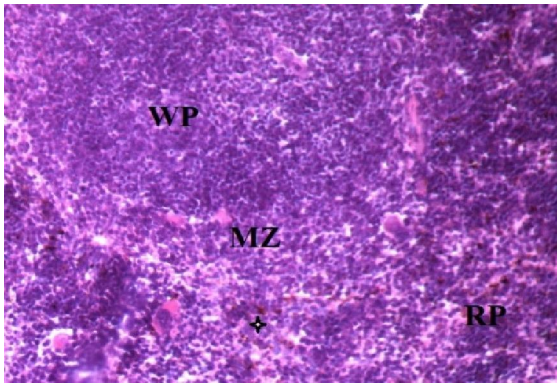

Fig. (21): A section of spleen of female mouse treated with *G. tenax* fruit for one week showing normal white pulp (WP), Red pulp (RP) with mild hypocellularity, marginal zones (MZ), Few hemosedrine (Star) (H & E stain, X200).

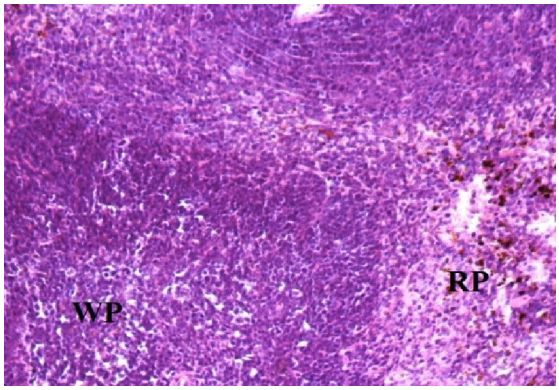

Fig. (22): A section of spleen of female mouse treated with *G. tenax* fruit for two weeks showing white pulp (WP) with many necrotic cells, hypocellularity and edema in red pulp (RP) with some haemosiderosis (H & E stain, X200).

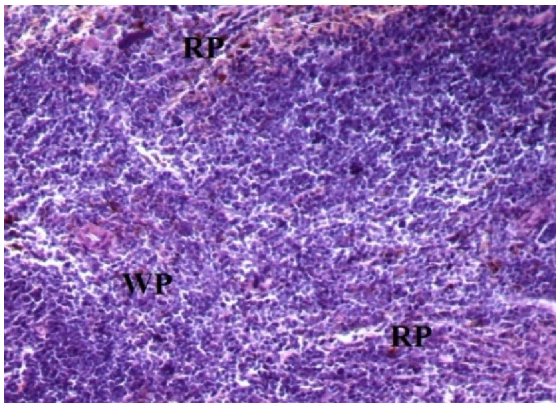

Fig. (23): A section of spleen of female mouse treated with formalin for one week showing hyperplasia and disorganization of lymphoid follicles in white pulp (WP), congestion and hemosedrine in red pulp (RP). Note diminished or disappeared marginal zones (H & E stain, X200).

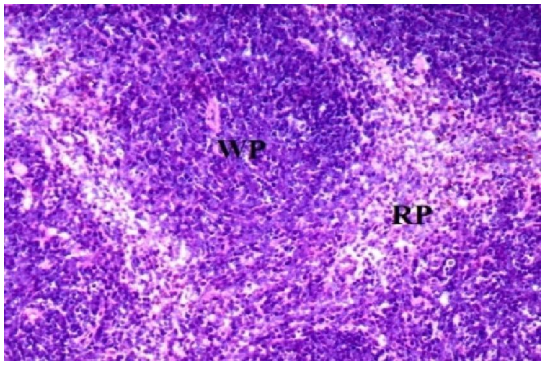

Fig. (24): A section of spleen of female mouse treated with formalin and aqueous extract of *G. tenax* fruit for one week showing hyperplasia and necrosis of some cells in spleen follicles of white pulp (WP), hypocellularity in red pulp and (RP) with few hemosedrine (H & E stain, X200).

