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Curcuma longa and *Nigella sativa* Modulate the Hepatotoxic Effect of Anti-Tuberculosis Drugs and Acetaminophen in Rats

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Abstract: *Liver plays a central role in the metabolism and excretion of xenobiotic which makes it highly susceptible to their adverse effects. The aim of the present work is to evaluate the possible protective effect of Curcuma longa powdered rhizome and /or Nigella sativa powdered seeds against hepatotoxicity of Rifampicin, Isoniazid and Acetaminophen combination in rats. Rats were randomly allocated into 10 groups, all groups were administered the drugs orally for 21 days and the treatments started one hour before drugs administration according to the following scheme:Group (1): Normal group received 2% tween 80.Group (2): Received Curcuma longa (200 mg/kg) .Group (3): Received Nigella sativa (250 mg/Kg) Group (4): Received Curcuma longa + Nigella sativa Group (5): Received Acetaminophen (1000 mg/kg). .Group (6): Received Rifampicin (100 mg/kg) + Isoniazid (50mg/kg). Group (7): Received Rifampicin +Isoniazid +Acetaminophen .Group (8): Received Curcuma longa before Rifampicin +Isoniazid +Acetaminophen. Group (9): Received Nigella sativa before Rifampicin +Isoniazid + Acetaminophen.Group (10): Received Curcuma longa +Nigella sativa before Rifampicin +Isoniazid +Acetaminophen. Biomarker of liver functions (serum levels of AST, ALT, and ALP, albumin, total protein and total bilirubin) and liver homogenate contents of oxidative stress biomarkers (MDA, GSH reduced GSH peroxidase and SOD) and inflammatory and apoptotic necrosis markers (TNF-α and Caspase-3) were measured, and Liver histopathological changes were examined. Administration of Acetaminophen- Rifampicin and INH resulted in significant increase in the levels of hepatic TNF-α &Casp-3, hepatic MDA and biomarker of liver functions and significantly decrease in reduced GSH, GSH peroxidase, SOD, serum albumin and total protein compared to normal control group. Curcuma longa significantly decreased AST, ALT, ALP and total bilirubin levels by (72.8%, 79.8%, 68.2%, 37.7%) respectively and significantly increased albumin and total protein levels by (61.5%, 56.1%) respectively. Nigella sativa significantly decreased AST, ALT, ALP and total bilirubin levels by (70.5%, 78.1%, 67.1%, 30.4%) respectively and significantly increased albumin and total protein levels by (51.9%,39.7%) respectively. Pretreatment of the animals with Curcuma longa and or Nigella sativa resulted in significant decrease in the level of hepatic TNF-α & Casp-3 and hepatic MDA and significant increase in serum reduced GSH, GSH peroxidase and SOD when compared to Rifampicin, INH and Acetaminophen treated group. It is concluded that Rifampicin and INH induce hepatotoxicity confirmed by changes in liver function and the oxidative stress parameters and using Curcuma longa and Nigella sativa modulate the hepatotoxic effect of anti-tuberculosis drugs and Acetaminophen.*

Key words: *Curcuma longa, Nigella sativa, Rifampicin, Isoniazid and Acetaminophen, Casp-3 , TNF-α hepatotoxicity.*

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I. Introduction

Liver is the largest organ of the human body. It plays a central role in the metabolism and excretion of xenobiotic which makes it highly susceptible to their adverse and toxic effects **(Singh *et al*., 2011)**.

Liver enzymes are usually raised in acute hepatotoxicity, but tend to decrease with prolonged intoxication due to damage to the liver **(Jens and Hanne, 2002).** Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) are mainly implicated in the diagnosis of biliary obstruction **(Jens and Hanne, 2002)** while Gamma Glutamyl Transpeptidase (GGT) is elevated in diseases that decrease or obstruct the flow of bile **(Adaramoye *et al*., 2008)**. Total protein reflects the synthetic

functions of the liver, albumin and ammonia concentration reflects detoxifying function of the liver **(Jens and Hanne, 2002)**. **Tuberculosis** TB is a commonly occurring respiratory infection and its incidence has increased globally in the past few years. It is displayed by infection with *Mycobacterium Tuberculosis* The first line drugs used for tuberculosis are Isoniazid (INH) and Rifampicin(RIF). Both drugs are potentially hepatotoxic independently, and when given in combination their toxic effects are enhanced in a synergistic manner **(Villemagne *et al.*, 2012)**.

Peripheral neuropathy and hepatotoxicity are the most frequently observed adverse effects of INH **(Duarte *et al.*, 2007),** more recent studies suggested that direct oxidation of INH leads to hepatotoxicity and severe injury that may include an autoimmune component, which makes it difficult for patients to recover even the drug is stopped **(Metushi *et al.*, 2016)**. Rifampicin is a semisynthetic derivative of Rifamycin antibiotics. In addition of its hepatotoxicity it may lead to acute hemolytic anemia and renal failure (nephrotoxicity). Acetaminophen (N-acetyl-p-aminophenol, APAP) is a widely used analgesic and antipyretic drug which in contrast to aspirin does not cause gastrointestinal irritation and micro bleeding **(Hodgman and Garrard, 2012)** but ingestion of high doses of acetaminophen is associated with acute liver injury **(Placke *et al.*, 1987).**

A small portion of a dose of Acetaminophen is converted to a highly reactive intermediate, N -acetyl-P-benzoquinoneimine (NABQI) by microsomal cytochrome P- 450, which is then detoxified by conjugation with glutathione and excreted as mercapturate and cysteine conjugates. Following acetaminophen over dosage, tissues stores of glutathione become depleted and the intermediate accumulates and cell damage **(Martindale, 1993 and Itoh *et al.*, 2002).** It is thought that it initiate the processes leading to cell death by covalently binding to cellular macromolecules **(Miller *et al.*, 1993).**

High acetaminophen concentration increase NAPQI which saturate GSH transferase and the excess of metabolites may covalently bind to electrophiles such as sulfhydryl-containing enzymes, causing enzyme inactivation and possibly cell death **(Jollow *et al.*, 1973).** Lipid peroxidation **(Wendel *et al.*, 1979**), protein sulfhydryl oxidation (**Moore *et al.*, 1985)**, and alteration in cellular calcium levels **(Boobis *et al.*, 1990)** have been postulated as mechanisms leading to the acetaminophen hepatotoxicity.

