EVALUATION OF THE POSSIBLE CURATIVE EFFECT OF NIGELLA SATIVA LINN OIL ON EXPERIMENTALLY ACETAMINOPHEN-INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN ADULT MALE ALBINO RATS

By

Hamed Mohammed Osman, Saad Mohammed Mohammed, Sayed Abd-El Reheim Sayed * and Ashraf Abd El-Aty Emara

Departments of Medical Physiology and Pathology*, Al-Azhar Faculty of Medicine

ABSTRACT

Background: There is a great realization that the use of herbs has been considered for the treatment of human diseases such as heptotoxicity and nephrotoxicity since ancient times. Nigella sativa (NS) is one of the most important medicinal plants having strong antioxidant properties.

Objectives: Study the possible curative effect of Nigella sativa linn (NSL) oil on experimentally acetaminophen-induced hepatotoxicity and nephrotoxicity in adult male albino rats.

Materials and Methods: Fifty adult male albino rats of local strain were chosen as an animal model for this study. They were divided into five equal groups: Negative control group, Positive control group, NsL oil group, Acetaminophen-treated group and Acetaminophen + NsL oil-treated group. Hepatotoxicity and nephrotoxicity was induced by oral administration of acetaminophen. At the end of the experiment (24 days), blood samples were obtained for measuring of liver functions (ALT, AST and total bilirubin), kidney functions (serum creatinine and blood urea) and TNFα. Animals were sacrificed; livers and kidneys were obtained for histopathological examination, immunofluorescence analysis for Transforming Growth Factor-β (TGF-β) and measurement of tissue level of malondialdehyde (MDA) in both liver and kidney.

Results: Acetaminophen-treated group and Acetaminophen + NsL oil-treated group showed significant increases in ALT, AST, total bilirubin serum creatinine,urea, TNFα and tissue MDA in both liver and kidney compared to Negative control group and Positive control group. Acetaminophen+NsL oil-treated group, when compared to Acetaminophen-treated group, showed a significant decrease in all previous parameters. Histopathological examination revealed appearance of partial improvement and evidence of some regeneration of both liver and kidney tissues of Acetaminophen + NsL oil-treated group in comparison with Acetaminophen-treated group in addition to immunofluorescence staining of expression of TGFβ in hepatocytes was improved in Acetaminophen + NsL oil-treated compared to Acetaminophen treated group, but there was no difference in the immunofluorescence staining of kidney sections of the previous groups.

Conclusion: Nigella sativa linn oil has a curative role in regeneration of liver and kidney tissues in acetaminophen-induced hepatotoxicity and nephrotoxicity.

Key words: Nigella sativa linn oil, acetaminophen, hepatotoxicity and nephrotoxicity.
INTRODUCTION

End-stage liver disease (ESLD) is a devastating condition with multiple etiologies. However, epidemiological data indicate an increasing worldwide prevalence of liver cirrhosis, related to chronic infection by hepatitis C or B viruses, alcohol consumption and non-alcoholic fatty liver disease (Parola et al., 2008). Currently, the only curative treatment is liver transplantation (Friedman, 2008). Many people around the world have been afflicted by some type of liver lesions including fatty liver, nonalcoholic steatohepatitis, hepatitis with its types (A, B, and C), cirrhosis and hepatocellular carcinoma (Kim et al., 2013). Fibrosis, and in certain, cirrhosis represent the final end point of chronic liver tissue injury, in which chronic inflammation leads to the formation of scar tissue, loss of tissue architecture, and organ failure (Trautwein et al., 2015). Liver fibrosis is one of the leading causes of morbidity and mortality worldwide (Kandeel et al., 2017).

Chronic renal failure is a complication defined as progressive disturbance of kidney function over time and is characterized by abolishing the ability of the body to remove soluble waste resulting in the accumulation of “uremic toxins” (Rafieian-Kopaei and Nasri, 2013). Acute kidney injury is commonly described as a sudden decline in kidney function, its clinical manifestation appears as a reversible acute rise in nitrogen waste products, measured as blood urea nitrogen and serum creatinine levels over a period of hours to weeks (Ghosh et al., 2014).