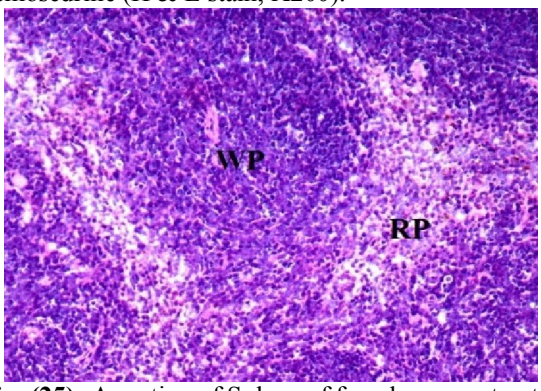

Fig. (25): A section of Spleen of female mouse treated with formalin and *G. tenax* fruit for one week showing hyperplasia and necrotic some spleen follicular cells in white pulp (WP), hypocellularity in red pulp (RP) with few hemosedrine (H & E stain, X200).

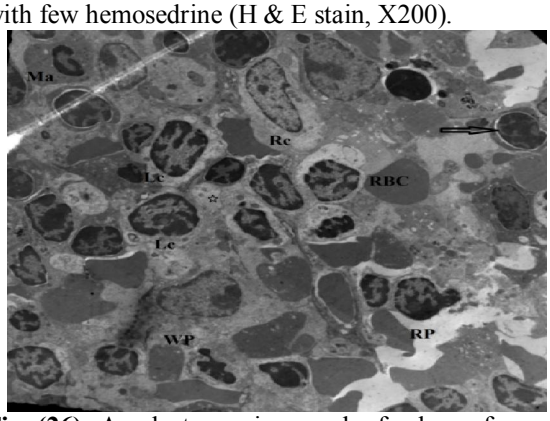

Fig. (26): An electron micrograph of spleen of mouse from control group showing splenic parenchyma with numerous cells in white pulp (WP) and red pulp (RP), lymphocytes (Lc) with free ribosomes and few small mitochondria, reticular cell (Rc), cytoplasmic processes of reticular cell (Star), red blood corpuscle (RBC), erytheroid element (Arrow).) Uranyl acetate and Lead citrate stain, X 13700).

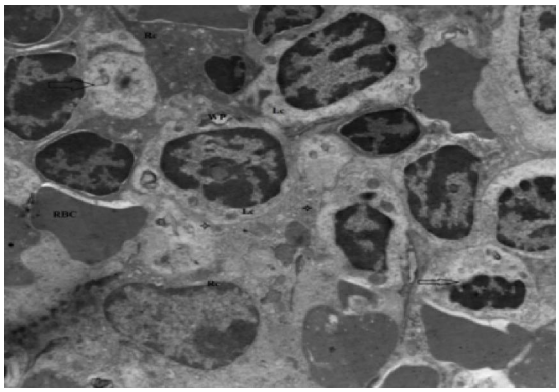

Fig. (27): An electron micrograph of spleen of mouse from control group showing numerous cells in white pulp (WP), lymphocytes (Lc) with free ribosomes and few small mitochondria, reticular cell (Rc) with cytoplasmic processes (Stars), red blood corpuscle (RBC). Necrotic cells with pyknotic nuclei (Arrows).) Uranyl acetate and Lead citrate stain, X 27500).

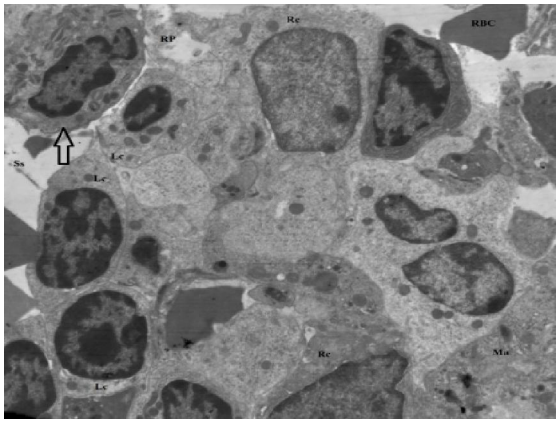

Fig (28): An electron micrograph of spleen of mouse treated with *G. tenax* showing numerous cells in red pulp (RP), lymphocytes (Lc) with free ribosomes and few small mitochondria with distinct cristae, reticular cell (Rc), macrophage (Ma), red blood corpuscle (RBC), granular leucocytes (Thick Arrow), Splenic sinus (Ss).) Uranyl acetate and Lead citrate stain, X 20600).

