PROTECTIVE EFFECT OF GARLIC OIL ALONE OR COMBINED WITH LOW-DOSE GAMMA IRRADIATION ON PARACETAMOL-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT: *We investigated the effect of garlic oil (GO), alone or combined with low dose total body gamma (γ)-irradiation (LDR) against paracetamol (APAP)-induced hepatotoxicity in rats. GO (100 mg/kg/day) was administered orally for 14 days before hepatotoxicity induction. Animals were irradiated 2 hours before hepatotoxicity induction by peroral APAP (1g/kg) administration, then animals were sacrificed 24 hours later. GO, alone or combined with LDR, ameliorated APAP-induced adverse effects as revealed by the reduction of serum liver marker enzymes activities and the histological examination. Hepatic microsomal cytochrome P2E1 activity was also reduced. Changes in hepatic redox balance were significantly attenuated by both treatments. Hepatic hydrogen peroxide was only reduced by LDR. APAP-induced mitochondrial dysfunction and apoptotic effect were also reversed. GO and/or LDR pretreatment, effectively protected against APAP-induced hepatotoxicity. Thus, the hepatoprotective effect of GO alone or combined with LDR could be of value in the protective management of APAP-induced hepatic damage.*

KEYWORDS: Low Dose (γ)-Radiation, Garlic, Hepatotoxicity, Oxidative Stress, Mitochondrial Dysfunction, Apoptosis.

INTRODUCTION

In order to achieve the goals of the present study, the following biochemical parameters will be investigated; hepatic mitochondrial activity of respiratory complex-I (NADH:ubiquinone oxido-reductase), mitochondrial contents of adenosine triphosphate (ATP) and B cell lymphoma-2 (Bcl-2) protein, liver contents of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and reduced glutathione (GSH), in addition to the activities of each of hepatic microsomal cytochrome P2E1 (CYP2E1) and serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH). Histological examination of the liver tissue will be carried out using light microscope to evaluate the degree of tissue damage/repair in different experimental groups of rats.

LITERATURE

Liver is functionally interposed between the site of absorption and the systemic circulation of xenobiotics and is a major site of their metabolism and elimination. These features render it a preferred target for drug toxicity **[Russmann et al., 2009]**. Most drugs are not intrinsically toxic to the liver but can cause injury secondary to the production of a hepatotoxic metabolite,

a process known as bioactivation **[Dahm and Jones, 1996].** CYP2E1, is one of the members of cytochromes P450 (CYPs) family; which are phase I enzymes, that metabolizes numerous small molecules of toxicological interest including ethanol, acetaminophen (APAP), halothane and carbon tetrachloride **[Gonzalez, 2005]**.

The widely used analgesic and antipyretic drug APAP undergoes the major elimination pathways through conjugation with glucuronide and sulfate **[Nelson, 1982]** at normal doses. The initial step in its toxicity its bioactivation by cytochrome P450 (CYP450 2E1) to an electrophilic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is efficiently detoxified by glutathione (GSH) and eliminated in the urine or bile as APAP–cysteine, APAP–N-acetylcysteine (APAP–NAC), and APAP–GSH **[Lauterburg and Velez, 1988]**. After an overdose of APAP, the glucuronidation and sulfation routes become saturated and more extensive bioactivation of APAP occurs, leading to rapid depletion of the hepatic GSH pool **[Thummel et al., 2000]**. Subsequently, NAPQI binds to cellular proteins, including a number of mitochondrial proteins **[James et al., 2003]**, which in turn causes oxidant stress. This mitochondrial oxidant stress is followed by redox-sensitive activation of c-jun-N-terminal kinases (JNK) **[Gunawan et al., 2006],** which has been found to trigger the mitochondrial membrane permeability transition (MPT) pore opening, ultimately causing the rupture of the outer mitochondrial membrane and release of cytochrome c and other proapoptotic factors into the cytosol with a consequent dramatic decrease in cellular ATP level. This pathway has been identified as a pivotal mechanism mediating APAP-induced cell death **[Reid et al., 2005].** In addition to its critical role in APAP-induced liver damage, the stress kinase JNK also activates members of the anti-apoptotic Bcl-2 protein family **[Latchoumycandane et al. 2007]**.