It is well detected that chronic renal failure is an inflammatory illness and uremic toxins have the main role in creating the inflammatory milieu (Amini et al., 2012 and Ghosh et al., 2014). Kidney fibrosis is generally considered as activation and proliferation of interstitial area and by excessive synthesis and aggregation of extracellular matrix components, containing fibronectin and collagen. Likewise, the phenotypic changes that attribute to acute renal failure comprise inflammatory response and oxidative stress. Slowing the rate of progression of chronic renal failure and promoting the recovery of acute kidney injury is a critical part of the management of these two diseases (Nasri, 2013).

Acetaminophen (Paracetamol; N-acetyl-p-aminophenol) is an active metabolite of phenacetin. It has analgesic and antipyretic effects, but has an only weak anti-inflammatory effect. It is a weak non-selective cyclooxygenase inhibitor which has an excellent oral bioavailability. Acetaminophen is well-tolerated at therapeutic doses but at overdose and chronic use may cause hepatic and renal damage (Grosser et al., 2011). Use of acetaminophen in therapeutic doses is safe, rapid and almost completely absorbed from the gastrointestinal tract, but overdose of the drug leads to life-threatening or fatal hepatic necrosis and renal failure (Bunchorntavakul and Reddy, 2013). Acetaminophen is now the most common drug in self-poisoning, with a high rate of morbidity and mortality (Pettie and Dow, 2013). Many cases of poisoning with N-acetyl-p-aminophenol (APAP) in humans and animals have been reported (Gulmez et al., 2013).

This use of herbal plants as alternative medicine over centuries remains a popular
choice for primary health care (Vanamala et al., 2012). Nigella (NS) sativa is a spice native to Mediterranean region. The used part of plant is the seeds which are utilized worldwide for edible and medicinal applications (Cemek et al., 2008).

Nigella sativa is one of the most popular, safe, non-detrimental, cytoprotective, and widely used as medicinal plant all over the world. NS is a flowering plant with dark-crescent shaped seeds and belongs to the family Ranunculaceae which commonly grows in North Africa and Asia (Kotb et al., 2017). The NS seeds have been used along many centuries as a medicinal plant and as a food additive (Ijaz et al., 2017).

A long list of studies has been conducted, and concluded that most of the anti-inflammatory effects are derived from the thymoquinone, which can be found abundantly in the seeds of Nigella sativa. Nevertheless, Nigella sativa along with its derivative products were used as treatment for liver diseases, rheumatism, as well as relevant inflammatory disorders (Al-Khalaf and Ramadan, 2013).

The present study aimed to understand the pathophysiology of liver and kidney failure, and also elucidating the effects and possible mechanisms of NsL oil in attenuation of hepatotoxicity and nephrotoxicity.

**MATERIALS AND METHODS**

**Experimental animals:** This study was done in the postgraduate laboratory of Medical Physiology Department, Faculty of Medicine, Al-Azhar University on 50 adult male albino rats of local strain obtained from Helwan animal house, used in the present work. The rats weighing from 120 to 140 grams, kept for 10 days to adapt to conditions in the lab before the start of any experimental procedure. Each two rats were housed in a cage (20X32X20 cm). They were fed the standard balanced diet with water ad lib with normal dark/light cycle.

**Experimental protocol:** The animals were divided into 5 equal groups (10 rats each) in this study as follows:

**Group I (Negative control group):** Each rat received only regular diet and tap water for 21 days to measure the basic parameters.

**Group II (Positive control group):** Each rat received 1 ml corn oil/day, orally for 21 days (Elkhateeb et al., 2015).

**Group III (NsL oil group):** Each rat received (1 ml/kg NsL oil dissolved in 1 ml corn oil/day), orally for 21 days (Assayed, 2010).

**Group IV (Acetaminophen-treated group):** Each rat was treated with Acetaminophen (500 mg/kg), orally for 3 days for induction of hepatotoxicity and nephrotoxicity (Gopi et al., 2010), then received 1 ml corn oil/day, orally for the next 21 days.

**Group V (Acetaminophen + NsL oil-treated group):** Each rat was treated with Acetaminophen for 3 days in the previous dose then received 1 ml/kg NsL oil dissolved in 1 ml corn oil/day orally for the next 21 days.