TEM examination of the spleen of control mice revealed that the spleen displayed splenic parenchyma composed of white and red pulps. The white pulp contained numerous cells including many polymorphic lymphocytes supported by reticular meshwork of reticular cells and reticular fibers. Few number of platelets, macrophage and occasional RBCs were present. Lymphocytes displayed varied size and showed large polymorphic nuclei with moderate amount of heterochromatin and prominent nucleoli in most of these nuclei were seen. These cells also displayed scant cytoplasm with many free ribosomes and few small mitochondria with distinct

cristae. Reticular cells were branched cells having ovoid nuclei with peripheral heterochromatin condensation. Most reticular cells showed less dense cytoplasm contained dense mitochondria and short strips of rER. Macrophage cells were irregular in shaped had large nuclei with loose peripheral heterochromatin and exhibited less dense cytoplasm with moderate amount of ribosomes and some dense materials. Also, few necrotic cells with pyknotic nuclei were noticed. The red pulp appeared as anatomizing fenestrated splenic sinusoids separated from each other by splenic cords composed of reticular cells, macrophage cells and granular leucocytes with segmented nuclei having peripheral heterochromatin patches and the cytoplasm contained many specific granules. Also, few platelets, RBCs and erytheroid elements were seen (Figs. 26 and 27). Administration of *G. tenax* for one week did not induce ulterastructure alterations in the spleen tissue of female mice. The cells of white and red pulps with normal features were demonstrated. Most cells displayed nuclei with normal chromatin features and exhibited cytoplasm with normal free ribosomes content and small mitochondria with distinct cristae (Figs. 28 and 29).

Ultrastructure examination of spleen of mice treated with formalin for one week showed sever changes in splenocytes of white and red pulps. Such changes in white pulp included depletions of lymphocytes and destructed or less distinct cell membrane in many cells. Such cells showed vacuolated cytoplasm and exhibited nuclei with abnormal chromatin features. In addition, some splenocytes with indistinct nuclei and displayed chromatin features in prophase stage of mitotic division were seen. However, accumulation of dense materials in cytoplasm of some seplenocytes were evident. In red pulp; marked congestion and increase granular leukocytes were detected. Many splenocytes in red pulp showed nuclei with abnormal chromatin features and exhibited cytoplasm with indistinct details. Also, some necrotic cells with pyknotic nuclei were frequently seen. Such cells contained few dense mitochondria with less distinct cristae. Furthermore, some splenocytes cytoplasm displayed destructed organelles and few short strips of rER with depilation of ribosomes (Figs. 30 - 32). Examinations of spleen of mice Co-treated with formalin and *G. tenax* for one week revealed that *G.tenax* fruit succeeded to lessen most abnormal ultrastructure alterations comparing to formalin only treated group. Most splenocytes showed obvious improvement in nuclear chromatin features and vacuolization in splenocytes. However, dense mitochondria and some necrotic cells were still presented. Some granular leukocytes and plasma cells with large eccentric nuclei and cytoplasm occupied with rER were seen (Figs. 33 and 34).

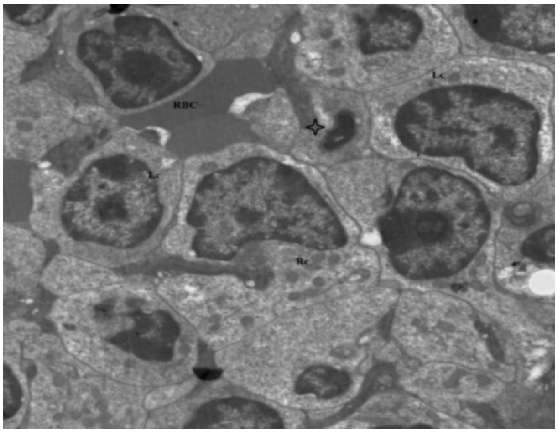

Fig. (29): An electron micrograph of spleen of mouse treated with *G. tenax* showing numerous cells in white pulp; lymphocytes (Lc) displaying nuclei with normal chromatin features and contains free ribosomes and few small mitochondria, Reticular cell (Rc), red blood corpuscle (RBC). Necrotic cells with pyknotic nuclei (Star).) Uranyl acetate and Lead citrate stain, X 27500).

