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# Synergistic Effect of *Zingiber officinale* and *Nigella sativa* Combinations in Ameliorating Carbon tetrachloride-Induced Liver Injury in Female Rats

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**Abstract** The objective of this study is to evaluate Synergistic effect of *Zingiber officinale* (Ginger) and *Nigella sativa* (black cumin) combinations in ameliorating Carbon tetrachloride- Induced Liver injury in Female Rats. Evaluation was done through measuring certain hematological parameters, hepatic function markers, lipid and protein profiles in serum as well as the lipid peroxidation and endogenous antioxidants content in the liver were analyzed. CCl4 diluted 1: 9 (v/v) in olive oil was injected intraperitoneally followed by *Ginger* (G) and *Nigella sativa* (N) mixed extracts (200 mg/kg body weight) were administered orally. The $CCl_4$-treated rats showed a significant decline in the studied hematological parameters, the serum levels of high-density lipoprotein (HDL), albumin (A) as well as the hepatic levels of glutathione (GSH) and activities of catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR). This was accompanied by a significant elevation in the levels of total lipids (TL), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), globulin (G), total bilirubin (TBil) and the activities of alanine and aspartate aminotransferase (ALAT and ASAT) and alkaline phosphatase (ALP) as well as the hepatic levels of malondialdehyde (MDA). In contrast, the administration of (G)and (N) mixed extracts, notably improved all the studied parameters. This study disclosed that $CCl_4$ administration to Wistar rats, at a high dose level, could induce a hepatic injury in addition to certain hematologic and metabolic alterations. The work was extended to examine tissue histopathology. Yet, the treatment with (G) and (N) mixed extracts could ameliorate these alterations via their antioxidative effect. In conclusion, ginger and Nigella mixed ethanolic extracts, resulted in an attractive candidate for ameliorating of hepatotoxicity induced by $CCl_4$ through scavenging free radicals, improved liver functions, and normalizing the liver histopathological architecture. Further studies are required in order to identify the molecules responsible of the pharmacological activities.

**Keywords** *Zingiber officinale, Nigella sativa*

## Introduction

The liver is responsible for metabolism and detoxification of the most of components that enter the body [1]. Carbon tetrachloride ($CCl_4$) is a highly toxic chemical agent, the most famous drug used to induce liver damage experimentally. Histopathological sectioning of the liver tissues indicated that, $CCl_4$ induced fibrosis, cirrhosis and hepatocarcinoma [2]. The toxic effect of $CCl_4$ is attributed to trichloromethyl radical produced during oxidative

stress [3]. The number of infiltrated neutrophils, macrophages, Kupffer cells, lymphocytes and natural killer cells are significantly increased after liver injury induced by hepatotoxins such as $CCl_4$. It induced activation of liver resident macrophages and/or chemoattraction of extrahepatic cells (e.g. neutrophils and lymphocytes; [4]). The activated macrophages are released and contributed to liver fibrosis, inflammation and injury [5]. Once the liver became injured, its efficient treatment with famous chemical drugs is limited [6]. Therefore, interest concerned the use of alternative medicines for the treatment of hepatic disease has been arisen. Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Medicinal use of spices/herbs has been gradually increasing in developed countries. *Zingiber officinale* roscoe, commonly known as ginger belongs to the Zingiberaceae family is one of the most commonly used spices in India and around the world. It is an indispensable component of curry, belongs to Zingiberaceae family. The rhizome of ginger has an aromatic pungent taste. It is consumed worldwide as a spice and flavoring agent, and is attributed to have many medicinal properties. It is used in traditional medicine as carminative, and antipyretic, and in the treatment of pain, rheumatism and bronchitis [7]. It has different pharmacological activities such as hepatoprotective [8], antiparasitic [9], antimalarial [10], antimicrobial [11], antidiabetic [12], and radioprotective effects [13]. The plant contains high level of phenolic and flavonoid compounds, responsible for its high antioxidant activities [14]. On the other hand, *Nigella sativa* Linn. *(N. sativa)* belongs to family Ranunculaceae is a herbaceous annual plant that commonly known as black cumin and black seed [15]. *N. sativa* has been traditionally used in India, Europe, Middle East, Far East, and South-East Asia as spices and natural remedy for several ailments including asthma, headache, infections, obesity, fever, vertigo, hypertension, influenza, and cough [16]. There is also an Islamic belief that black cumin is a remedy for all illnesses, except ageing and death [17]. *N. sativa* major chemical components are 36-38% fixed oil, 0.4-2.5% essential oil, alkaloid, saponin, mineral elements, proteins, vitamins, and carbohydrates [18]. It has been reported that *N. sativa* exhibits many pharmacological effects, including antioxidant [19], anti-inflammatory [20], antimicrobial [21], antidiabetic [22], antihypertensive [23], Neuroprotective [24], and anticarcinogenic [25] properties. It has also been shown that *N. sativa* seeds and/or its constituents have protective effects against nephrotoxins. Yaman and Balikci reported that *N. sativa* oil (0.2 ml/kg) protected the rats from gentamicin-induced nephrotoxicity [26]. Furthermore, another study revealed a protective effect of thymoquinone against doxorubicin-induced cardiotoxicity [27].

Based on these findings, we hypothesized that the combination of these medicinal plants may produce a higher protective effect against Carbon tetrachloride- induced liver and kidney injuries. Therefore, in the present study, we investigated the synergistic effect of *Zingiber officinale* and *Nigella sativa* combinations in ameliorating Carbon tetrachloride- induced hepatotoxicity and nephrotoxicity in female rats through assaying liver and kidney functions, lipid profiles and histopathology of liver tissues.

## Material and Methods

Chemicals: All chemicals in the present study were of analytical grade, products of Sigma (US), Merck (Germany), and BDH (England).

### Plant Collection

*Zingiber officinale* (Ginger rhizomes) [G] and *Nigella sativa* (black cumin seeds) [N] were purchased froma local market (Hyper One Market, 6th October City, Egypt). Dried Ginger rhizomes were ground in a grinder with 2 mm diameter mesh. Five hundredgrams of dry powder was kept in tightly closed container until needed.

### Plant Extraction

1. ***Zingiber officinale*** **(Ginger) [G]:**

The dried powered rhizome (125 grams) was sequentially extracted in a Soxhlet (Toshiba, India) apparatus using 70 % ethanol for 72 h. Solvent removal was carried out under vacuum using rotatory evaporator for drying at 40◦C, producing a semisolid residue of 9.8 grams.

**2. *Nigella sativa* (black cumin) [N]:**

Seeds (125 grams) were sequentially extracted in a Soxhlet (Toshiba, India) apparatus using 70 % ethanol for 72 h. Solvent removal was carried out under vacuum using rotatory evaporator for drying at 40◦C, producing a semisolid residue of 10.7 grams.

The produced (Ginger) [G] and (black cumin) [N] ethanolic extracts were mixed as equal W/W.

***Doses of Administration***

Administration regime was twice a week for six consecutive weeks. Five hundred microliters of $CCl_4$ diluted 1: 9 (v/v) in olive oil was injected intraperitoneally [28], followed by [G] and [N] alone or mixed extracts (200 mg/kg body weight) were administered orally [9].