Antioxidants are substances that react with reactive oxygen species and reactive nitrogen species and stop tissue oxidation by processes involving free radical scavenging; sequestration of transition met also, enzymatic hydrolysis of ester bonds, and enzyme-catalyzed reduction of peroxides **(Lachance *et al.*, 2001).**

Curcuma longa rhizome is a herb from ginger family (Zingiberaceae) and is cultivated extensively in Asia, India, China and other tropical climate countries **(Dobelis, 1990)**. Curcumin is the active constituent of *Curcuma longa*, which has been shown to have hepatoprotective activity (**Park *et al.*, 2000**) through scavengimg and preventing formation of reactive oxygen species (ROS) (**Betancor *et al.*, 2003**) and reactive nitrogen species (RNS) (**Kim *et al.*, 2003**). In addition, *Curcuma longa* was shown to induce several enzymatic anti-oxidants, such as glutathione transferase, catalase (**Iqbal *et al.*, 2003**), and hemeoxygenase-1 (**Motterlini *et al.*, 2000**). Also inhibits nuclear binding of hepatic nuclear factor kappa B (NFkB) in a rat model of ethanol-induced hepatotoxicity (**Nanji *et al.*, 2003**) and NFkB-mediated expression of pro-inflammatory molecules, such as inducible nitric oxide (iNOS) is partially prevented (**Singh and Aggarwal 1995**).

Nigella sativa L. (Ranunculaceae family) seeds, commonly known as black seed or black cumin, have been employed for thousands of years as a spice and food preservative, as well as a protective for numerous disorders (**Nadkarni., 1976**).

The pharmacological investigations of the seed extracts reveal abroad spectrum of activities including immunopotentiation (**Khan, 1999)** and antihistaminic (**Mahfouz *et al.*, 1965),** antidiabetic (**Al-Hader *et al.*, 1993**), anti-hypertensive (**El Tahir *et al.*, 1993**), anti-inflammatory (**Houghton *et al.*, 1995**), and antimicrobial activities (**El-Alfy *et al.*, 1975**). Many of these activities have been attributed to thymoquinone constituents of the seed (**Ali and Blunden, 2003**). Black seed preparations may have a cancer chemo preventive potential and may reduce the toxicity of standard antineoplastic drugs (**Salomi and Nair, 1992**). In addition, others have reported an antitumor activity of some crude and purified components of *N. sativa*, and other investigators have reported that TQ triggers apoptotic cell death in human colorectal cancer cells (**Gali-Muhtasib *et al.*, 2004**).

The present work is therefore carried out to further investigate the possible possible protective effect of *Curcuma longa* powdered rhizome and /or *Nigella sativa* powdered seeds against hepatotoxicity of Rifampicin, Isoniazid and Acetaminophen combination in rats.

II. Materials and Methods

2. Materials

2.1. Animals

Adult albino rats of Sprague - dawely strains weighing 150 ± 200g were obtained from the animal house of National Organization of Drug Control and Research (NODCAR) Cairo, Egypt. The animals were housed in hygienic metal cages and kept in a clean, well ventilated room. Animals were left for two weeks of

acclimatization under standard laboratory conditions with temperature at 23±2 c, and 12:12 light / dark cycle. The animals were allowed free access to Food and water.

2.2. Drugs

Acetaminophen was obtained from El-Nasr Company for pharmaceutical Chemicals, Cairo. It was made into suspension in distilled water by the use of 1% tween-80 as suspending agent. The dose used was 1000 mg/kg body weight **(Madkour *et al*., 2013)**.

Rifampicin and Isoniazid were obtained from Novartis Pharma Company, Cairo. They were made into suspension in distilled water by the use of 1% tween-80 as suspending agent just before administration. The doses used in the present study were (100&50mg/kg body weight) respectively. RIF. & INH were administered orally for 21 consecutive days according to (**Bhupinder *et al*., 2007).**

2.3. Natural herbs

Curcuma longa

Curcuma longa powder was purchased from local market. It was made into suspension in distilled water by the use of 1% tween-80 as suspending agent. The dose used was 200 mg/kg body weight **(Singh *et al*., 2012)**.

Nigella sativa

Nigella sativa was purchased from local market, freshly grinded just before used and made into suspension in distilled water by the use of 1% tween-80 as suspending agent. The dose used was 250 mg/kg body weight **(Hadjzadeh *et al*., 2009)**.

2.4. Chemicals

Albumin kit was obtained from Diamond Diagnostics Company, Cairo. Tween 80 was obtained from Sigma Company, USA .(Alanine aminotransferase kit, Alkaline phosphatase kit, Aspartate aminotransferase kit, Bilirubin Kit, Glutathione kit, Malondialdehyde kit, Superoxide dismutase kit, Total protein kit, Tumor necrosis factor kit) were obtained from Biodiagnostic Company, Cairo.

2.5. Experimental design

Rats were randomly allocated into 7 groups, all groups were administered the drugs orally for 21 days and the treatments started one hour before drugs administration according to the following Group(1) vehicle (1% tween 80 & distilled water) orally for 21 days, Group(2) Acetaminophen (1000 mg/kg), Group(3) Rifampicin (100 mg/kg) +Isoniazid (50mg/kg), Group(4) Rifampicin (100mg/kg) +Isoniazid (50 mg/kg) +Acetaminophen (1000mg/kg) for 3 days, Group(5) *Curcuma longa* (200mg/kg) before Rifampicin (100mg/kg) +Isoniazid (50 mg/kg) +Acetaminophen (1000mg/kg), Group(6) *Nigella sativa* (250mg/kg) before Rifampicin (100mg/kg) +Isoniazid (50 mg/kg) + Acetaminophen (1000mg/kg), Group(7) *Curcuma longa* (200mg/kg) +*Nigella sativa* (250mg/kg) before Rifampicin (100mg/kg) +Isoniazid (50mg/kg) +Acetaminophen (1000mg/kg). 24 hours after the last dose, the animals weighed, blood was collected from the retro-orbital plexus (**Schermare, 1967)** using a heparinized capillary tube. Blood was then centrifuged for serum separation and stored at -80°C for ALT, AST, ALP total protein, albumin and total bilirubin levels estimation. The animals were sacrificed; livers were removed, washed with saline, dried and weighed to assess their relative weights. Finally, they were divided into 2 parts; a piece of the right lobe of the liver was taken and preserved in -80**o**c refrigerator for MDA, GSH reduced as antioxidant biomarkers, glutathione peroxidase (Gpx) enzyme activity as oxidative stress biomarkers, tumor necrosis factor-alpha TNF-α as inflammatory marker and caspase-3 as apoptotic marker estimation. The remnant of the liver preserved in 10% formalin at room temperature till processed for histopathological examination.