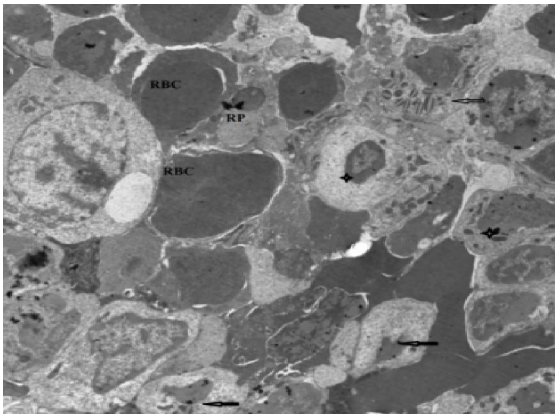

Fig. (30): An electron micrograph of spleen of mouse treated with formalin for one week showing congested red pulp (RP), granular leukocyte (Arrow), necrotic cells (Thick Arrows). Note many splenocytes displaying nuclei with abnormal chromatin features and small dense mitochondria with less distinct cristae, destructed organelles and short strips of rER with depletion of ribosomes (Stars), Red blood corpuscles (RBCs).) Uranyl acetate and Lead citrate stain, X20600).

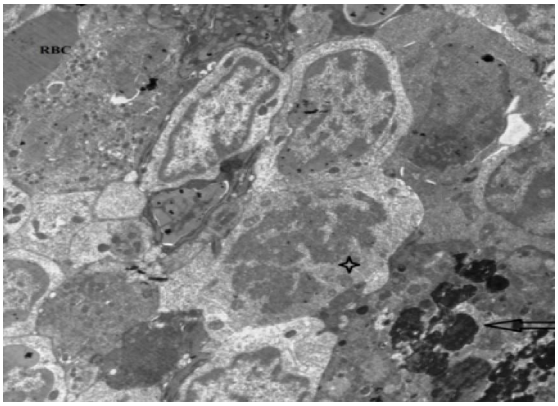

Fig. (31): An electron micrograph of spleen of mouse treated with formalin for one week showing depletions of lymphocytes, many cells with destructed or less distinct cell membrane and vacuolated cytoplasm displaying nuclei with abnormal chromatin features. Splenocytes in prophase stage of mitotic division (Star), Seplenocyte with accumulation of dense materials in the cytoplasm (Arrow), red blood corpuscle (RBC).) Uranyl acetate and Lead citrate stain, X 27500).

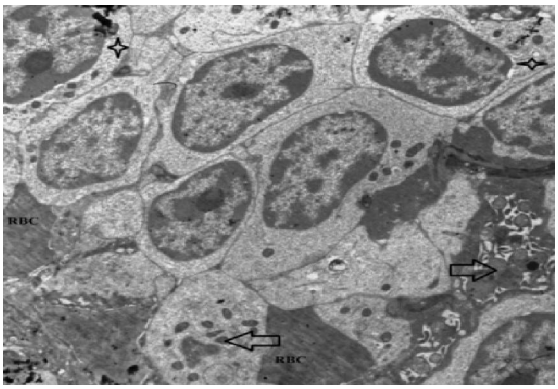

Fig. (32): An electron micrograph of spleen of mouse treated with formalin for one week showing necrotic splenocytes with destructed cytoplasmic rganelles (Arrows), lymphocytes with small dense mitochondria with less distinct cristae (Stars), Red blood corpuscles (RBCs).) Uranyl acetate and Lead citrate stain, X 27500).

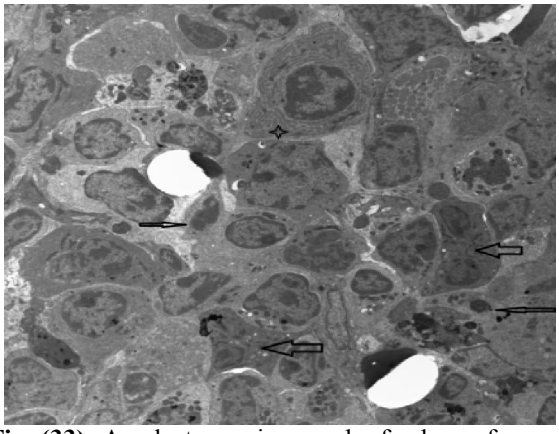

Fig. (33): An electron micrograph of spleen of mouse Co- treated with formalin and *G. tenax* showing splenocytes with improvement in nuclear chromatin features. Note dense mitochondria, necrotic cells (Arrows), granular leukocytes (Thick Arrows), plasma cells with large eccentric nuclei and cytoplasm occupied with rER (Star).) Uranyl acetate and Lead citrate stain, X 13700).