At the time of sacrification, rats were anesthetized with isoflurane and then, blood samples were collected from the retro-orbital plexuses. The liver and kidneys of each rat were dissected out carefully. Each specimen was divided into
two parts; one part was kept frozen at −80°C till they were used for measurement of MDA content, and the other part was preserved for histopathological examination.

Methods: Hepatotoxicity and nephrotoxicity were induced by oral administration of acetaminophen 500 mg/kg for 3 days according to (Gopi et al., 2010). Acetaminophen was dissolved in boiling water (USP31, 2007).

Preparation of Nigella sativa linn oil:

Extraction of N. sativa oil from N. sativa seeds was done according to the method described by (Pal et al., 2011).

Biochemical analysis of liver and kidney functions:

The blood samples were used for estimation of serum alanine transaminase (ALT) (Reitman and Frankel, 1957), aspartate transaminase (AST) (Thomas, 1998), serum total bilirubin (Mallay and Evelyn, 1937), blood urea (Orsonneau et al., 1992) serum creatinine (Fossati et al., 1983), and (TNF α) determined by Enzyme Linked Immuno-Sorbent Assay (ELISA) according to the manufacturer’s instructions of ELISA kits (Abcam, UK).

Preparation of liver and kidney homogenate:

Liver and kidney tissues were homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates were used to determine Malondialdehyde (MDA) was assayed (Ohkawa et al., 1979).

Immunofluorescence Analysis for Transforming Growth Factor-β (TGF-β):

Slides containing liver and kidney tissue sections were handled and prepared for detection of TGF-β by immunofluorescence microscope “Leica DM 5500B” (Abdel-Bakky et al., 2011).

Histopathological examination of liver and kidney tissues:

Autopsy samples taken from the liver and kidney of rats in different groups were prepared and stained by hematoxylin & eosin, and Masson’s trichrome stains for routine examination the light electric microscope (Banchroft et al., 1996).

Quantitative morphometric analysis:

The area percentage of collagen fibers was measured in Masson’s trichrome stained sections. The measurements were obtained using computer-based image analysis software (Leica Qwin 500; Imaging Systems, Cambridge, UK).

Statistical Analysis:

All the statistical analysis were processed using Statistical Program of Social Sciences (SPSS) for windows (version 17, SPSS Inc., Chicago, IL, USA). Values of the measured parameters were expressed as mean value ± standard deviation (SD), and the differences and significance were verified by one-way ANOVA followed by the Fisher’s least significant difference (LSD) post hoc test. P values ≤ 0.05 were considered statistically significant.
RESULTS

Changes in liver functions ALT, AST and total bilirubin (TB) in different groups (figure 1):

In group I, the mean ± SD of ALT, AST and TB were 51 ± 3.65 units /l, 40.4 ± 2.5 units /l and 0.37 ± 0.058 mg/dl respectively.

In group II, the mean ± SD of ALT, AST and TB were 60.5 ± 3.63 units /l, 50.8 ± 4.26 units /l and 0.48 ± 0.035 mg/dl respectively. There was an insignificant increase in ALT & a significant increase in AST and TB in group II when compared with group I.

In group III, the mean ± SD of ALT, AST and TB were 41.8 ± 5.16 units /l, 35.8 ± 5.14 units /l and 0.33 ± 0.048 mg/dl respectively. There was an insignificant decrease in ALT, AST and total bilirubin in group III when compared with group I and a significant decrease in ALT, AST and total bilirubin in group III when compared with group II.

In group IV, the mean ± SD of ALT, AST and TB were 147.8 ± 13.21 units /l, 142.1 ± 13.77 units /l and 0.86 ± 0.14 mg/dl respectively. There was a significant increase in ALT, AST and TB in group IV when compared with group I, II and III.

In group V, the mean ± SD of ALT, AST and TB were 107.5 ± 18.8 units /l, 99.8 ± 17.79 units /l and 0.79 ± 0.055 mg/dl respectively. There was a significant increase in all parameters in group V when compared with group I, II and III, also there was a significant decrease in all parameters in group V when compared with group IV.

Changes in kidney functions serum creatinine and blood urea in different groups (figure 1):

In group I, the mean ± SD of serum creatinine and blood urea were 0.61 ± 0.077 mg/dl and 36.1 ± 3.6 mg/dl respectively.