2.6. Determination of biochemical parameters

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined colourimetrically by the method described by **(Reitman and Frankel, 1957)** using commercial kits. The intensity of the colour which is developed by coupling pyruvate or oxaloacetate with 2, 4 dinitrophenyl-hydrazine (2, 4 DNPH) was measured spectrophotometrically at 505 nm. Whereas ALP level was determined colourimetrically by the method described by (**Belfield and Goldberg, 1971)** using commercial kits. Albumin, was determined according to method described by **(Gendler and Kaplan, 1984)** while total protein and total bilirubin were determined according to method described by **(Gornal *et al*., 1949**) and **(Walter and Green., 1970)** respectively using commercial kits. The lipid peroxidation products MDA content was estimated in liver homogenate according to the method of **(Satoh, 1978)** and **(Ohkawa *et al*., 1979)** while reduced glutathione (GSH) was estimated according to the method of **(Beutler *et al.*, 1963)** using test reagent kits

(Biodiagnostics,Egypt). In addition, the activities of some antioxidant enzymes including SOD (U/g tissue) and GSH-Px (U/g tissue) were estimated using test reagent kits (Biodiagnostics, Egypt).
The liver homogenate content of TNF- α (pg/ml) as inflammatory marker was estimated by solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle using test reagent kits (Biodiagnostics,Egypt) and liver homogenate content of caspase-3 as apoptotic marker was also estimated by the method described by (**Wolf B. B., Green D. R. 1999).**

2.7. Preparation of sections for histopathological examination

Liver samples were removed, rinsed in formalin, dehydrated, cleared, impregnated and embedded in paraffinto facilitate ease of cutting for assessment of histopathological changes using haematoxylin, eosin **(Afifi, 1986**) as following: Dehydration: was carried out by bringing the liver specimens through succession of alcohol of increasing strengths in order to remove water from the specimen. Clearing: Xylene was used as clearing agent, liver was immersed in xylene. Impregnation and embedding: Tissues were impregnated with paraffin by using automatic tissue processing machine, following impregnation; the tissue was embedded in molten wax. Section cutting: Before cutting, the wax block was trimmed then sections were cut in thickness of 4 microns using rotary microtome. Staining: The sections were stained by Haematoxylin and Eosin stain. The stained sections were examined under the light microscope and were photographed at magnification (X 400).

2.8. Statistical Analysis

Data were statistically analyzed and expressed as mean ± standard error (SEM) using one way ANOVA test and Post-hoc Tukey's multiple comparisons of mean tests using IBM SPSS (2011). $P \leq 0.05$ was used as criteria for significance.

III. Results

3.1. Effect of *Curcuma longa* and/or *Nigella sativa* on serum AST, ALT ALP albumin, total protein and total bilirubin serum levels

Acetaminophen treated animals showed significant increase in serum AST, ALT ALP and total bilirubin levels approximately by 205%, 171%, 127% and 205% respectively, more significant increase in these enzyme levels were shown in animals that treated with RIF. And INH. In addition of more and more increase in these levels in animal group that treated by acetaminophen RIF. And INH. combination. It reached about 605%,680, 436% and390% respectively when compared to normal control.

Curcuma longa significantly decreases AST, ALT, ALP and total bilirubin levels by (72%, 79%, 68%, 37%) respectively when compared to RIF, INH and acetaminophen combination. Meanwhile, *Nigella sativa* significantly decrease these parameters levels by (70%, 78%, 67%, 30%) respectively when compared to RIF, INH and acetaminophen combination. On the other hand, *Curcuma longa* and *Nigella sativa* combination significantly decrease their levels by (68%, 76%, 66%, 29%) respectively when compared to RIF, INH and acetaminophen combination.

Acetaminophen treated animals showed significant decrease in serum albumin and total protein levels by 30% and14.15% respectively compared to normal control rats. Also RIF. and INH. showed significant decrease in these parameters by 42% and 23% respectively, in addition to more significant decrease in these parameters in animal group treated by their combination. It reached about 46% and 41% respectively when compared to normal control rats.

Curcuma longa significantly increase in albumin and total protein levels by (61%, 56%) respectively when compared to RIF, INH and acetaminophen combination. Meanwhile, *Nigella sativa* significantly increases albumin and total protein levels by (51%,39%) respectively when compared to RIF, INH and acetaminophen combination. On the other hand, *Curcuma longa* and *Nigella sativa* combination significantly increase in albumin and total protein levels by (39%, 38%) respectively when compared to RIF, INH and acetaminophen combination.

We can notice that the mean values of biochemical serum analysis show significant improvement which is more prominent in pre-treatment with *Curcuma longa* than pre-treatment with *Nigella sativa* or pre-treatment with *Curcuma longa* and *Nigella sativa* combination. Fig.(1)