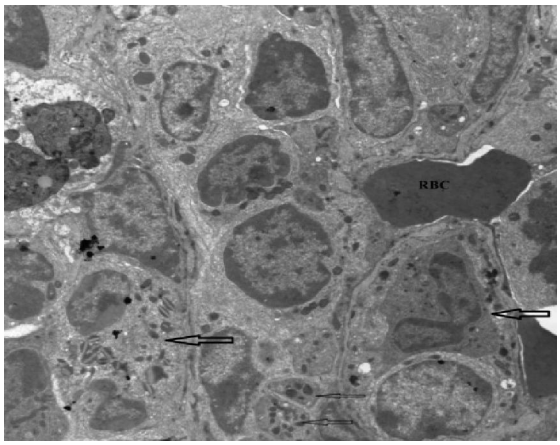

Fig. (34): An electron micrograph of spleen of mouse Co- treated with formalin and *G. tenax* showing splenocytes with improvement in nuclear chromatin features. Note dense mitochondria, granular leukocytes (Arrrows), Red blood corpuscle (RBC), platelets (Thin Arrows).) Uranyl acetate and Lead citrate stain, X 27500).

4. Discussion

In the present work the results of morphological study were in agreement with previous study which demonstrated that no deaths or remarkable changes in general appearance or behavior and external features were observed in male rats fed diet containing 10% *G. mollis* stem bark daily for four weeks **(Obidah *et al.*, 2010)**. No clinical signs and symptoms of toxicity were recorded in mice administered *G. tenax* extract dissolved in distilled water via oral route at various doses, ranging from 50 to 2000 mg/kg to different groups of mice **(Al-Said *et al.*, 2011)**. **Pitten *et al.* (2000)** reported that inhaled formaldehyde had been shown to cause behavioral disorders in rats and had been classified as probably neurotoxic.

It was reported in preliminary phytochemical studies that plant extracts of Grewia in different solvents were found to contain diterpines, glycosides, fats, alkaloids, glycosides, triterpenoids, sterols flavonoids, saponins, tannins **(Patil *et al.*,2011 and Siddiqi *et al.*,2011). Sini *et al.* (2010)** reported that the presence of tannins and other compounds may be

interferes with absorption of nutrient such as proteins and minerals resulting in weight loss which may explain decrease body weight in mice treated with aqueous extract of *G. tenax*.

Formaldehyde inhalation was observed to cause a reversible decrease in food and water consumption, and body weight gain in rats **(Zararsiz *et al.*, 2006)**. Male and female rats exposed to formaldehyde in drinking-water or by inhalation showed lower food and liquid intake and lower body weights **(Rusch *et al.*, 1983 and Til *et al.*, 1989)**. The reduction in body weight gains may be due to the combined action of cholinergic and oxidative stress and/or due to the increased degradation of lipids and proteins as direct effects of toxic compound exposure **(Goel *et al.*,2005; Mansour and Mossa,2010 and Saafi *et al.*, 2011)**. Significant reduction in food and water intake was suggested as being responsible for the observed decrement in body weight gain. Loss of appetite is often synonymous with weight loss due to disturbances in carbohydrate, protein or fat metabolisms (**Klaassen, 2001)**. Moreover, decreased body weight gain may result from interfere with gastric function and decreased food conversion efficiency **(Chokshi, 2007)**. The body weight changes serve as a sensitive indication of the general health status of animals (**El Hilaly *et al.*, 2004)**.