-Animals**.** Female Wistar albino rats (100 to 120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet ad libitum**.**

-Ethics**.** Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

- Doses of Administration**.** Administration regime was twice a week for six consecutive weeks. Five hundred microliters of $CCl_4$ diluted 1: 9 (v/v) in olive oil were injected intraperitoneally (0.1 ml). G and N mixed extracts (200 mg/kg bodyweight) were administered orally after intraperitoneal injection of $CCl_4$.

**Experimental Design**

24 male rats were used in this study. Animals were divided into 4 groups (6 rats each) as following:

-Group 1 served as normal healthy control rats.

-Group 2: Rats were intraperitoneally injected with $CCl_4$ alone.

-Group 3: Rats were intraperitoneally injected with $CCl_4$ followed by oral administration of (Ginger) [G] extract (200 mg/kg bodyweight).

-Group 4: Rats were intraperitoneally injected with $CCl_4$ followed by oral administration of (black cumin) [N] (200 mg/kg bodyweight).

-Group 5: Rats were intraperitoneally injected with $CCl_4$ followed by oral administration of (Ginger) G and (black cumin) N mixed extracts (200 mg/kg bodyweight).

**Hematological and Biochemical studies**

***1- Sample Preparations:***

Blood was collected from each animal by puncture of sublingual vein. Blood samples were divided into two parts. The first part was collected on EDTA for hematological analyses. The second part was collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum. The sera were kept at -20 ºC for further biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected. The liver was homogenized with 10% w/v ratio in ice-cold 50 mMTrisHCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4EC. The supernatant was collected and kept in deepfreeze at -20 ºC for further analyses.

***Estimation of hematological parameters:***

The hematological parameters including red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, hemoglobin (Hb) content and packed cell volume (PCV) were analyzed using Medonic M-Series analyzer (Clinical Diagnostics solutions Inc, Florida, USA).

***Estimation of serum biochemical parameters:***

1-In the serum of all the experimental groups: the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline

phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured colorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt). Lipid peroxide assay: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the technique described by Ohkawa et al [29]. The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in acidic medium.

2-Non-enzymatic and enzymatic antioxidant assay: The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats. The method by which GSH content was measured was based on the reaction of 5,5'-Dithiobis-2-nitrobenzoic acid with GSH [30]. The CAT activity was estimated in accordance to the method described by Aebi et al. [31]. The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitrobluetetrazolium dye mediated by phenazinemethosulphate [32]. The principle for measuring the GR activity was based on its ability to catalyze the reduction of glutathione (GSSG) as described by Goldberg and Spooner [33].

***The Comet Assay:***

Comet assay was performed referring to the protocol developed by Blasiak et al [34], with minor modifications. Rats liver cells of each treatment were mixed with low-melting-point agarose (ratio of1:10v/v), then pipetted to precoated slides with normal-melting-point agarose. The slides were kept flat at 4°C for 30 min in dark environment. The third layer of low melting point agarose was then pipetted on slides, left to solidify at for 30 min 4°C. The slides were transferred to pre-chilled lysis solution, kept for 60min at 4°C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300mAmps at 4°C for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (510–560 nm, barrier filter 590 nm) with a magnification of ×400. 100 cells per animal were scored then analyzed with DNA damage analysis software (Comet Score, TriTek corp., Sumerduck, VA22742).

***Statistical analysis:***

Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA). Two-way analysis of variance (ANOVA) was used to study the effect of the type of treatment on tested groups (control; Rats were intraperitoneally injected with $CCl_4$ alone; Rats injected with $CCl_4$ followed by oral administration of different tested extracts. Data were expressed as a mean ± standard error of mean (SEM).

***Histopathological Study***

After blood sampling, animals were dissected and brains, liver and kidney of each group were removed carefully. The organs were fixed in buffer formalin for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax. Sections of 6μm thickness were prepared and stained with Haematoxylin and eosin [35].

**Results and Discussion**

**Effect on hematological parameters:** The results of hematological parameters (Table 1) revealed that the type of treatment significantly affected all the studied blood parameters except for the PLT count that did not show any significant differences among all the studied groups. Rats of $CCl_4$-administered group showed a notable decline in the RBC and WBC counts, Hb content and PCV, as compared to the controls. As compared to the rats of $CCl_4$-treated group, the rats administered *Nigella, Ginger* and mixed extracts after $CCl_4$ administration exhibited significant elevations in the RBC, WBC counts, Hb content and PCV. This data is in accordance with Meral

and Kanter [36], who reported that rats treated with $CCl_4$ for 45 days significantly decreased the red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), and Hb levels while *Nigella sativa* treatment significantly increased the reduced RBC, WBC, PCV, and Hb levels.

**Effect on serum biochemical parameters:** The lipid profile of the experimental animals as affected by the administration of $CCl_4$ alone, *Nigella, Ginger*and mixed extracts plus $CCl_4$ are shown in Table 2. The serum levels of TL, TC, TG, LDL-C and HDL-C of the rats were markedly influenced by the type of treatment. In comparison to control group, all the studied lipid profile parameters of $CCl_4$-treated group were significantly elevated except the levels of HDL-C that were notably reduced. On the other hand, rats treated with *Nigella, Ginger* and mixed extracts plus $CCl_4$ exhibited a marked reduction in the levels of TL, TC, TG and LDL-C, as compared with the $CCl_4$-treated group.

The results of the present study have also established that $CCl_4$ treatment could have affected the lipid metabolism of liver (triglyceride and cholesterol levels). This is evidenced from the present observations in which $CCl_4$ caused a significant ($p < 0.05$) increase in the levels of lipid parameters. In this connection, $CCl_4$ intoxication is similar to hepatitis in case of the triglycerides catabolism. This situation could be also attributed to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis [37]. On the other hand, it can be assumed that hypercholesterolemia in $CCl_4$ intoxicated rats was resulted from damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in liver [38]. However, rats treated with *Nigella, Ginger* and mixed extracts plus $CCl_4$ showed a significant ($p < 0.05$) decline in triacylglycerol and cholesterol values compared to $CCl_4$-intoxicated rats. The mechanism of lipid lowering effects of *Nigella, Ginger* and mixed extracts might be attributed to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferase in vitro. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver [39].

Serum protein profile of different groups of rats (Table 3) was noticeably affected by the type of treatment as rats administered $CCl_4$ alone exhibited marked reductions in the levels of albumin simultaneous with a significant increase in the levels of globulin, as compared to the controls. Thus, the A/G ratio of this group was remarkably reduced. On the other hand, the rats of *Nigella, Ginger* and mixed extracts plus $CCl_4$ -treated groups displayed a marked increase in the levels of albumin and A/G ratio but a marked decrease in the levels of globulin, as compared to the $CCl_4$-treated group.

In this study the significant ($p < 0.05$) decrease in serum albumin of rats treated with $CCl_4$ as compared to control may indicates poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition, or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis [40]. On the other hand, a significant ($p < 0.05$) increase in concentration of serum albumin was observed in rats received *Nigella, Ginger* and mixed extracts plus $CCl_4$ in comparison to rats received $CCl_4$ alone. The increase of albumin concentration after treatment with *Nigella, Ginger* and mixed extracts may be attributed to the decrease in lipid peroxidation processes and increase in the activities of plasma protein thiols as a result of the treatment [41].