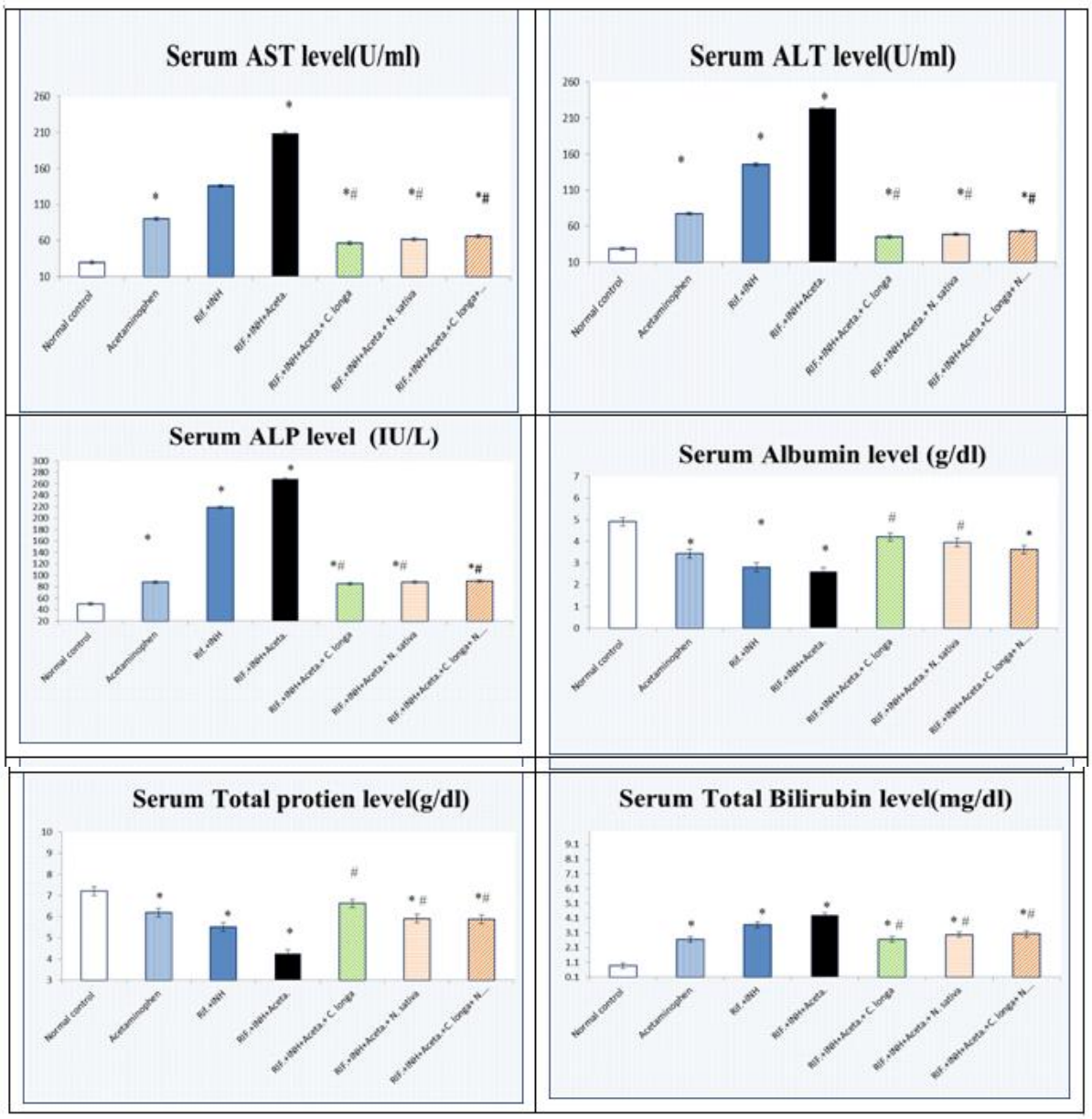

Fig.(1) Effect of pretreatment of *Curcuma longa* (200mg/kg) and/or *Nigella sativa* (250mg/kg) on the changes produced by RIF.(100mg/kg), INH (50mg/kg) and Acetaminophen (1000mg/kg) on serum levels of AST, ALT, ALP , albumin, total protein and total bilirubin in rats.

* Significantly different from normal control group at $p \leq 0.05$.

Significantly different from Rif. + INH+ Acetaminophen group.

3.2. Effect of *Curcuma longa* and/or *Nigella sativa* on liver homogenate contents of MDA, reduced GSH, GSH peroxidase and SOD in rats.

Acetaminophen treated animals showed significant increase in liver homogenate content of MDA by 127%, while RIF&INH and RIF&INH acetaminophen combination showed significant increase by 687% and 809% respectively when compared to normal control rats. Treatment with *Curcuma longa* , *Nigella sativa* and their combination significantly decreased liver MDA content b by 79%, 77% and 77%, respectively when compared to RIF&INH and acetaminophen combination.

Acetaminophen treated animals showed significant decrease in liver homogenate content of reduced GSH, GSH peroxidase and SOD by 48%,70% and47% respectively when compared to normal control rats. Also, RIF. &INH showed significant decrease in these parameters by 69%74% and 55% respectively. In addition to more significant decrease in these parameters in animal group treated by their combination. It reached about 73% 79%and 60% respectively when compared to normal control rats.

Curcuma longa significantly increases liver homogenate content of reduced GSH, GSH peroxidase and SOD by (47%, 283% and 133%,) respectively when compared to RIF&INH and acetaminophen combination. Meanwhile, *Nigella sativa* significantly increases these parameters by (66%, 267% and 132%) respectively when compared to RIF& INH and acetaminophen combination. On the other hand, *Curcuma longa* and *Nigella sativa* combination significantly increase their liver content by (80%, 254% and 127%) respectively when compared to RIF& INH and acetaminophen combination.

The mean values of antioxidant analysis show significant improvement which is more prominent in pre-treatment with *Curcuma longa* than pre-treatment with *Nigella sativa* or pre-treatment with *Curcuma longa+ Nigella sativa* combination.

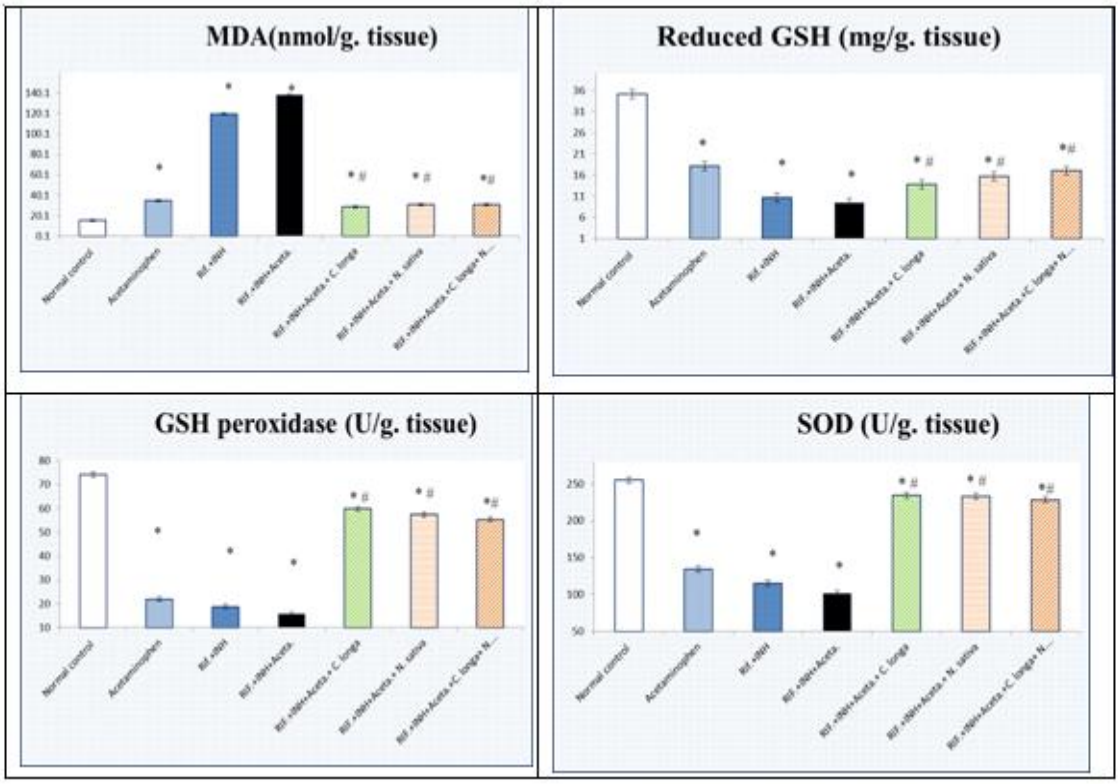

Fig.(2)Effect of pre-treatment of *Curcuma longa* (200mg/kg) and/or *Nigella sativa* (250mg/kg) on the changes produced by RIF. (100mg/kg), INH (50mg/kg) and Acetaminophen (1000mg/kg) on liver homogenate contents of MDA, GSH reduced, GSH peroxidase and SOD in rats.

Data were analyzed by one-way ANOVA followed by post-hoc Tukey's test and expressed as mean ± SEM. N= 6. $P \leq 0.05$ was used as criteria for significance.

* Significantly different from Normal control group.

Significantly different from Rif.+ INH+ Acetaminophen group.

Tab.(1): Effect of pre-treatment of *Curcuma longa* (200mg/kg) and/ or *Nigella sativa* (250mg/kg) on the changes produced by RIF.(100mg/kg), INH (50mg/kg) and Acetaminophen (1000mg/kg) on liver homogenate contents of TNF-α and Casp-3 in rats.