Oral administration of aqueous extract of *G.tenax* with formalin in the present study induced improvement in the final body weight comparing to formalin only treated group. While the statistical analysis revealed that the final body weight of mice treated with *G. tenax* alone or with formalin decreased significantly comparing to control group. This was found to be consistent with other studies where food intake decreased significantly in animals fed diet containing 10% *G. mollis* stem bark powder daily for four weeks as compared to control animals while, no remarkable changes were observed in the mean body weight gain **(Obidah *et al.*, 2010)**.

In the present work treatment of mice with *G. tenax* for one week exhibited insignificant alterations in most hematological parameters. While, significant increase in RBCs, platelets count and insignificant increase in Hb were recorded. This finding supports the traditional use of *Grewia spp* in the treatment of anaemia. This was found to be consistent with other previous studies where **Al-Said *et al.* (2011)** reported that oral administration of ethanol extract of *G.tenax* at doses of 250 and 500 mg/kg for 3 weeks induced significant increase in the hemoglobin level in rats and the same author validates its folkloric use in anemic and other conditions. Also, analysis of the nutritional composition of fruits of three species of *Grewia* (G. *tenax, G. flavescens* and *G. villosa*) showed that the three *Grewia* spp contained remarkably high amounts of iron and all *Grewia* spp had similar distribution of amino acids but in varying amounts **(Mohammed Elhassan and Yagi, 2010).** In contrast repeated oral administration of aqueous extract of *G. tenax* for 2 weeks induced obvious disturbance in the hematological parameters; significant decrease in Hb, RBCs and PCV values compared to corresponding values in control group. It was reported that the decrease in RBCs count may be returned to the reduction in erythropoiesis in bone marrow and faster rate of destruction of peripheral RBCs in spleen **(Coles 1986)**. Decreased Hb can be related to reduction in size of RBCs, impaired biosynthesis of heam in bone marrow or due to reduction in the rate of formation of RBCs **(Mahmoud and Elbessoumy, 2013)**. Such disturbance in the hematological parameters may be related to iron overload. Although, iron deficiency anemia represents a common nutritional problem which affects many societies all over the world and iron fortified diet had been suggested as one of possible tools to combat and solve such problem **(El-Seweidy *et al.*, 2010)**. Moreover, iron is an essential element in the body, being found in functional form in hemoglobin, myoglobin, cytochromes and enzymes. At the same time, excess iron in the body is associated with toxic effects and posses health problems **(Lebda, 2014)**.

In addition, present study clearly demonstrated that administration of of *G. tenax* fruit for two weeks induced significant decrease in the platelets count. Reduction in platelets number had also been associated with some herbal remedy **(Cheesbrough, 2005)**. Other studies have reported the toxic effects of herbal medicines **(Calixto,2000 and Jaouad *et al.*, 2004).** Also, toxicity of high dose of aqueous extract of *G. tenax* fruit was explained by **Khemiss *et al.* (2006)**. In the present work significant increase in the platelets count were recorded in mice treated with formalin only or aqueous extract of *G. tenax* fruit only for one week. Megakaryocytes give rise to circulating platelets and there is a relationship between the increase of megakaryocytes and platelets **(Deutsch and Tomer, 2006)**. However, abnormal proliferation of megakaryocytes can cause malignant diseases like megakaryocytic leukemia, characteristics of which include proliferation of abnormal megakaryocytes and myelofibrosis **(Oki *et al.*,2006).** It was also reported that occupational formaldehyde exposure was associated with a decrease in platelets counts **(Tang *et al.*, 2009)**.

In the present study obvious disturbance in hematological parameters was also recorded in formalin only treated group whereas Hb, RBCs and PCV decreased significantly indicate that formalin induce anemia. In addition, administration of formalin caused significant decrease in total WBCs count. Our