A liver function marker, as influenced by the administration of $CCl_4$ - Nigella and Ginger extracts alone and mixed, were presented in Table 4. The activities of ASAT, ALAT and ALP and TBil, in serum of rats were significantly affected by the type of treatment, whereas the serum levels of DBil were not affected by any of the studied factors. In comparison to the controls, the $CCl_4$-treated rats showed significant elevations in the activities of ASAT and ALAT and ALP as well as the levels of TBil. On the contrary, the activities of ALP, ASAT and ALAT as well as the levels of TBil and DBil of *Nigella, Ginger* and mixed extracts plus $CCl_4$ -treated rats were not significantly different from those of the control group.

In the present study serum hepatic biomarkers, AST and ALT activities were greatly increased ($p < 0.05$) in rats treated with the $CCl_4$ compare to control. As in the present investigation, previous studies have shown that $CC1_4$ increased significantly serum ALP levels, and total protein and albumin levels [42-43]. The increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are found in cytoplasmic area of the cell and they are released into circulation in case of cellular damage [44]. On the other hand, treatment

with *Nigella, Ginger* and mixed extracts plus $CCl_4$ was found to suppress ($p < 0.05$) the increase of serum AST and ALT activities. In accordance with the present results, previous investigations on $CCl_4$ experimentally-induced hepatotoxicity in rats indicated that Ginger improves liver functional factors, including serum ALT, AST and LDL levels [45-46]. Moreover, many other plant extracts were reported to have considerable therapeutic effects on liver injury induced by chemical agents, for example, administration of poly phenolic extracts from chicory (Cichoriumintybus) resulted in wholly normalization of the serum AST and ALT levels in mice exposed to thioacetamide, a hepatotoxic organosulfur compound [47]. Rafiei et al. have also reported similar effects from barberry extract upon administration to $CCl_4$ induced hepatotoxic animals [48]. These finding implies that challenge to protect liver tissue from $CCl_4$ injury.

**Effect on the hepatic lipid peroxidation and endogenous antioxidants**

The effects of $CCl_4$ alone or with *Nigella* and *Ginger* mixed extracts administrations on the levels of hepatic MDA and GSH and the activities of endogenous antioxidant enzymes were shown in Table 5. The hepatic levels of MDA and GSH as well as the activities of CAT, SOD and GR were significantly influenced by the type of treatment. In the liver of rats administered $CCl_4$ alone, there was a meaningful elevation in the levels of MDA accompanied by a marked reduction in the GSH content, SOD and GR activities as compared to those of controls. In the rats of *Nigella, Ginger* and mixed extracts plus $CCl_4$ -treated groups, the mean values of hepatic MDA concentration were significantly lower than those of $CCl_4$-treated rats and were not significantly different from those of the controls. On the other hand, the mean values of hepatic GSH content of *Nigella, Ginger* and mixed extracts plus $CCl_4$ -treated rats were significantly higher than those of $CCl_4$-treated group. As compared to the $CCl_4$-treated group, the rats administered *Nigella* and *Ginger* mixed extracts plus $CCl_4$ showed a marked elevation in the activities of CAT and SOD and GR that did not significantly differ from those of the controls.

Data of the present study is in accordance with the findings of other workers such as Park et al. [49] who reported that hepatotoxic effects by $CCl_4$ are lipid peroxidation origin, and are largely due to its active metabolite $CCl_3$ (This metabolite can abstract hydrogen from fatty acids, initiating the lipid peroxidation), lead to cell injury, and finally liver damage. Moreover, Palanivel et al. [50], stated that the efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. In this connection, the present study revealed that *Nigella* and *Ginger* mixed extracts decreased ($p < 0.05$) $CCl_4$ induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. Furthermore, the present results are in agreement with the work done [51], who found that *Nigella* seeds have notable amounts of antioxidant substances with hepatoprotective effects and their antioxidant properties may be attributable to its flavonoid and polyphenolic contents. On the same basis, the protective effect of *N. sativa* extract may be related to its antioxidant and cytoprotective effects [19, 22, 52]. As previously noted and similar to the results achieved for other plants in the literature [53-55], our observations and findings can be attributed to the antioxidant ingredients of *Nigella*,and *Ginger* and their mixed extracts that probably inhibit lipid peroxidation and consequently inhibition of oxidative stress. Therefore, the cell membranes remain intact and as a result cells are prevented to enter the necrosis step.

**Determination of percent of DNA damage by comet assay in liver tissues**

The data in table (6) and figures 1& 2 revealed that $CCl_4$ liver intoxication produced a significant elevation in tail moment compared to control group of rats. On the other hand, administration of either *Nigella, Ginger* or their mixed extracts plus $CCl_4$ significantly reduced tail moment and consequently significant reduction in the percent of DNA damage as compared to $CCl_4$-intoxicated group in comparison to the control group. These results are in connection with a recent study reporting that quercetin decreased the severity of acrylamide-induced DNA damage in the rat liver [56].

**Histopathological results**

**Brain**

Microscopic investigation of control brain sections of rats show highly active neurons which having huge pale-stained nuclei, nuclear chromatin and prominent nucleoli disappeared. The glial cells surrounded the neurons and support it. These cells have small densely stained nuclei with condensed chromatin and no visible nucleoli. Neuropil or background substances are shown in the cortex (Figure 3). Examination of sections of brain cortex of rats administered with $CCl_4$ alone showed dark neurons with irregular shape and glial cells that appearedinside white vacuoles. Neurofibrillary tangles stained with magenta color and looking like flames were founded. The tangle appears as long pink filaments in the cytoplasm. The neuropil is appeared vacuolated (Figure 4). Photomicrograph of section in brain cortex of rat administered with $CCl_4$ and Ginger showing the structure of neurons appeared more or less like normal and regular shape (Figure 5). Photomicrograph of section in brain cortex of rat administered with $CCl_4$ and Nigella showing dark neuronswith irregular shape and surrounded by pericellular halos. No extracellular vacuoles are found in the neuropil (Figure 6).

Photomicrograph of section in brain cortex of rat administered with $CCl_4$ and Ginger and Nigella mixed extract showing the structure of neurons appeared more or less like normal and regular shape. Small and large extracellular vacuoles are found .Few neuronsare irregular in shape and surrounded by pericellular halos (Figure 7).

**Liver**

Microscopic examinations of sections of liver from normal control rats show the normal architecture of hepatic lobules. The central veins lies at the center of the lobules surrounded by cords of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Figure 8). Histopathological investigation of liver from rats administered with $CCl_4$ alone showing disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein with mononuclear cells, vacuolar degeneration and necrosis of hepatocytes (Figure 9). Liver sections of rats administered with $CCl_4$ and Ginger showing mild inflammatory cells infiltrations around central vein, vacuolar degeneration, and necrosis of hepatocytes. Binucleated and activated Kupffer cells were noticed (Figure 10). In case of rats administered with $CCl_4$ and Nigella it was observedthat liver section maintained hepatic architecture, with only few inflammatory cells infiltrations around central vein, and centrilobular hepaticnecrosis with mild vacuolar degeneration of hepatocytes (Figure 11). Examination of liver sections in rats administered with with $CCl_4$ and Ginger and Nigella mixed extract showed a more or less like normal and regular shape of the hepatic lobule structure (Figure 12).

In the present investigation, the biochemical findings were also confirmed by the above mentioned histpathological observations. The changes mostly include hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells infiltration and other histological manifestations which were also consistent with the findings of other authors [43].