In group II, the mean ± SD of serum creatinine and blood urea were 0.82 ± 0.2 mg/dl and 41.9 ± 5.7 mg/dl respectively. There was an insignificant increase in group II when compared with group I in both serum creatinine and blood urea.

In group III, the mean ± SD of serum creatinine and blood urea were 0.64 ± 0.12 mg/dl and 35.3 ± 4.2 mg/dl respectively. There was an insignificant decrease in group III when compared with group I and II in both serum creatinine and blood urea.

In group IV, the mean ± SD of serum creatinine and blood urea were 6.63 ± 0.77 mg/dl and 116.5 ± 22.67 mg/dl respectively. There was a significant increase in group IV when compared with group I, II and III in both serum creatinine and blood urea.

In group V, the mean ± SD of serum creatinine and blood urea were 5.88 ± 0.08 mg/dl and 94.2 ± 14.4 mg/dl respectively. There was a significant increase in group V when compared with group I, II and III in both serum creatinine and blood urea, also there was a significant decrease in group V when compared with group IV in the same parameters.
Changes in TNFα in different groups (figure 1):

In group I, the mean ± SD of TNFα was 1.9 ± 0.2 ng/ml. In group II, the mean ± SD of TNFα was 2.74 ± 0.52 ng/ml. There was an insignificant increase in group II when compared with group I.

In group III, the mean ± SD of TNFα was 1.76 ± 0.51 ng/ml. There was an insignificant decrease in group III when compared with group I and II.

In group IV, the mean ± SD of TNFα was 10.69 ± 2.67 ng/ml. There was a significant increase in group IV when compared with group I, II and III.

In group V, the mean ± SD of TNFα was 6.85 ± 1.02 ng/ml. There was a significant increase in group V when compared with group I, II and III, also there was a significant decrease in group V when compared with group IV.

Changes in liver and kidney tissue levels of MDA in different groups (figure 2):

In group I, the mean ± SD of liver and kidney tissues levels of MDA were 12.4 ± 1.86 nmol/g tissue and 10.26 ± 1.65 nmol/g tissue respectively.

In group II, the mean ± SD of liver and kidney tissues levels of MDA were 11.17 ± 1.75 nmol/g tissue and 11.97 ± 1.42 nmol/g tissue respectively. There was an insignificant decrease in group II when compared with group I.

In group III, the mean ± SD of liver and kidney tissues levels of MDA were 9.89 ± 1.91 nmol/g tissue and 10.29 ± 1.68 nmol/g tissue respectively. There was an insignificant decrease in group III when compared with group I and II.

In group IV, the mean ± SD of liver and kidney tissues levels of MDA were 94.39 ± 9.45 nmol/g tissue and 98.86 ± 7.43 nmol/g tissue respectively. There was a significant increase in group IV when compared with group I, II and III.

In group V, the mean ± SD of liver and kidney tissues levels of MDA were 57.61 ± 4.88 nmol/g tissue and 42.67 ± 3.77 nmol/g tissue respectively. There was a significant increase in group V when compared with group I, II and III, also there was a significant decrease in group V when compared compared with group IV.
Figure (1): Changes in liver functions (ALT, AST, TB) and kidney functions (serum creatinine, blood urea) and TNFα in different groups (mean ± SD).
Figure (2): Changes in liver and kidney tissue levels of MDA in different groups (mean ± SD).

* Significant with Gr I  
φ Significant with Gr III  
# Significant with Gr II  
¥ Significant with Gr IV

Histopathological examination of the liver:

Group I (Negative control group) showed average hepatic architecture and hepatocytes arranged in single-cell cords (Fig 3a).

Group II (Positive control group) showed average hepatic architecture, minimally dilated central veins and average portal tracts (Fig 3b).

Group III (NsL oil group) showed average hepatic architecture, minimally dilated and congested central veins, average portal tracts and hepatocytes arranged in single-cell cords (Fig 3c).

Group IV (Acetaminophen-treated group) showed expanded portal tracts with markedly dilated blood vessels, dilated central vein with peri-venular cellular infiltrate, scattered apoptotic hepatocytes and scattered hepatocytes with micro-vesicular steatosis. No underlying fibrosis (Fig 3d).

Group V (Acetaminophen + NsL oil-treated group) showed expanded portal tracts with markedly dilated blood vessels, markedly dilated central veins, markedly congested blood sinusoids and vacuolated hepatocytes (Fig 3e).