findings was found consistent with other studies where the total leucocytes count significantly declined in male mice exposed to formaldehyde gas at levels 28 ppm, during 8 hours/day over a period of 30 consecutive days **(Louei Monfared *et al*., 2013).** The alterations in hematological changes serve as the earliest indicator of toxic effects on tissues **(Barber *et al*., 2011)**. Our results were in agreement with previous studies which had demonstrated that exposure of male mice to formaldehyde at dose 0.5 and 3.0 mg/m^3 by nose-only inhalation for 8 hours/day over a two weeks period induced significant decrease in counts of WBCs, RBCs, lymphocytes. Moreover, the counts of monocytes and granulocytes in exposed groups were not significantly altered relative to controls **(Zhang *et al*., 2013)**. It was also reported that occupational formaldehyde exposure was associated with a decrease in WBCs count **(Tang *et al*., 2009)**. **Zhang *et al*. (2010)** found that formaldehyde-exposed workers had lower counts of WBCs, granulocytes, platelets, RBCs, and lymphocytes than did non-exposed workers. Decreased erythrocytes and leukocytes count, hemoglobin concentrations, and hematocrits had also been reported in Japanese quail fed formalin at 10 and 20 ml/kg **(Khan *et al*., 2005)**. Birds fed formalin mixed homogeneously in food at dose 10 ml/kg showed significant decrease in RBCs count, hemoglobin concentration, PCV, and leukocytes count **(Khan *et al*., 2006)**. A decreasing trend in erythrogram in birds given higher levels of formalin suggests that formalin might have an inhibitory effect on the synthesis of these cells in bone marrow.

Administration of aqueous extract of *G. tenax* fruit with formalin induced ameliorating changes and inhibited the decrease in Hb, RBCs and PCV compared to formalin only treated group. Also, an improvement in platelets count was demonstrated. The ameliorating changes in most hematological parameters of mice treated with *G. tenax* and formalin may be related to antioxidant properties of the contents of *G. tenax* extract. Animals treated with *G. tenax* ethanol extract fruits effectively improved the hemoglobin level. These findings substantiate the folkloric use of *G. tenax* ethanol extract fruits for the prevention and treatment of anemic conditions **(Al-Said *et al*., 2011)**. Reactive oxygen species are known to initiate, promote, or amplify oxidative damage **(Ramesh *et al*., 2010)**.

Histopathological methods are commonly used for detecting and evaluating organ-specific effects related to chemical exposure **(Travlos *et al*.,1996 and Crissman *et al*.,2004)**. In the present work no obvious histological alterations in spleen sections of mice treated with *G. tenax* for one week. While, administration of *G. tenax* for two weeks induced some changes include hypocellularity in white pulp and red pulp, many necrotic cells in white pulp, edema and hemosedrine in red pulp. This observation is in line with hematological finding in the present work. These abnormal observations may be attributed to severe toxic effect of excess iron on different organs; since acute iron overload induced significant deposition of iron in rat's organs associated with exacerbated oxidative stress status and remarkable alterations of antioxidants **(Lebda, 2014)**. Moreover, adult male rats which were fed on biscuits fortified with iron daily for 10 weeks showed increased serum iron, ferritin, tumor necrosis factor alpha, nitric oxide and decreased testosterone level. Testicular tissues showed significant increase of iron content and decreased glutathione as compared to control group **(El-Seweidy *et al*., 2010)**. This reduction of glutathione level leads to elevation of lipid peroxidation **(El-Maraghy *et al*.,2001)**.

In this study, spleen of mice treated with formalin for one week showed many abnormal lesions include disorganization of lymphoid follicles and depletions of lymphocytes in white pulp, disappeared marginal zones,, marked congestion, hemosedrine and increase granular leukocytes in red pulp, all of these changes may be attributed to a loss of infiltration efficiency and may be related to oxidative stress of formalin. Also, the cellularity of spleen was affected by the formalin administration. Splenic immunosuppression may be attributed to the decreased different lymphatic cells numbers in the spleen as well as other immune organ **(Monfared *et al*., 2014)**. Formaldehyde is identified as one of the causative agent of oxidative stress **(Rasyidah *et al*., 2014)**. Some epidemiological studies of industrial workers, embalmers and pathology anatomists have indicated association of formalin exposure with elevated cancer risks at various sites, including the brain, nasal cavities and lungs **(Coggon *et al*., 2003)** and lymphohematopoietic system **(Hauptmann *et al*., 2003 and Pinkerton *et al*.,2004)**. Histological study, histometrical analysis and peripheral blood cells counting indicate that inhalation of formaldehyde gas has a harmful impact on the immunocompetent organs in mice **(Louei Monfared *et al*., 2013)**. According to **Elmore (2006)** formaldehyde may act as antigens. A severe immune reaction to antigens could result in an increased cellularity in the B-cell areas and an increase in secondary follicles with major germinal centers. These effects lead to an increase in the white pulp area, follicle diameter, germinal centre area and marginal zone diameter **(Golalipour *et al*.,2008).** Other studies have shown that formaldehyde can increase lymph node weight during oral consumption, although there is no effect on the cellularity of lymphoid tissue (**Vargova *et al*.,1993**). **Medical**