**Conclusion**

Hepatoprotective effects of *Nigella, Ginger* and their mixed extracts on $CCl_4$-induced hepatic damage in male Wistar rats were observed in the present study. Probably, antioxidative properties of the extract helped hepatic cells to obviate $CCl_4$-induced necrosis and inflammation which can be also observed in histopathological findings. The results obtained here and the reports from previous studies suggest that *Nigella, Rosemary and Ginger* mixed extracts may function as a good candidate for the treatment or prevention of liver failure. However, further investigations are required to unveil the molecular identification of the active ingredients and elucidation of the mechanisms involved in the effect.

**Table 1:**Rate of DNA damage in liver tissues of control and $CCl_4$ induced female albino rats treated with different extracts using comet assay

| Treatment | *No. of cells | | ¥Class of comet | | | | DNA damaged cells |
|---|---|---|---|---|---|---|---|
| | Analyzed | Total comets | 0 | 1 | 2 | 3 | (mean ± SEM) |
| **Control** | 500 | 36 | 464 | 26 | 10 | 0 | 7.2 ± 0.13 |
| **$CCl_4$** | 500 | 117 | 383 | 35 | 39 | 43 | 23.4 ± 0.84 |
| **$CCl_4$ + G** | 500 | 69 | 431 | 23 | 25 | 21 | 13.8 ± 0.41 |
| **$CCl_4$ + N** | 500 | 51 | 449 | 21 | 17 | 13 | 10.2 ± 0.76 |
| **$CCl_4$ + G + N** | 500 | 57 | 443 | 17 | 22 | 18 | 11.4 ± 0.52 |

¥: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.

(*): No of cells analyzed were 100 per an animal.

**Table 2:** Effect of administration of $CCl_4$ alone or with different extracts combinations, on certain hematological parameters of female albino rats

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | $CCl_4$ | $CCl_4$ + G | $CCl_4$ + N | $CCl_4$ + G + N |
| Red blood cell count (×1012 $L^{-1}$) | 6.99 ± 0.71 | 5.01 ± 1.02 * | 6.91 ± 1.06# | 6.56 ± 0.93# | 6.06 ± 0.85# |
| White blood cell count (×109 $L^{-1}$) | 12.9 ± 0.94 | 9.5 ± 2.82* | 14.7 ± 2.07# | 13.1 ± 1.89# | 14.4 ± 2.73# |
| Platelet count (×109 $L^{-1}$) | 471 ± 31.5 | 783.5 ± 43.3* | 583.3 ± 50.3# | 551.3 ± 33.6# | 505.1 ±54.84# |
| Hemoglobin content (g d $L^{-1}$) | 12.6 ± 0.98 | 10.3 ± 1.38* | 12.36 ±1.29# | 11.7 ± 1.11# | 11.6 ± 0.89# |
| Packed cell volume (%) | 34.2 ± 2.23 | 40.1 ± 2.91* | 36.7 ± 2.78# | 35.3 ± 2.83# | 36.1 ± 2.46# |

Data are represented as mean standard error( ± SE).

*: Represent significant differences ($p<0.05$) in comparison to the control group.

#: Represent significant differences ($p<0.05$) in comparison to the $CCl_4$ - treated group.

**Table 3:** Effect of administration of $CCl_4$ alone or with different extracts combinations, on the concentrations of serum total lipid (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) of female albino rats

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | $CCl_4$ | $CCl_4$ + G | $CCl_4$ + N | $CCl_4$ + G + N |
| **TL (mgd$L^{-1}$)** | 512.5±24.6 | 658.8±20.3* | 488.4±23.7# | 440.8±21.7# | 432.20±18.2# |
| **TC (mgd$L^{-1}$)** | 118.2±2.9 | 228.8±10.3* | 122.4±8.6# | 109.8±6.5# | 113.8±4.4# |
| **TG (mgd$L^{-1}$)** | 104.4±7.4 | 164.8±1.5* | 105.9±7.3# | 110.6±8.6# | 106.4±9.3# |
| **LDL-C (mgd$L^{-1}$)** | 61.2±4.8 | 159.7±6.7* | 55.6±3.3# | 63.8±4.4# | 59.8±3.9# |
| **HDL-C (mgd$L^{-1}$)** | 36.60±1.40 | 27.2±0.9* | 40.1±2.5# | 39.8±1.7# | 37.8±2.3# |

Data are represented as mean standard error( ± SE).

*: Represent significant differences ($p<0.05$) in comparison to the control group.

#: Represent significant differences ($p<0.05$) in comparison to the $CCl_4$ - treated group.

**Table 4:** Effect of administration of $CCl_4$ alone or with different extracts combinations, on the concentrations of serum total protein (TP), albumin (A), globulin (G) and A/G ratio of female albino rats

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | $CCl_4$ | $CCl_4$ + G | $CCl_4$ + N | $CCl_4$ + G + N |
| **TP (g d$L^{-1}$)** | 6.68±0.22 | 6.52±0.3* | 6.24±0.05# | 6.24±0.05# | 6.24±0.05# |
| **A (g d$L^{-1}$)** | 4.42±0.13 | 3.42±0.1* | 4.12±0.09# | 4.65±0.19# | 4.2±0.08# |
| **G (g d$L^{-1}$)** | 2.46±0.24 | 3.71±0.8* | 2.35±0.17# | 2.74±0.11# | 2.62±0.09# |
| **A/G ratio** | 1.7±0.06 | 0.85±0.05* | 1.53±0.04# | 1.8±0.08# | 1.62±0.07# |

Data are represented as mean standard error( ± SE).

*: Represent significant differences ($p<0.05$) in comparison to the control group.

#: Represent significant differences ($p<0.05$) in comparison to the $CCl_4$ - treated group.

**Table 5:** Effect of administration of $CCl_4$ alone or with different extracts combinations, on the activities of serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkalinephosphatase (ALP) and the levels of total bilirubin (TBil) and direct bilirubin (DBil) of female albino rats

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | **Control** | **$CCl_4$** | **$CCl_4$ + G** | **$CCl_4$ + N** | **$CCl_4$ + G + N** |
| ASAT ($UL^{-1}$) | 33.7±4.3 | 158.9±8.4* | 48.2±5.7# | 39.6±3.5# | 42.4±3.1# |
| ALAT ($UL^{-1}$) | 25.60±3.5 | 75.60±2.7* | 32.9±1.7# | 31.8±1.5# | 23.6±1.2# |
| ALP ($UL^{-1}$) | 55.30±6.8 | 70.02±1.8* | 59.8±3.4# | 61.6±3.2# | 53.2±2.7# |
| TBil (mg $dL^{-1}$) | 0.66±0.02 | 0.89±0.03* | 0.61±0.05# | 0.7±0.03# | 0.69±0.04# |
| DBil (mg d $L^{-1}$) | 0.11±0.03 | 0.23±0.06* | 0.14±0.08# | 0.13±0.09# | 0.12±0.02# |

Data are represented as mean standard error( ± SE).

*: Represent significant differences ($p<0.05$) in comparison to the control group.

#: Represent significant differences ($p<0.05$) in comparison to the $CCl_4$ - treated group.