Expression and localization of TGFβ of the liver:

It was observed that the expression is located in the cell membrane of hepatocytes. It was also noted that the expression occurred in areas with maximal lesions as necrosis and steatosis as follows:

Group I (Negative control group) showed mild cell membrane expression of TGFβ in hepatocytes (Fig 5a).

Group II (Positive control group) showed moderate cell membrane reactivity in peri-venular hepatocytes (Fig 5b).

Group III (NsL oil group) showed mild cell membrane reactivity in hepatocytes (Fig 5c).

Group IV (Acetaminophen-treated group) showed marked cell membrane reactivity in hepatocytes and cytoplasmic reactivity in stellate-shaped cells (Fig 5d).

Group V (Acetaminophen + NsL oil-treated group) showed mild cell membrane expression of hepatocytes, but
the reaction was cytoplasmic and increased in apoptotic hepatocytes.

Figure (3): Histopathological examination of the liver by H&E: Figs. (3a, b, c) related to GI, II, III showed average hepatic architecture, minimally dilated central veins (black arrow), average portal tracts (blue arrow) and hepatocytes arranged in single-cell cords (green arrow). Fig. (3d) related to GIV (Acetaminophen-treated group) showed dilated central vein with peri-venular cellular infiltrate (blue arrow) and scattered apoptotic hepatocytes (black arrows). Fig. (3e) related to GV (Acetaminophen + NsL oil-treated group) showed expanded portal tract with markedly dilated blood vessels (blue arrow), and vacuolated hepatocytes (green arrow).

Figure (4): Histopathological examination of the liver by Masson trichrome stain: Figs. (4a, b, c, e) related to GI, II, III, GV showed portal tract with no fibrosis (black arrow).
arrow). **Fig. (4d)** related to GIV (Acetaminophen-treated group) showed expanded portal tract with no fibrosis (black arrows).

**Fig. (5) Immunofluorescence staining of liver by TGFβ:** Figs. (5a, b, c) related to GI, II, III showed mild to moderate cell membrane reactivity in hepatocytes (red arrow). **Fig. (5d)** related to GIV (Acetaminophen-treated group) showed marked cell membrane reactivity in hepatocytes (green arrows) and cytoplasmic reactivity in stellate-shaped cells (red arrows). **Fig. (5e)** related to GV (Acetaminophen + NsL oil-treated group) showed cytoplasmic reactivity in spindle-shaped cells (red arrow) and stellate-shaped cells (green arrow) with mild cell membrane reactivity (black arrow).

**Histopathological examination of the kidney:**
- **Group I (Negative control group)** showed average glomeruli, tubules and interstitium (**Fig. 6a**).
- **Group II (Positive control group)** showed average glomeruli with markedly dilated thin-walled blood vessels in the interstitium (**Fig. 6b**).
- **Group III (NsL oil group)** showed less cellular glomeruli with mild vacuolar degeneration of tubular epithelium (**Fig. 6c**).
- **Group IV (Acetaminophen-treated group)** showed distorted hypercellular glomeruli with interstitium showing fibrous band with minimal cellular infiltrate and marked vacuolar degeneration of tubular epithelium (**Fig. 6d**).
- **Group V (Acetaminophen + NsL oil-treated group)** showed distorted hypercellular glomeruli with mild vacuolar degeneration of tubular epithelium (**Fig. 6e**).

**Expression and localization of TGFβ of the kidney:**
- **Group I (Negative control group)** showed no reactivity in glomeruli or tubules (**Fig. 8a**).
- **Group II (Positive control group)** showed mild reactivity in glomeruli and tubules (**Fig. 8b**).
- **Group III (NsL oil group)** showed mild reactivity in glomeruli, with no reactivity in tubules (**Fig. 8c**).
Group IV (Acetaminophen-treated group) showed moderate reactivity in glomeruli and tubules (Fig. 8d).

Group V (Acetaminophen + NsL oil-treated group) showed moderate reactivity in glomeruli and tubules (Fig. 8e).

Masson trichrome stain: showed mild glomerular and peri-tubular fibrosis in group IV (Fig. 7d).