Treatment	TNF-α (pg/ml)	Caspase 3(ng/ml)
Normal control	**154.4 ± 7.0**	**2.85 ± 0.28**
Acetaminophen	**665.4 *± 29.80**	**20.28 *±0.49**
Rif.+INH	**805.5 * ± 17.85**	**27.41 *±0.75**
Rif.+INH+Aceta.	**865 * ± 17.75**	**30.55 *±0.36**
RIF.+INH+Aceta.+*C.longa*	**237.9*#±25.63**	**7.61 *# ±0.21**
RIF.+INH+Aceta.+*N.sativa*	**246.5 *#±5.41**	**9.6 *# ±0.31**
RIF.+INH+Aceta.+*C.longa*+ *N. sativa*	**240.4 *#±2.61**	**8.08 *# ±0.26**

Data were analyzed by one-way ANOVA followed by post-hoc Tukey's test and expressed as mean ± SEM. N= 6. $P \leq 0.05$ was used as criteria for significance.

* Significantly different from Normal control group.

Significantly different from Rif.+ INH+ Acetaminophen group.

Acetaminophen treated animals showed significant increase in liver homogenate content of TNF-α and Caspase 3 by 332% and 612% respectively compared to normal control rats. Also RIF.&INH. showed significant increase in these parameters by 421% and 861% respectively. In addition to more significant increase in these parameters in animal group treated by their combination. It reached about 460 % and 972% respectively when compared to normal control rats.

Curcuma longa significantly decreases liver homogenate content of TNF-α and Caspase3 by (73% and 75%) respectively when compared to RIF, INH and acetaminophen combination group. Meanwhile, *Nigella sativa* significantly decrease liver homogenate content of TNF-α and Caspase 3 by (72% and 69%) respectively when compared to RIF, INH and acetaminophen combination. On the other hand, *Curcuma longa* and *Nigella sativa* combination significantly decrease liver homogenate content of TNF-α and Caspase3 by (72% and 74%) respectively when compared to RIF, INH and acetaminophen combination group. Tab. (1)

Liver histopathological findings:

Histopathological examination of liver sections of acetaminophen treated rats showed inflammatory cells infiltration and pigmentation in the portal area associated with degeneration in the hepatocytes, while examination of liver sections of Rifampicin and Isoniazid treated rats showed congestion in the portal vein with inflammatory cells infiltration and newly formed bile ducts in the portal area. On the other hand, examination of liver sections of rats treated by their combination showed congestion in the central vein with degeneration in the hepatocytes in diffuse manner as well as inflammatory cells infiltration in the portal area. However, pretreatment with *Curcuma longa* alone showed diffuse kupffer cells proliferation in between the hepatocytes, while pretreatment with *Nigella sativa* showed congestion in the central vein associated with diffuse kupffer cells proliferation in between the hepatocytes. Pretreatment with combination of *Curcuma longa* and *Nigella sativa* showed fatty changes in few hepatocytes in the hepatic parenchymas (**Fig. 3).**

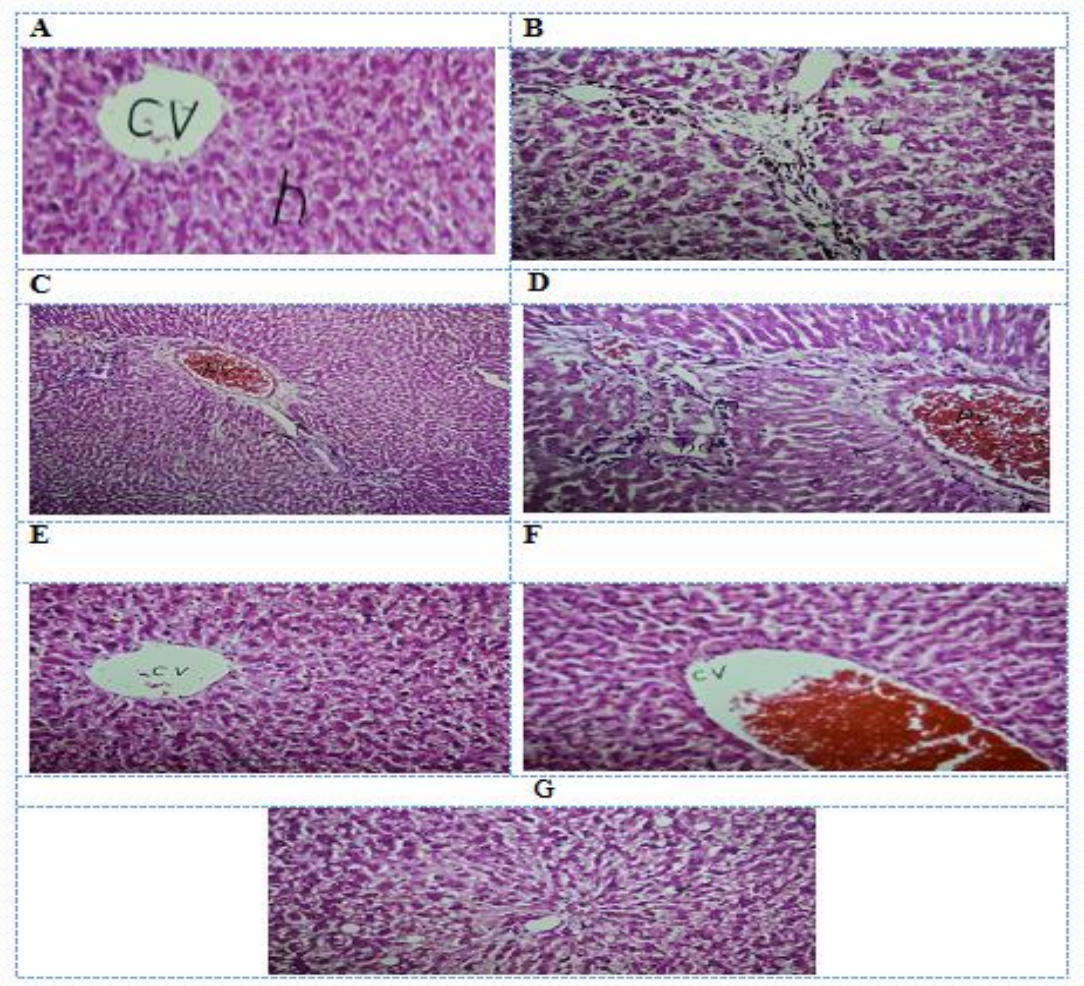

Fig.(3)Effect of pretreatment treatment with *Curcuma longa* (200mg/kg) and/ or *Nigella sativa* (250mg/kg) on liver rat structure. Tissues were stained with hematoxylin and eosin (magnification · 400). (A) Normal hepatic tissue showing normal histological structure (typical hexagonal hepatic lobules formed of readily arranged hepatocytes and central vein. (B) acetaminophen hepatic tissue with inflammatory cells infiltration and pigmentation in the portal area associated with degeneration in the hepatocytes (C) Rifampicin &Isoniazid treated rats showed congestion in the portal vein with inflammatory cells infiltration and newly formed bile ducts in the portal area (D) Rifampicin &Isoniazid and acetaminophen combination showed congestion in the central vein with degeneration in the hepatocytes in diffuse manner as well as inflammatory cells infiltration in the portal area. (E) Pretreatment with *Curcuma longa* alone showed diffuse kupffer cells proliferation in between the hepatocytes. (F) Pretreatment with *Nigella sativa* showed congestion in the central vein associated with diffuse kupffer cells proliferation in between the hepatocytes. (G) Pretreatment with combination of *Curcuma longa* and *Nigella sativa* showed fatty changes in few hepatocytes in the hepatic parenchymas .