**Table 6:** Effect of administration of $CCl_4$ alone or with different extracts combinations, on the levels of hepatic malondialdehyde (MDA) and glutathione (GSH) and the activities of catalase (CAT),superoxide dismutase (SOD) and glutathione reductase (GR) of female albino rats

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | **Control** | **$CCl_4$** | **$CCl_4$ + G** | **$CCl_4$ + N** | **$CCl_4$ + G + N** |
| MDA (nmol $g^{-1}$ liver) | 4.48±0.11 | 9.18±0.4* | 4.15±0.21# | 4.26±0.26# | 4.37±0.3# |
| GSH (mg $g^{-1}$ liver) | 40.04±5.10 | 19.72±0.9* | 38.32±4.5# | 39.42±2.8# | 37.34±2.8# |
| CAT (U $g^{-1}$ liver) | 104.30±8.16 | 39.40±2.7* | 99.1±7.3# | 101.2±9.4# | 100.6±10.3# |
| SOD (U $g^{-1}$ liver) | 9.56±0.17 | 4.36±0.2* | 9.78±0.9# | 10.45±1.3# | 9.41±1.2# |
| GR (U $g^{-1}$ liver) | 73.20±2.71 | 27.80±1.9* | 70.4±8.4# | 69.7±6.3# | 68.5±7.8# |

Data are represented as mean standard error( ± SE).

*: Represent significant differences ($p<0.05$) in comparison to the control group.

#: Represent significant differences ($p<0.05$) in comparison to the $CCl_4$ - treated group.

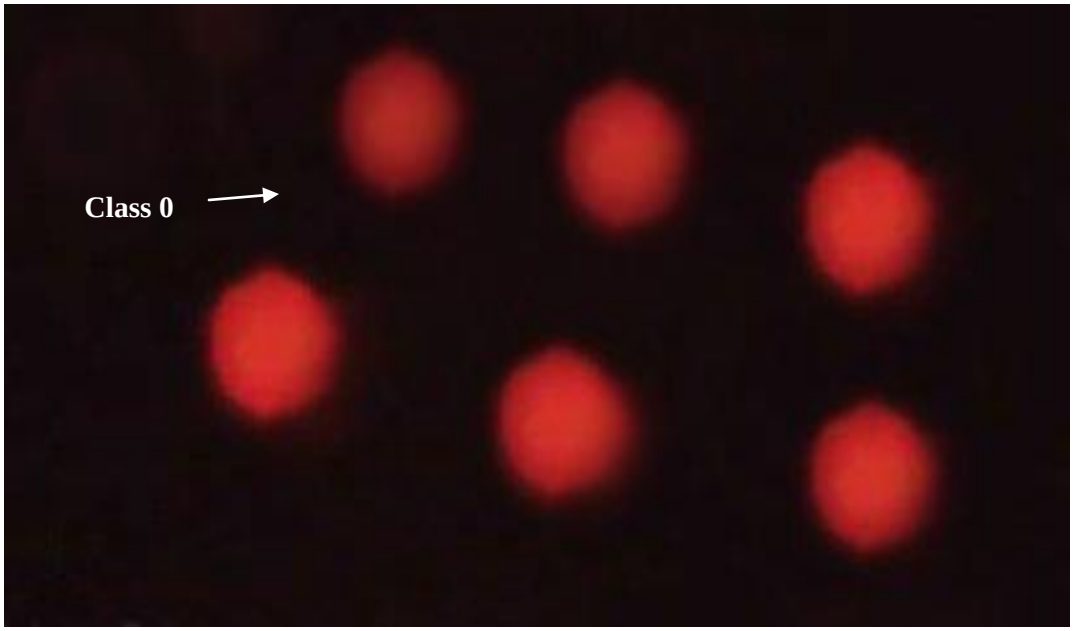


*Figure 1: Visual score of DNA damage (class 0) using comet assay in liver tissues of female rats.*

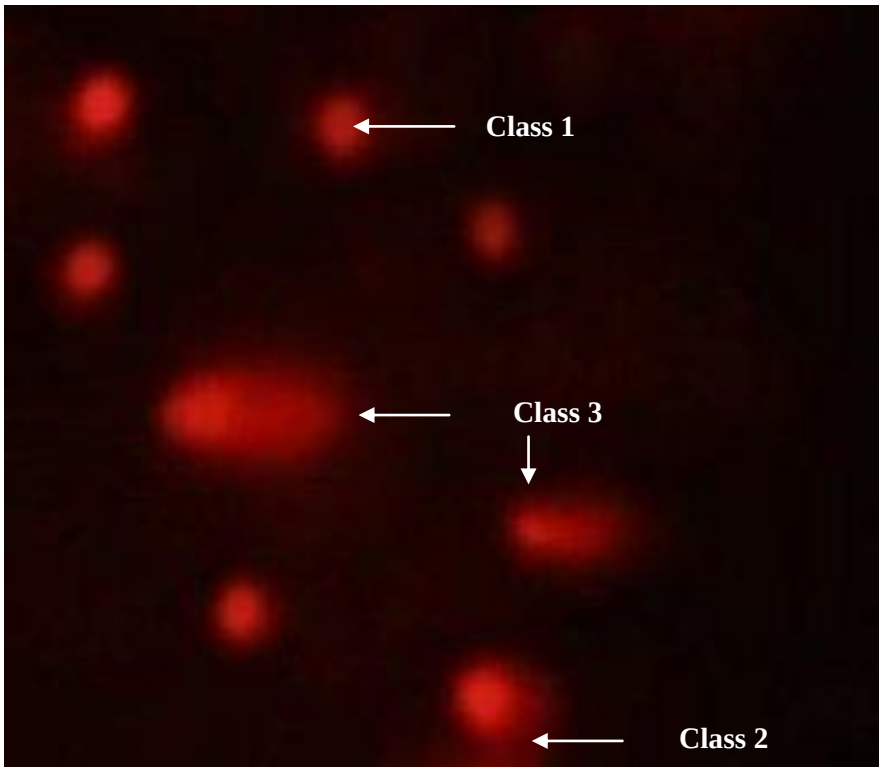


*Figure 2: Visual score of DNA damage (classes 1, 2 and 3) using comet assay in liver tissues of female rats.*

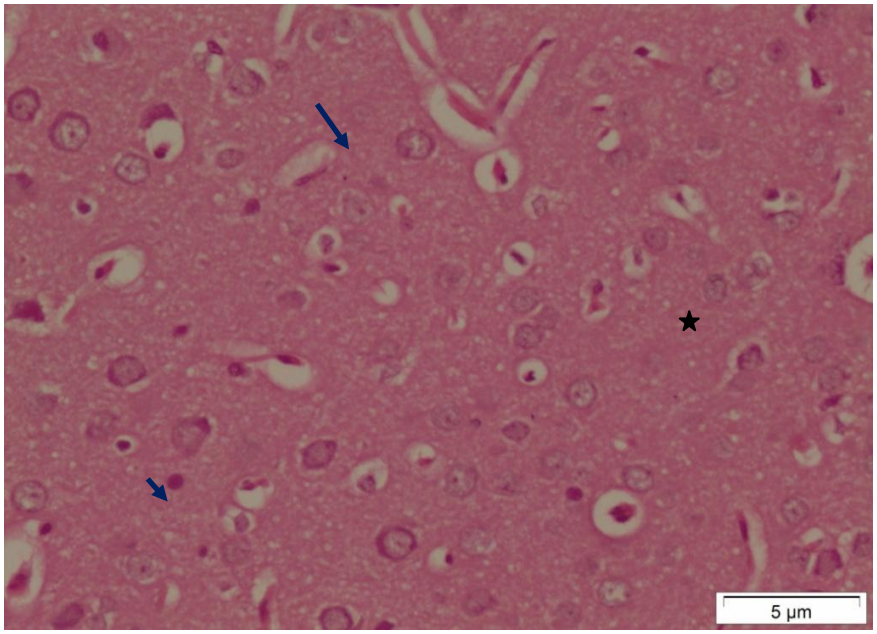


*Figure 3: Photomicrograph of section in brain cortex of control rat shows the nerve cells (neurons) (arrow) that having pale-stained huge nuclei, disappeared nuclear chromatin and prominent nuclei, surrounding support cells (glial cells) (arrow head) having small nuclei with densely stained, condensed chromatin with no visible nucleoli, background substance (neuropil) (asterisk) and perivascular space are shown in the cortex (blue arrow) (H and E, Scale bar 5 µm).*