Figure (6) Histopathological examination of the kidney by H&E: Fig. (6a) related to GI showed average glomeruli (black arrow) with average proximal (blue arrow) and distal tubules (green arrow). Figs. (6b, c) related to G II, III showed average glomeruli (black arrow) with dilated thin-walled blood vessel (blue arrow). Fig. (6d) related to GIV (Acetaminophen-treated group) showed distorted hypercellular glomeruli (black arrow) with marked vacuolar degeneration of tubular epithelium (blue arrows). Fig. (6e) related to GV (Acetaminophen + NsL oil-treated group) showed distorted hypercellular glomeruli (black arrows) with mild vacuolar degeneration of tubular epithelium (blue arrow).

Figure (7) Histopathological examination of the kidney by Masson trichrome stain: Figs. (7a, c, e) related to GI, II, III, GV showed renal tissue with no fibrosis. Fig. (7b)
showed average renal tissue with mild positivity (green arrow). Fig. (7d) related to GIV (Acetaminophen-treated group) showed glomerular (red arrow) and peri-tubular fibrosis (green arrows).

Figure (8) Immunofluorescence staining of kidney by TGFβ: Fig. (8a) related to GI showed no reactivity in glomeruli (red arrow) or tubules (blue arrow). Figs. (8b, c) related to GII, III showed mild reactivity in glomeruli (green arrow) or tubules (red arrow). Figs. (8d, e) related to GIV (Acetaminophen-treated group) and GV (Acetaminophen + NsL oil-treated group) showed moderate reactivity in glomeruli (green arrow) or tubules (red arrow).

**DISCUSSION**

The major objective of this study was to assess the possible benefit of Nigella sativa linn oil administration on acetaminophen-induced tissue injury including hepatotoxicity and nephrotoxicity. This study investigated the ability of Nigella sativa linn oil to alleviate the acetaminophen-induced hepatic and renal functions impairments and oxidative stress in rats. Exposure to acetaminophen at their recommended dose to rats led to an alteration of hepatic and renal parameters and antioxidant capacities.

Acetaminophen has a reasonable safety profile in therapeutic doses, its overdose remains the most important cause of liver injury and even death in many parts of the world among all drug toxicities (Yayla et al., 2014). Hepatotoxic and nephrotoxic effects of Acetaminophen overdose occur by a complex sequence of events (Hinson et al., 2010). Acetaminophen toxicity in animals and man produces hepatic necrosis and a depletion of both mitochondrial and cytosolic pools of reduced glutathione. Kidney is the second target organ of Acetaminophen toxicity although nephrotoxicity may exist in the absence of hepatotoxicity following Acetaminophen overdose (Gulnaz et al., 2010).

The acetaminophen-treated group which was subjected to oral administration of 500 mg / kg of acetaminophen for induction of
EVALUATION OF THE POSSIBLE CURATIVE EFFECT OF NIGELLA...

hepatotoxicity and nephrotoxicity showed significant increase in liver enzymes (ALT, AST and TB) and renal biomarkers (serum creatinine and blood urea) when compared to positive control group. These results agreed with (Gopi et al., 2010 and Pal et al., 2011).

Serum ALT is considered a highly sensitive and specific biomarker of hepatotoxicity (Schefer et al., 2011). Also, increase in serum transaminases (AST and ALT) levels after acetaminophen administration highly signifies hepatocellular injury (Pratt et al., 2012). Moreover, elevation in liver enzymes reflected liver cell damage and could be attributed to tissue breakdown, permitting the escape of intracellular enzymes from cytosol into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation (Jaswal and Shukla, 2015).

Renal biomarkers (urea and serum creatinine) significantly increased as compared with the positive control group, a reactive metabolite of acetaminophen that caused oxidative damage to tissues. This might be the reason for its reno-toxic effects (Gopi et al., 2010).

Creatinine, a more reliable renal function marker, is a breakdown product of creatine phosphate in muscle metabolism at a constant rate. Increased creatinine concentration reflects the reduced glomerular filtration rate. An indication of renal dysfunction which is greatly reinforced our results as the acetaminophen-treated group showed an elevation in creatinine level which this also reflects the ameliorative role of N. sativa in reducing this dysfunction by decreasing creatinine level in acetaminophen + NsL oil-treated group (Ismail et al., 2014). On the same basis, urea, a major nitrogenous end product of protein/amino acid catabolism, also acts as a renal function marker as its elevation indicates impaired glomerular filtration and this also coincides with our results.