IV. Discussion

In the present study, giving Sprague - dawely rats acetaminophen, Rifampicin and Isoniazid elicited a full array of hepatotoxicity as previously reported.

As regards the high dose of Acetaminophen for 3 days resulted in significant increase in biochemical parameters (serum AST, ALT, ALP and total bilirubin) levels and significant decrease in serum albumin and total protein levels in comparison to normal control. Fig.(1). These results agreed with **(Larson *et al*., 2005, Sener *et al*., 2005 and Kanbur *et al*. 2009)** who said that it is well known that acute Acetaminophen - induced liver injury, usually involves dramatic increases in plasma aminotransferase with moderate rises in plasma bilirubin. Also **Hassanin *et al*., (2013)** results showed that paracetamol caused a significant increase in activities of liver enzymes (ALT, AST and ALP) when compared with rats of control group. In addition, paracetamol administration caused a marked decrease in total proteins.

Administration of Rifampicin & INH resulted in significant increase in serum AST,ALT, ALP and total bilirubin levels and significant decrease in serum albumin and total protein levels in comparison to normal control group. Fig. (1). **Christiane and Peter, (2006)** reported that rifampicin coadministered with INH in treatment of tuberculosis, is toxic to hepatocytes**. Chowdhury *et al*., (2006)** reported that reactive oxygen species play an important role in INH and rifampicin induced liver injury. Our findings agreed with those of **Anbarasu *et al* ., (2011)** who showed that the levels of serum AST, ALT, ALP and total bilirubin were markedly elevated and that of total protein decreased in rifampicin &INH treated group indicating liver damage. Low level of serum total protein and albumin indicates alarming liver damage in rats treated with INH and rifampicin as the reactive metabolites of these drugs result in liver dysfunction (**Jeyakumar *et al*., 2009 and Sankar *et al*., 2015**).

Rifampicin, INH & Acetaminophen administration resulted in significant increase in serum AST, ALT, ALP and total bilirubin levels and significant decrease in serum albumin and total protein levels in comparison to normal control. Fig.(1).These results agreed with **Larson, (2007**) who observed that concomitant use of medications that induce the cytochrome P system, such as INH and Rifampicin, may predispose to acetaminophen hepatotoxicity by increased production of NAPQI by way of the oxidative pathway.

Pre-treatment with *Curcuma longa* in the Rifampicin, INH & acetaminophen combination group reflected significant improvement of liver functions as it decreases serum AST, ALT, ALP and total bilirubin levels and significant increase in serum albumin and total protein levels Fig.(1).

Our result was in agreement with **Moghadam *et al*., (2015**) who found that rats pre-treated with turmeric before methotrexate exposure showed a significant decrease in blood ALT, AST, ALP, bilirubin and albumin. Also, our results were in agreement with **Granados *et al*., (2016)** who found that acetaminophen-induced liver histological damage and increment in plasma ALT and AST activity, were attenuated with *Curcuma longa* and associated with attenuation of mitochondrial dysfunction.

Administration of *Nigella sativa* with Rifampicin, INH &Acetaminophen resulted in significant decrease in biochemical parameters (serum AST, ALT, ALP, total bilirubin levels and significant increase in serum albumin and total protein levels in comparison to Rifampicin, INH &Acetaminophen combination group. Fig.(1).These results reflected a significant improvement of liver functions as a result of *Nigella sativa* administration.

Concomitant administration of *Curcuma longa* and *Nigella sativa* with Rifampicin, INH & Acetaminophen resulted in significant decrease in serum AST, ALT, ALP, total bilirubin levels and significant increase in serum albumin and total protein levels in comparison to Rifampicin, INH &Acetaminophen combination group. Fig.(1).Our results were in agreement with **Mourad *et al*.,** (**2006**) who found that daily intake of Curcumin or *Nigella sativa* seeds in CCl4-treated rats was associated with significant reduction in the

rise of serum activity of hepatic AST and ALT comparing to control group, and **Jadhav and Mateenuddin., (2013)** who found that concurrent administration of *Nigella sativa* oil given along with antitubercular drugs significantly prevented the rise in the enzyme levels such as ALT, AST and ALP. It also reduced the serum bilirubin and the fall in serum total protein level as compared to group receiving antitubercular drug alone.

Administration of Acetaminophen resulted in significant increase in the level of hepatic MDA and significant decrease in reduced GSH, GSH peroxidase and SOD when compared to control group. Fig. (2).

These findings come in agreement with **Hassanin *et al*., (2013)** who found that paracetamol caused a significant increase in MDA level, depletion of GSH contents and decreased reduced GSH activity as compared to that of rats of control group. In addition, paracetamol administration caused a significant increase in SOD activities.

Administration of Rifampicin and INH resulted in significant increase in the level of hepatic MDA when compared to control group. On the other hand, GSH reduced; GSH peroxidase and SOD were significantly decreased when compared with control treated group. Fig. (2). These results agreed with **Anbarasu *et al* ., (2011) and Sankar *et al*., (2015)** who said that the levels of serum SOD, reduced GSH, GSH peroxidase were significantly decreased while lipid peroxidase level showed a significant increase in Rifampicin& INH treated rats .

Hydrazine, the principal metabolite of INH is highly reactive with sulfhydryl group, which results in depletion of GSH in liver cell and induces oxidative stress to the hepatic cell (**Wessam *et al*., 2013)**. More over elevation of MDA**,** which is a clear manifestation of excessive free radical formation and lipid peroxidation (**Viswanthaswamy *et al*., 2010** and **Zeinab *et al*., 2012**).

Administration of Rifampicin, INH and Acetaminophen resulted in significant increase in the content of hepatic MDA and a significant decrease in reduced GSH, GSH peroxidase and SOD when compared to control group. Fig. (2).

Concurrent administration of *Curcuma longa* with Rifampicin, INH &Acetaminophen resulted in significant decrease in the content of hepatic MDA when compared with Rifampicin, INH &Acetaminophen treated group. On the other hand, reduced GSH, GSH peroxidase and SOD contents were significantly increased when compared to Rifampicin, INH and Acetaminophen group. Fig. (2).

These results were in agreement with **(Adhvaryu *et al*., 2007**) who said that turmeric powder was used as hepatoprotectant in INH+Rif.+pyrazinamide induced hepatotoxicity in guinea pigs. It suppresses the production of superoxide by macrophages, has a potent anti-inflammatory action that inhibits the production of tumor necrosis factor alpha (TNF-α), in human monocytic derived cells.