Garlic (*Allium sativum L.*) has been hypothesized to be beneficial to human health, including cardioprotective, chemotherapeutic, antidiabetic, antioxidant and hepatoprotective properties **[Siddique and Afzal, 2004; Ohaeri and Adoga, 2006; Mirunalini et al., 2010].** Garlic oil (GO) has been demonstrated to contain more than 30 organosulfur compounds including diallyl trisulfide (DATS), diallyl disulfide (DADS) and diallyl sulfide (DAS) **[Amagase et al., 2001].** Sulfur containing compounds in garlic have been used as an anticancer **[Na et al., 2012]**, a lipid lowering agent **[Asgari et al., 2012]**, anti-ulcerogenic agent **[Tope et al., 2014],** and has been demonstrated to provide protective effects against chemically-induced oxidative stress **[Agarwal et al., 2007]**.

Cellular stimulatory effects are observed following low-dose gamma irradiation at dose level range of 0.01-0.5 Gy. These effects include adaptive responses **[Wang et al., 2004]**, activation of immune functions **[Ina et al., 2005],** enhancement of resistance to high-dose radiation **[Yonezawa et al., 1996],** regulation of mitochondrial dysfunction caused by a higher dosage of radiation **[Lu et al., 2011]** and induction of endogenous antioxidant defense in the liver **[Avti et al., 2005],** the latter effect could be beneficial in protecting the liver cells from oxidative stress.

The aim of the current study was to investigate the possible protective effect of low-dose total body (γ)-irradiation (LDR) alone or combined with GO against APAP-induced hepatotoxicity. Secondly, investigation of the possible mechanisms through which GO and LDR exerts their hepatoprotective effects against APAP-induced hepatotoxicity.

METHODOLOGY

Animals

Adult male Wistar rats weighing 150-200 g were obtained from the National Research Centre, Dokki, Cairo, Egypt and were fed a standard pellet diet. Rats were acclimatized in the animal facility of the National Centre for Radiation Research and Technology (NCRRT)-Atomic Energy Authority, Cairo, Egypt, at a temperature of 25 ± 5ºC, humidity of $60 \pm 5\%$ and 12/12-hour light-dark cycle. The study was conducted in accordance with the guidelines set by the EEC regulations (Revised Directive 86/609/EEC) and approved by the Ethics Committee at the Faculty of Pharmacy, Cairo University.

Chemicals

Paracetamol (acetaminophen, APAP) and N-acetyl cysteine (NAC) were purchased from Sigma-Aldrich Chemical Company (Saint Louis, Missouri, USA).Garlic oil (GO) was purchased from El-Captain Company (Cairo, Egypt). Garlic oil quality parameters described in the accompanying technical report are: yellow to red-orange color, specific gravity of 1.05-1.095 g/cm^3 at 25˚C, refraction index of 1.55-1.58 at 20˚C, $\leq$ 3ppm of arsenic, $\leq$ 1 ppm of cadmium, $\leq$ 1ppm of mercury and $\leq$ 1 ppm of lead. HPLC analysis of garlic oil were performed on a 1100 HPLC instrument (Agilent Technologies, California, USA) equipped with a binary pump, a UV detector, a manual sampler, and a column thermostat. Chromatographic separations were carried out on a C-l8 column (5.0 mm, 250 mm, 4.6 mm, Japan). The HPLC mobile phase was prepared by combining equal volumes of HPLC grade methanol and distilled water; the mobile phase was degassed in ultrasonic bath (Shimadzu) for 30 minutes. The HPLC conditions were as follows: column temperature of 280˚C; wavelength of 240 nm; flow rate of 0.8 ml min^{-1}; injection volume of 20 μl; run time of 30 minutes; chart speed of 2 mm min^{-1}.

HPLC chromatographic technique showed that garlic oil contains diallyl sulfide (46.6%), diallyl disulfide (20.3%), diallyl trisulfide (11.5%), diallyl tetrasulfide (4.3%), allyl methyl sulfide (8.9%), allyl propyl disulphide (6%), alliin (1.1%), allicin (0.78%) and selenium (0.4%).