In the present work, the acetaminophen-treated group showed significant increase in TNFα when compared to positive control group. These results agreed with the results of Singh and (Chowdhary, 2014). These findings supported the induction of oxidative stress in rat kidney by acetaminophen which was in concomitant with (Ilbey et al., 2009). (Ghosh et al., 2010) reported increased oxidative stress and TNF-alpha production in rat tissues.

In the present work, the acetaminophen-treated group showed significant increase in MDA in the liver and kidney tissues when compared to positive control group which indicated occurrence of oxidative stress in this group. These results agreed with (Kleniewska et al., 2013).

The histopathological results of the current work supported the toxic effect of acetaminophen on the liver administered with previous dose as the acetaminophen-treated group showed expanded portal tracts with markedly dilated blood vessels, dilated central vein with peri-venular cellular infiltrate, scattered apoptotic hepatocytes and scattered hepatocytes with micro-vesicular steatosis, without underlying fibrosis. These changes coincide with (Hussain et al., 2014).

Also, a histopathological evidence of renal damage was observed after
administration of acetaminophen in the previous dose in the form of distorted hypercellular glomeruli with interstitium showing fibrous band, minimal cellular infiltrate and marked vacuolar degeneration of tubular epithelium. These results were in agreement with (Canayakin et al., 2016).

Moreover, the results of the current work revealed mild glomerular and peritubular fibrosis due to increased collagen fibers in Masson’s trichrome-stained sections of kidney in acetaminophen-treated group. These results agreed with (Elkhateeb et al., 2015).

Surazynski et al. (2008) stated that collagen is not only a structural component of extracellular matrix. It has been recognized as a ligand for integrin receptors, which play an important role in signaling that regulate ion transport, lipid metabolism, kinase activation and gene expression. Therefore, changes in the quantity, structure and distribution of collagens in tissues may affect cell signaling, metabolism and function.

In the present work, the immunofluorescence staining of liver sections of acetaminophen treated group showed marked cell membrane expression of TGFβ in hepatocytes and cytoplasmic reactivity in stellate-shaped cells compared to positive control group that showed moderate cell membrane reactivity in peri-venular hepatocytes these results agreed with (Elsakka et al., 2016).

The immunofluorescence staining of kidney sections of acetaminophen-treated group showed moderate reactivity in glomeruli and tubules compared to positive control group that showed mild reactivity in glomeruli and tubules. These results agreed with (L?pez-Hern?ndez and L?pez-Novoa, 2012).

In the current work, acetaminophen + NsL oil-treated group showed significant increase in liver enzymes (ALT, AST and TB) and renal biomarkers (serum creatinine and blood urea) when compared to positive control group. These results agreed with (Hasan et al., 2016), but these parameters when compared to acetaminophen treated group showed significant decrease.

The therapeutic and protective effects of NS in models of hepatotoxicity failure have been elucidated in various studies. The NS effect on alanine aminotransferase (ALT), aspartate aminotransferase (AST), LDH, total antioxidant capacity (TAC), catalase (CAT), myeloperoxidase (MPO), total oxidative status (TOS) and oxidative stress index (OSI) denotes its hepatoprotective activity (Islam et al., 2017).

Ahmed and Eid (2010) reported the role of Nigella sativa in preventing carbon tetrachloride (CCl4)-induced liver toxicity. The results showed that Nigella sativa oil decreased the levels of the elevated serum of liver enzymes considerably, and could improve the state of oxidative stress induced by CCl4. Regarding renal failure, early studies have shown that the predominant recovery mechanism of the acute kidney injury was mediated through therapeutical and protective effects of NS. (Saleeem et al., 2012) reported that nephroprotective effect of NsL oil against gentamycin-induced nephrotoxicity in rabbits where NsL oil lowers the values of serum creatinine and blood urea nitrogen,
decreases the tubular necrosis score, and prevents the degenerative changes in kidney tissues.