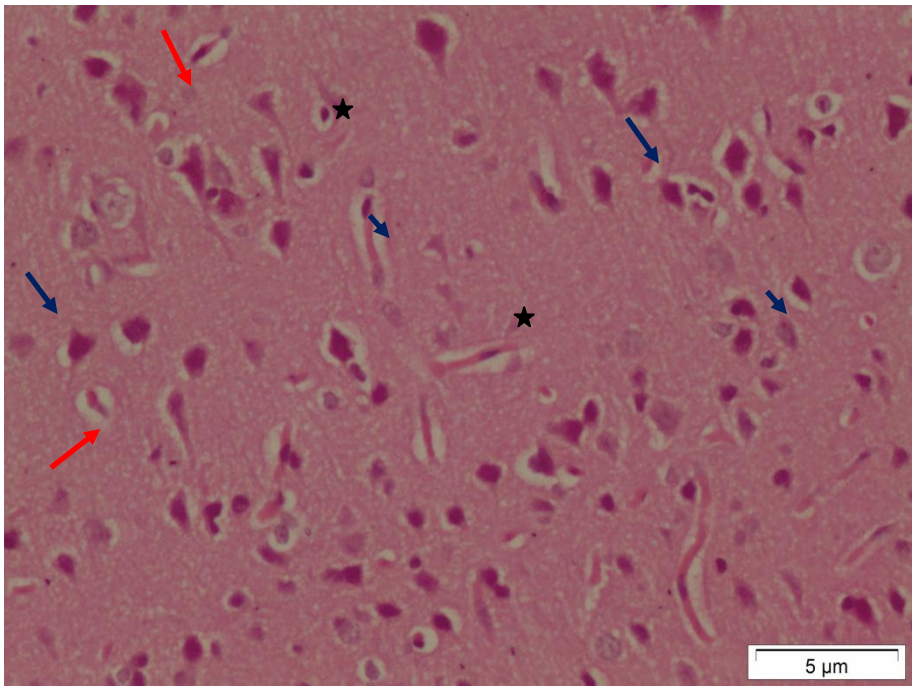


*Figure 4: Photomicrograph of section in brain cortex of rat administered with $CCl_4$ alone showing dark neuron with irregular shape (arrows) and glial cells that appearedinside white vacuoles (arrowheads). Neurofibrillary tangles stained with magenta color and looking like flames were founded (red arrows). The tangle appears as long pink filaments in the cytoplasm (red arrows). The neuropil is appeared vacuolated (asterisk) (H and E, Scale bar 5 µm).*

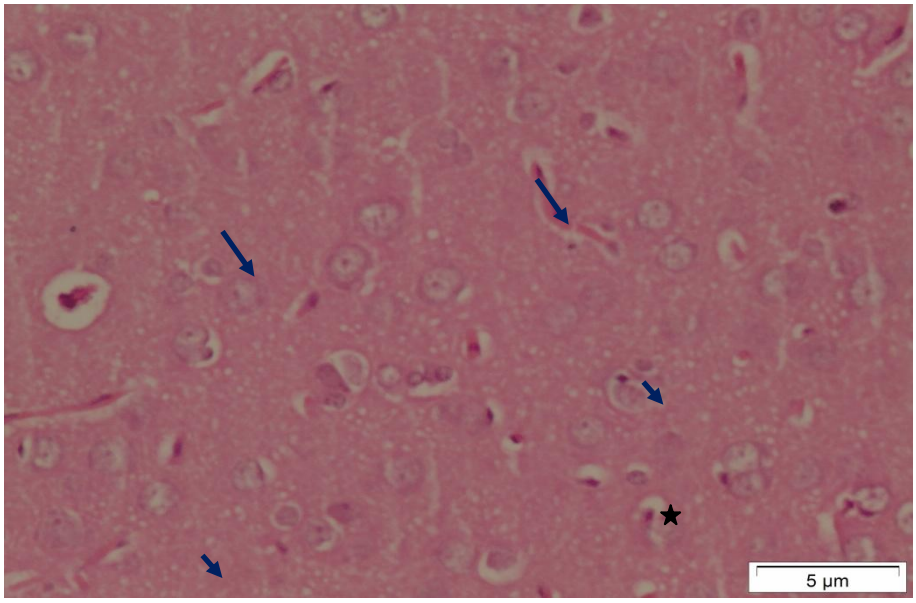


*Figure 5: Photomicrograph of section in brain cortex of rat administered with $CCl_4$ + G showing dark neuron with irregular shape (arrows) and glial cells(arrowheads). No neurofibrillary tangles are found. The neuropil is appeared vacuolated (asterisk) (H and E, Scale bar 5 µm).*

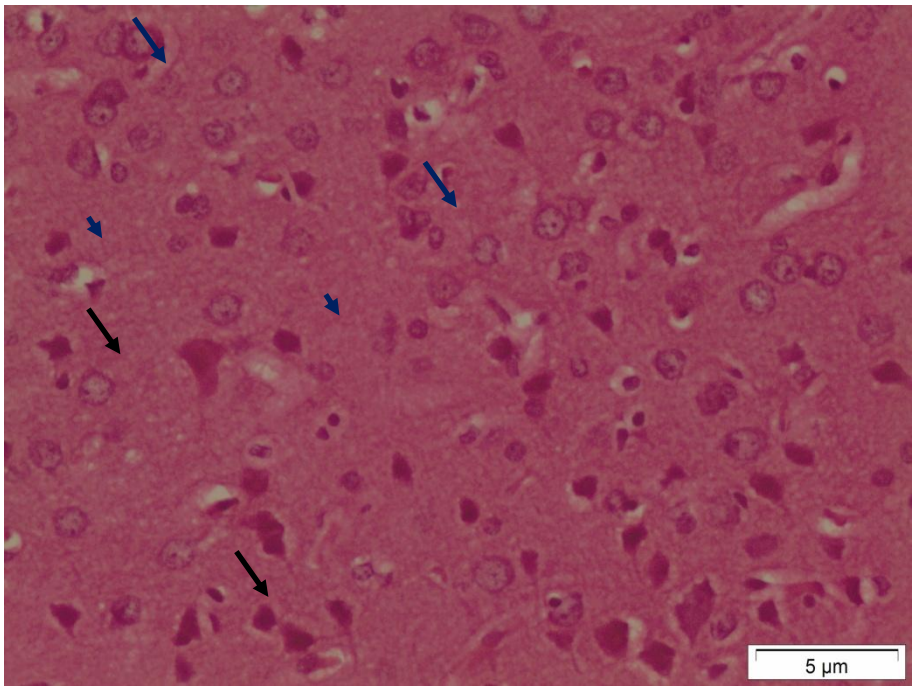


*Figure 6: Photomicrograph of section in brain cortex of rat administered with $CCl_4$ + N showing the normal structure of neuron with regular shape (arrows) and glial cells(arrowheads). Notice few dark neurons (black arrows) are found (H and E, Scale bar 5 µm).*