Management Guidelines for Formaldehyde Agency for Toxic Substance and Disease Registry (2002) reported that formaldehyde can interact with molecules on cell membranes and in body tissues and fluids and disrupt cellular functions. High concentrations cause precipitation of proteins, which results in cell death. Absorption from the respiratory tract is very rapid; once absorbed, formaldehyde is metabolized to formic acid, which may lead to acid-base imbalance and a number of other systemic effects.

Formalin also was found to induce destructed or less distinct cell membranes of splenocytes and vacuolated cytoplasm were also seen, Interpretation of vacuolar formation following chemical treatments had been subjected to wide speculations by many investigators. **Robbins and Angell (1976)** regarded such vacuolation to represent primary morphologic response to many forms of cell injury. Lipid peroxidation is a multistep reaction that can result in destruction of cellular membranes.

Regarding ulterastructure examination in the current work formalin caused the presence of abnormal and irregular nuclear shapes in the spleen and some necrotic cells with pyknotic nuclei. Also, few dense mitochondria with less distinct cristae and destructed organelles. It was previous reported that, the nucleus is one of the most prominent cellular organelles within an eukaryotic cell, and altered nuclei shape is considered to be important for cell function **(Webster *et al*.,2009)**. The detection of pyknosis in the spleen may be related to an increase in T cell susceptibility to apoptosis, which may be an important mechanism of autoimmune diseases and immune senescence **(Hsu and Mountz, 2003).** It had been speculated that changes in nuclei shape might lead to changes in chromosome organization, which in turn can affect gene expression **(He *et al*.,2008).** On the other hand, an abnormal nuclei shape is also associated with cancer **(Zink *et al*.,2004).** The condensation of nucleus is believed to be one of the two major morphological features of apoptosis, the cell suicide program **(Wang *et al*.,2011).** Mitochondrial permeability transition is a key mechanism underlying both apoptosis and necrosis. Mitochondrial function is important in the regulation of cellular life and death, including disease states, and the disturbance in mitochondrial function and distribution can be accompanied by significant morphological alterations **(Mumcuoglu *et al*.,2012).**

Light and TEM studies of spleen of mice Co-treated with formalin and *G. tenax* for one week revealed that the damage found in the spleen of group receiving formalin was still detected. Although, *G. tenax* succeeded to lessen most of the abnormal alterations comparing to formalin only treated group. Grewia fruits are a rich source of nutrients such as proteins, amino acids, vitamins, and minerals and contain various bioactive compounds, like anthocyanins, tannins, phenolics and flavonoids **(Zia-Ul-Haq *et al*.,2013)**. The plant species has free radical scavenging activities which may be responsible for the therapeutic action against tissue damage **(Martins *et al*., 2008)**. The role of aqueous extract of *G. tenax* fruit in restoring the tissue and ameliorating the toxic and hazardous disorders induced on the spleen organelles may be due to a high antioxidant activity of this extract (**Kshirsagar and Upadhyay,2009**). In human and animal bodies, reactive oxygen species can be neutralized by antioxidant defense systems including antioxidant enzymes (**Fang *et al*., 2002**) and antioxidant compounds **(Catapano *et al*.,2000).**

Conclusion

Administration of aqueous extract of *Grewia tenax* fruit for one week lessened toxicity in mice intoxicated with formalin. However, some abnormalities were still detected in mice receiving both formalin and aqueous extract of *G. tenax* fruit. The improvement in hematological, histological and ultrastructure findings may related to flavonoids and other antioxidant constituents in this plant. However, the present study suggested carefully that administration of *G.tenax* fruit for two weeks in traditional treatment and further studies concerned with aqueous extract of *G. tenax* fruit with different doses and durations and its effects on other organs will be need to prove the effective of aqueous extract of *G. tenax* fruit (guddaim) in the inhibition of oxidative effects resulted in formalin exposure.

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