In the present work, the acetaminophen + NsL oil-treated group showed significant decrease in serum TNFα compared to acetaminophen treated group which supported that NS exerted anti-inflammatory and antioxidant effects. These results agreed with (Islam et al., 2017). Volarevic et al. (2011) demonstrated that the secreted anti-inflammatory cytokines were associated with decrease in secretion of inflammatory cytokines like TNFα. Also, the acetaminophen + NsL oil-treated group showed significant decrease in MDA in the liver and kidney tissues compared to acetaminophen-treated group. These results agreed with (Canayakin et al., 2016).

In the present work, the histopathological results of the liver tissue of acetaminophen + NsL oil-treated group showed expanded portal tracts with markedly dilated blood vessels, markedly dilated central veins, markedly congested blood sinusoids and vacuolated hepatocytes. These results agreed with Ghadlinge et al. (2014) who stated that co-administration of NS oil with paracetamol significantly reduces scores of degeneration, necrosis and fibrosis. Also, the histopathological results of the kidney tissue of the same group showed distorted hypercellular glomeruli with mild vacuolar degeneration of tubular epithelium. These results agreed with the findings of Canayakin et al. (2016).

It was noted that the histopathological results of the current work supported the biochemical results of acetaminophen + NsL oil-treated group through appearance of partial improvement and evidence of some regeneration of both liver and kidney tissues when compared to acetaminophen-treated, and expression of TGFβ in hepatocytes was improved in acetaminophen + NsL oil-treated compared to acetaminophen-treated group which also supported both partial improvement of the biochemical results and evidence of some regeneration of liver tissues through the histopathological results.

CONCLUSION

Our results indicated that the NsL oil alleviated the toxic effects of acetaminophen on the liver and kidney including the histopathological and biochemical changes as well as the oxidative damage. The curative effect of NsL oil could be due to its antioxidant potential by scavenging the free radicals. So, patients using acetaminophen for long times should be checked regularly for their liver and kidney functions. Our study revealed that usage of TGF β as a candidate marker for diagnosis and prognosis of both chronic liver and kidney diseases also as a target for their treatment. Also, other studies on dose-dependent effects of NSL oil are also recommended.

REFERENCES


EVALUATION OF THE POSSIBLE CURATIVE EFFECT OF NIGELLA…

Tقييم التأثير العلاجي المحتمل لزيت الحبة السوداء على كل من التسمم الكبدى والتمسم الكلوى المحدث معملاً باستخدام عقار الأسيتامينوفين في ذكور الجرذان البيضاء البالغة

حامد محمد عثمان - سيد محمد سيد - سيد عبد الرحيم سيد - أشرف عبد العاطى عمار

قسم الفسيولوجيا الطبية والباثولوجيا* - كلية طب الأزهر

خلفية البحث: هناك إدراك كبير أن إستعمال الأعشاب وإعتبارها في علاج الأمراض البشرية مثل التسمم الكبدى والتمسم الكلوى. وتعتبر الحبة السوداء واحدة من أهم النباتات الطبية التي لها تأثير قوى كمضاد للأكسدة.

الهدف من البحث: دراسة التأثير العلاجي المحتمل لزيت الحبة السوداء على كل من التسمم الكبدى والتمسم الكلوى المحدث معملاً باستخدام عقار الأسيتامينوفين في ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: استخدم في هذا البحث خمسون جرذًا أبيضًا بالغًا من فصيلة محلية، وقد تم إحداث التسمم الكبدي الكلوى باستخدام عقار الأسيتامينوفين. وفي نهاية التجربة (أربعة وعشرين يومًا) تم أخذ عينات من الدم لقياس بعض وظائف الكبد والكلي وأيضاً قياس TNFα وكذلك تم أخذ عينات من نسيج كل من الكبد والكلي لفحصها مجهرياً وعمل بعض القياسات الخاصة بحساب عملية الأكسدة داخل هذه الأنسجة.

النتائج: أحدث عقار الأسيتامينوفين زيادة في وظائف الكبد والكلي أيضًا في كل المقاييس التي تم دراستها في هذا البحث وتأكيد هذه النتائج بفحص نسيج كلاً من الكبد والكلي مجهرياً، بينما أحدث زيت الحبة السوداء نقصًا في المقاييس التي تم دراستها.

الاستنتاج: العلاج بزيت الحبة السوداء له دور فعال في إتنم كل من نسيج الكبد والكلي في الفشل الكبدي الكلوي المحدث تجريبياً بعقار الأسيتامينوفين.