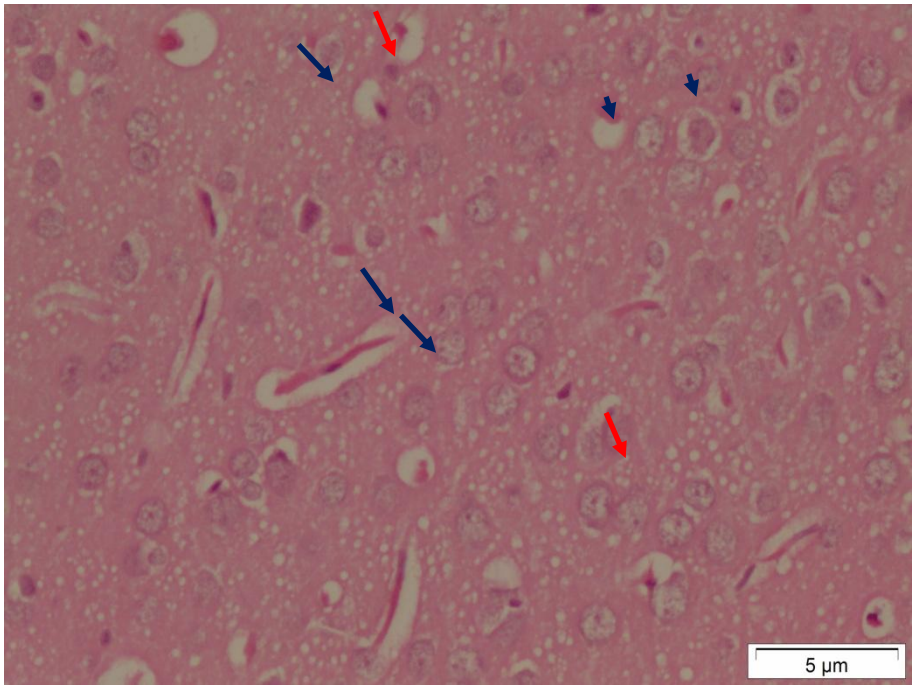


*Figure 7: Photomicrograph of section in brain cortex of rat administered with $CCl_4$ + G + N showing the structure of neurons appeared more or less like normal and regular shape (arrows). Small and large extracellular vacuoles are found (red arrows). Few neuronsare irregular in shape and surrounded by pericellular halos (arrowhead) (H and E, Scale bar 5 µm).*

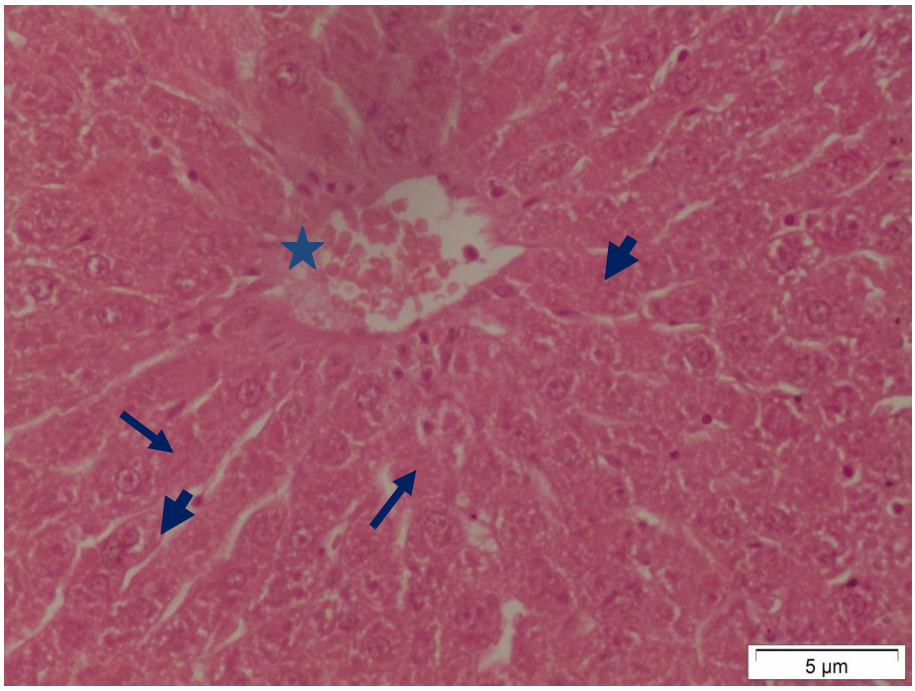


*Figure 8: Photomicrograph of section in liver of normal control rat shows the normal architecture of hepatic lobule. The central vein (asterisk) lies at the center of the lobule surrounded by cords of hepatocytes (arrow). Between the strands of hepatocytes, the hepatic sinusoids are shown (arrowhead) (H and E, Scale bar 5 µm).*

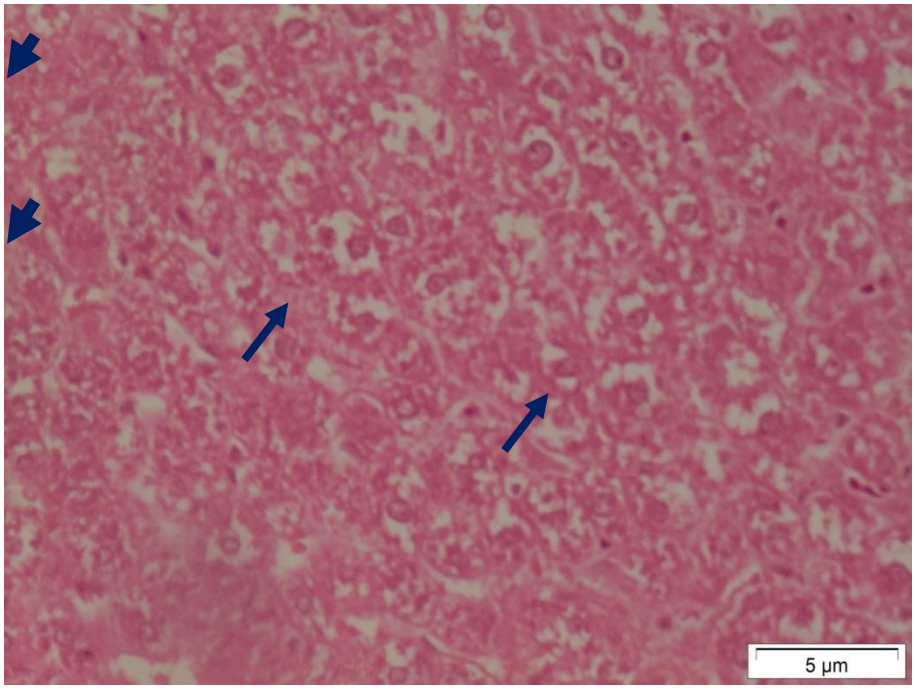


*Figure 9: Photomicrograph of section in liver of rat administered with $CCl_4$ alone showing the disturbance of normal architecture of hepatic lobule. Notice the hydropic degeneration (blue arrows), foci of nectotic hepatocytes (blue arrowheads)(H and E, Scale bar 5 µm).*