Also, the result was in agreement with **Kumar *et al.*, (2013)** who studied the effect of powder of *Curcuma longa* rhizomes in INH, Rifampicin and pyrazinamide -induced hepatic injury and found that it suppresses the production of superoxide by macrophages, has a potent anti-inflammatory action that inhibits the production of tumor necrosis factor alpha (TNF-α) in guinea pigs and recognize a decrease in hepatic peroxidation and these properties clearly explain the hepatoprotective potential of *Curcuma longa* in the experimental study. Also, **Li *et al*., (2013)** found that both pre- and post-treatment with *Curcuma longa* resulted in a significant reduction in serum ALT, MDA and hepatocyte necrosis.

The protection of *Curcuma longa* may be related to inhibition of lipid peroxidation and oxidative stress by increasing SOD activity to neutralize and scavenge the free radical in order to prevent oxidative damage to cells. In addition , our results was in agreement with **Moghadam *et al*., (2015**) who said that rats pre-treated with turmeric before Methotrexate exposure, showed a significant decrease in MDA and significant increase in SOD and GSH peroxidase.

Curcuma longa has several mechanisms as hepatoprotectant against acetaminophen hepatotoxicity. *Curcuma longa* either decrease the concentration of acetaminophen toxic active metabolite or increase hepatic GSH concentration and maintain concentration of GSTase which accelerate acetaminophen toxic metabolite execretion **(Kalantari *et al*., 2007)**.

Administration of *Nigella sativa* with Rifampicin, INH & Acetaminophen showed a potent antioxidant effect where there is a significant decrease in the content of hepatic MDA when compared with Rifampicin, INH &Acetaminophen treated group. On the other hand, reduced GSH, GSH peroxidase and SOD hepatic contents were significantly increased when compared to Rifampicin, INH and Acetaminophen group. Fig. (2). This agreed with (**Mourad *et al* ., 2006**) who found that daily intake of Curcuma longa or *Nigella sativa* seeds in CCl4-treated rats was associated with significant reduction in the rise of serum activity of hepatic transaminases (AST and ALT) and liver content of MDA and caspase-3 activity compared to CCl4-treated mice. Our results agreed with **El-Beshbishy *et al*., (2010)** who said that pretreatment with *Nigella sativa* or *Curcuma longa* in tamoxifen –induced liver injury rats caused a significant decline in MDA and a significant increase in reduced GSH, GSH peroxidase and SOD when compared to tamoxifen treated group.

Administration of Curcuma longa and Nigella sativa with Rifampicin, INH & Acetaminophen resulted in significant decrease in the content of hepatic MDA when compared with Rifampicin, INH &Acetaminophen

treated group. On the other hand, reduced GSH, GSH peroxidase and SOD contents were significantly increased when compared to Rifampicin, INH and Acetaminophen group. Fig. (2).

The data and results from the present study clearly indicate that *N. sativa* may play an important role as antioxidant and may efficiently act as a protective agent against drug-induced hepatic damage. These protective effects of *N. sativa* probably related to its antioxidant and leukocyte inhibiting property, which might be due to its active constituent, thymoquinone (TQ) (**Kushwaha *et al*., 2013**).

Administration of acetaminophen resulted in significant increase in the level of hepatic TNF-α when compared to control group. Tab. (1). **Knight *et al*., (2002)** suggested that covalent binding of NAPQI (the reactive metabolite of paracetamol) to macromolecules lead to formation of hepatocyte soluble products such as tumor necrosis factor-α (TNF-α). Administration of Rifampicin & INH resulted in significant increase in the level of hepatic TNF-α when compared to control group, Tab. (1). **(Nicoletti *et al*., 2014)** found that Rifampicin & INH administration resulted in augmented levels of pro-inflammatory cytokines TNF-α and IL-10 which have been previously correlated with liver injury by several drugs such as CCL4 and acetaminophen. Administration of Rifampicin & INH & Acetaminophen resulted in significant increase in the level of hepatic TNF-α when compared to control group, Tab. (1).

Administration of *Curcuma longa* with Rifampicin, INH& acetaminophen resulted in significant decrease in the level of hepatic TNF-α when compared to Rifampicin , INH & Acetaminophen treated group. Table (1).

Administration of *Nigella sativa* with Rifampicin, INH & Acetaminophen resulted in significant decrease in the level of hepatic TNF-α when compared to Rifampicin , INH & Acetaminophen treated group. Tab. (1). This result agreed with **El-Beshbishy *et al*., (2010)** who said that TNF-α is pro-inflammatory cytokine associated with liver injury.

Administration of *Curcuma longa* and *Nigella sativa* with Rifampicin, INH & acetaminophen resulted in significant decrease in the level of hepatic TNF-α when compared Rifampicin, INH & acetaminophen treated group.Tab (1). These results were confirmed by data from **Suddek G.M. (2014)** showing that pretreatment with *Nigella sativa* or *Curcuma longa* caused a significant decline in hepatic TNF-α level in tamoxifen –induced liver injury in rats. These findings indicate the anti-inflammatory effect of *Curcuma longa* and *Nigella sativa*.

Administration of acetaminophen resulted in significant increase in the level of hepatic Casp-3 when compared to control group. Tab. (1) .This agreed with **Hassanin *et al*., (2013)** who showed that acetaminophen administration markedly increased the expression of active caspase-3 in rat liver tissues.

Administration of Rifampicin & INH resulted in significant increase in the level of hepatic Casp-3 when compared to control group Tab. (1).This result agreed with (**Sankar *et al*., 2015**) who said that rats treated with INH and RIF show significant up regulation of caspase-3. Also, **Chen *et al*., (2011**) found that the number of hepatocytes with active caspase-3 was significantly increased in mice administered with INH and Rifampicin.

Administration of Rifampicin, INH & Acetaminophen resulted in significant increase in the level of hepatic Casp-3 when compared to control group. Tab. (1)

Administration of *Curcuma longa* with Rifampicin, INH &acetaminophen resulted in significant decrease in the level of hepatic Casp-3 compared to Rifampicin, INH & Acetaminophen group. Tab.(1)

These results agreed with **Mourad *et al*., (2006)** who observed that the raised levels of the caspase enzymes in the liver homogenate of the CCl4-treated rats indicated an enhanced apoptotic activity. While pretreatment with *Curcuma longa* resulted in significant decrease in Caspase-3 activity. This indicates a hepatocellular protective effect.

Administration of *Nigella sativa* with Rifampicin, INH &Acetaminophen resulted in significant decrease in the level of hepatic Casp-3 when compared to Rifampicin, INH &Acetaminophen group.Tab. (1).This indicates a hepatocellular protective effect. These results agreed with **Mourad *et al*., (2006**) who observed that the raised levels of the caspase enzymes in the liver homogenate of the CCl4-treated rats indicated an enhanced apoptotic activity. While pre-treatment with *Nigella sativa* resulted in significant decrease in Caspase-3 activity.