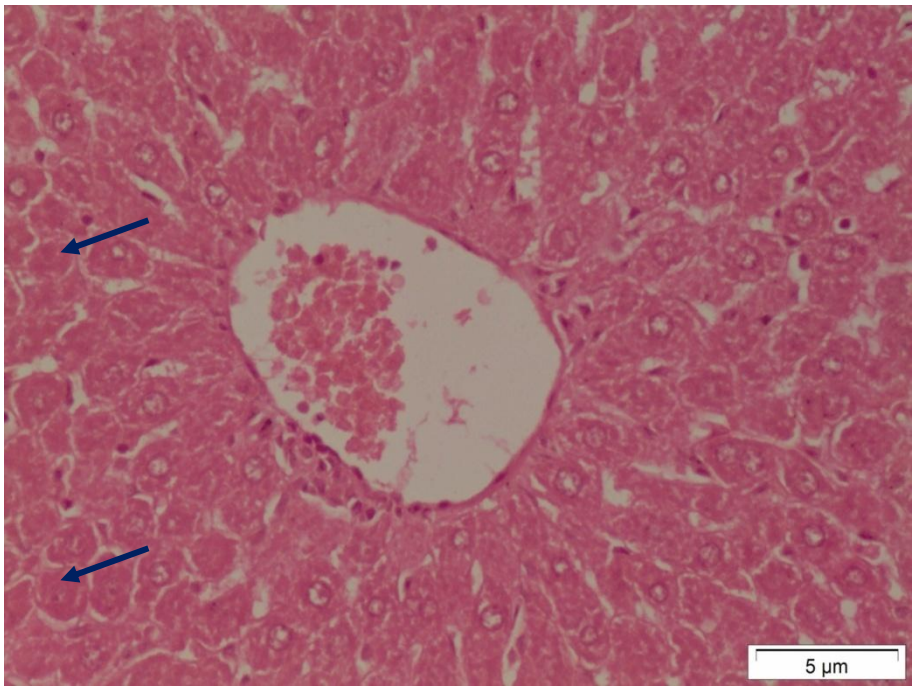


*Figure 10 Photomicrograph of section in liver rat administered with $CCl_4$ + Gshowing the normal structure of the hepatic lobule. Notice the regenerative hepatocytes (arrows) (H and E, Scale bar 5 µm)*

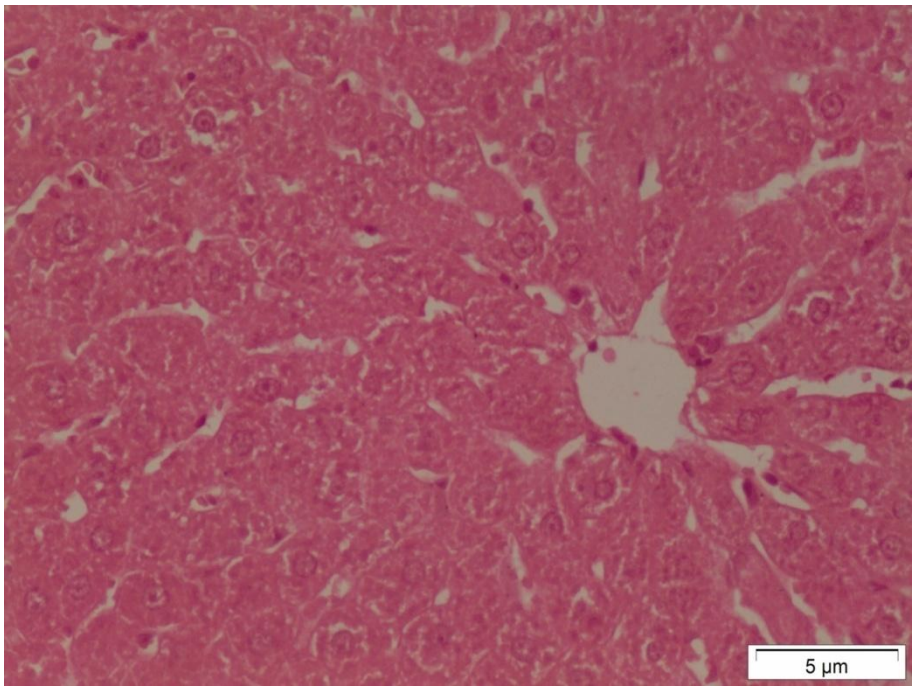


*Figure 11: Photomicrograph of section in liver of rat administered with $CCl_4$ + N showing improvement in the histological feature of the hepatic lobule (H and E, Scale bar 5 µm).*

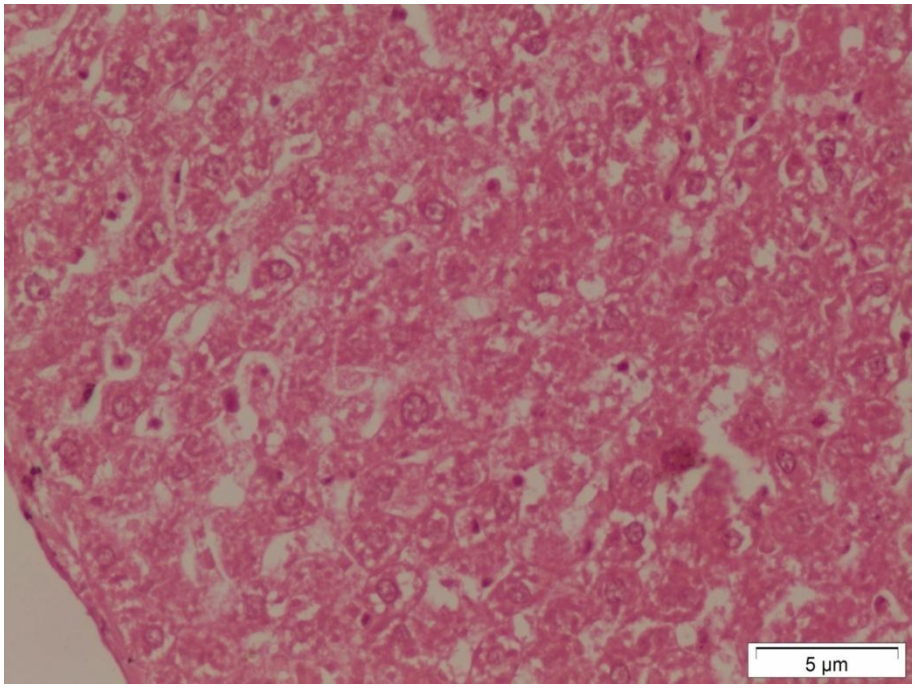


*Figure 12: Photomicrograph of section in liver of rat administered with $CCl_4$ + G + N showing a more or less like normal and regular shape of the hepatic lobule structure (H and E, Scale bar 5 µm).*

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