Administration of *Curcuma longa* and *Nigella sativa* with Rifampicin, INH &Acetaminophen resulted in significant decrease in the level of hepatic Casp-3 when compared to Rifampicin, INH &Acetaminophen group. Tab.(1) This indicates a hepatocellular protective effect.

In the present study histopathological findings in the liver of rats treated with acetaminophen showed inflammatory cells infiltration in the portal area associated with degeneration in the hepatocytes. Fig. (3 B).This result agreed with (**Hassanin *et al*., 2013)** who found that paracetamol treated group were showing dilated congested central vein (CV) degenerated hepatocytes and marked morphological changes. It exhibited disrupted lobular architecture with hemorrhage. There was marked hepatic injury, especially in the pericentral region, characterized by hepatocellular degeneration and central vein seemed to be congested.

In our study histopathological findings in the liver of rats administered Rifampicin & INH showed congestion in the portal vein with inflammatory cells infiltration and newly formed bile ducts in the portal area. Fig. (3 C). These results agreed with (**Anbarasu *et al*., 2011**) who said that Rifampicin & INH produced extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Also agreed with **Devendra *et al*., (2013)** who found that administration of Rifampicin caused mono nuclear infiltration, degenerative changes in hepatocytes, briding necrosis extended from one portal tract to another along with microvesicular steatosis.

Rifampicin is a powerful inducer of CYP2E1 and activation of CYP2E1 that lead to oxidative stress **(Thattakudian *et al*., 2011).** Moreover hydrazine depletes the reserve sources of GSH in hepatocytes, which results in altered mitochondrial permeability and induces apoptosis. Oxidative stress in the hepatocytes results in apoptosis one of the attributing mechanisms of liver dysfunction caused by INH and Rifampicin (**Chowdhury *et al*., 2006**). This also agreed with **Anbarasu *et al*., (2011)** who suggested that INH is converted to mono acetyl hydrazine (toxic metabolite) via cyp450 leading to hepatotoxicity.

The higher incidence of liver necrosis by Rifampicin &INH combination can be explained from drug interaction point of view as rifampicin induces cyp450 enzyme leading increased production of acetyl hydrazine (toxic metabolite), The plasma half-life of acetyl hydrazine (toxic metabolite of INH) is shortened by Rifampicin and acetyl hydrazine is quickly converted to its active metabolite by increasing its oxidative elimination rate **(Hussain *et al*., 2013)** .

In addition, rats administered Rifampicin, INH & acetaminophen showed congestion in the portal vein with inflammatory cells infiltration and newly formed bile ducts in the portal area. Fig. (3D).

While rats administered *Curcuma longa* with Rifampicin, INH &Acetaminophen showed diffuse kupffer cells proliferation in between the hepatocytes. Fig.(3E). These results agreed with (**Mourad *et al*., 2006**) who said that daily feeding on *Curcuma longa* in the diet showed an evident protective effect against the hepatic injury induced by CCL4 compared to control and CCL4 groups. Almost complete recovery of the normal hepatocyte appearance was observed both in the central as well as in the peripheral zones of the hepatic lobules.

Moreover, histopathological findings in the liver of rats administered *Nigella sativa* with Rifampicin, INH &Acetaminophen showed congestion in the central vein associated with diffuse kupffer cells proliferation in between the hepatocytes. Fig. (3 F). Our results agreed with **Devendra *et al*., (2013)** who observed that pretreatment with *Nigella sativa* in Rifampicin treated groups showed near normal regaining of architecture of liver with persistiting fatty degeneration in the portal tract .Also *Nigella sativa* inhibited leucocyte infiltration, which may lead to its hepatoprotective activity (partial protection of hepatocellular damage).

Rats administered Curcuma longa and Nigella sativa with Rifampicin, INH & acetaminophen in the present study showed fatty degenerative changes in few hepatocytes. Fig. (3G).

Nigella sativa has been for long time determined among the hepatoprotective herbs. Its active constituent thymoquinone, has been well documented as a potent antioxidant, particularly against the CCl4-induced free radical species 40 (**Al-Ghamdi**., **2003**). Chemical results proved that there was a significant reduction in the serum and liver homogenate indices of the oxidative stress measured in the CCl4 intoxicated rats receiving *Nigella sativa* seeds. This group revealed also recovery of the liver glutathione. In fact, *Nigella sativa* has been proved to preserve the natural antioxidants in the cells by scavenging the superoxide anions. (**Shen *et al*.**, **2005**).

Despite the antioxidative hepatic protection by *Nigella sativa*, yet generalized vacuolation of hepatocytes was a consistent feature to *Nigella sativa* ingestion whether in the intoxicated or in the positive control rats.

On one hand, the finding could be attributed to failure of *Nigella sativa* to achieve complete restoration of the liver. Thymoquinone was proved to activate the caspase pathway of apoptosis, which coincided with the obtained biochemical findings, specifically in cancer cells. The underlying mechanism was related to the antioxidant action of thymoquinone on the mitochondria causing the release of cytochrome C in the cytoplasm (**Mourad *et al*., 2006). Devendra *et al*., (2013)** suggested that Rifampicin-treated rats associated with liver cell damage due to increased lipid peroxidation. *Nigella sativa* combined with Rifampicin prevented significant lipid peroxidation either directly or through non-protein thiols (GSH) by scavenging free radicals.

According to **Mourad *et al*., (2006)** the results in the present study were generally in favor of *Curcuma longa*. These results were progressively promoting *Curcuma longa* over *Nigella sativa* as a reliable, safe heptoprotective herbal supplement. *Curcuma longa* as a powerful antioxidant possessing a LD50 (half-the lethal dose) with a wide range of safety. In comparison to *Nigella sativa*, *Curcuma longa* proved to exert a more pronounced antifibrotic action against the CCL4-induced liver fibrogenesis. *Curcuma longa* acted on multi-directional axes to reduce the deposition of excessive collagen and to accelerate its degradation. In one way, *Curcuma longa* has been proved to clear the excess of hepatic stellate cells from the stroma through apoptosis thus accelerating the reversal of fibrosis, even in liver cirrhosis.

The vast majority of researches concerned with the evaluation of the antioxidant property of *Curcuma longa* in comparison with other natural herb said that *Curcuma longa* is promoted over *Nigella sativa* as a safe hepatoprotective herbal dietary supplement that carries powerful antioxidative, antifibrogenic and proliferative potentials essential for the recovery of the liver from a particular damage (**Kempaiah and Srinivasan ,2004).**

V. Conclusions

From all the above mentioned results, it could be concluded that:

Rifampicin and INH induce hepatotoxicity confirmed by changes in liver function and the oxidative stress parameters. Using *Curcuma longa* and *Nigella sativa* modulate the hepatotoxic effect of anti-tuberculosis drugs and Acetaminophen.

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