Colorimetric kits for the determination of AST, ALT and ALP activities as well as H_2O_2 concentration were purchased from Biodiagnostics (Giza, Egypt). ELISA kits for ATP and Bcl-2 were purchased from WUHAN EIAab® Science Co, LTD (Wuhan, China) and Biovendor-Laboratorní medicína a.s. (Modrice, CZECH REPUBLIC), respectively. All other chemical reagents were purchased from Sigma-Aldrich Chemical Company (Saint Louis, Missouri, USA).

Experimental Design

In the current study, eight groups of rats, each consisting of eight animals, were used:

Vehicle-treated group, received only the vehicle (isotonic saline solution containing gum of acacia (10 mg/ml).

APAP-treated group received paracetamol (1 g/kg body weight), suspended in isotonic saline solution containing gum of acacia (10 mg/ml).

GO-treated group, treated only with garlic oil in a dose of 100 mg/Kg/day orally for 14 days **[Zhang et al., 2010]**. Hepatotoxicity was induced 30 minutes after the last dose.

Reference drug treated group, received NAC intra-peritoneally in a single dose of 0.39 g/Kg **[Acharya and Lau-Cam, 2010]**, 30 minutes before APAP administration.

Irradiated group, was irradiated at γ-irradiation dose of 0.5 Gy.

Irradiated-paracetamol treated group, received paracetamol dose two hours following irradiation **[Kojima et al., 2000]**.

The last two groups of animals were irradiated-treated groups; where rats were exposed to 0.5 Gy γ-irradiation dose , 90 minutes prior to the last treatment dose of either GO or NAC. Paracetamol was administered 30 min after the administration of the last treatment dose.

The animals were sacrificed by decapitation 24 hours after paracetamol treatment. Serum and liver tissue were used for biochemical analysis.

METHODS

Induction of Hepatotoxicity

Experimental hepatotoxicity was induced in animals by a single oral administration of paracetamol at a dose of 1 g/kg body weight suspended in an isotonic saline solution containing gum of acacia (10 mg/ml) **[Banerjee et al., 1998]**.

Irradiation of animals

Total body irradiation of rats was carried out at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt; using Gamma cell-40 biological irradiator furnished with a Caesium^{-137} irradiation unit manufactured by the Atomic Energy of Canada Limited (Sheridan Science and Technology Park, Mississauga, Ontario, Canada). Different groups of animals were irradiated at a single radiation dose level of 0.5 Gy **[Avti et al., 2005]**, at a rate of 0.012 Gy/sec.

Tissue Sampling

The liver tissue was removed on ice, weighed and homogenized in ice cold saline and then the homogenate was divided as follows; an aliquot of the tissue homogenate was added to 9 volumes of the collecting buffer (pH 7.8): 10 mM Tris-HCl, 1 mM ethylene glycol tetracetic acid (EGTA) and 0.32 M sucrose using Glas-Col® homogenizer (Terre Haute, Indiana, USA). Two aliquots of homogenate were mixed with ice cold 6% w/v metaphosphoric acid (1:2 ratio) and 2.3% w/v KCl (1:1 ratio), centrifuged at 1,000xg for 15 min at 4ºC, and the resulting supernatant was used for the assay of liver contents of malondialdehyde (MDA) **[Uchiyama and Mihara, 1978]** and GSH **[Loven *et al*., 1986]**, respectively.

Another aliquot of liver homogenate was used for separation of liver mitochondrial fraction according to the method described by **Turpeenoja *et al.* (1988)** which was used for the determination of complex I (NADH:ubiquinone oxido-reductase) activity and the levels of ATP and Bcl-2.

The last aliquot of liver homogenate was used for the separation of microsomal fraction according to the method described by **Dorababu et al. (2006)** which was used for the determination of CYP2E1 enzyme activity.

- **Assessement of serum enzyme activities:** the activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined according to the manufacturers' instructions of each of the kits used. Serum malate dehydrogenase (MDH) was determined according to the method described by **Cox et al. (2005)**.

- **Assessment of hepatic microsomal cytochrome P2E1 (CYP2E1) activity:** the microsomal CYP2E1activity was determined according to the method of **Allis and Robinson (1994).**

- **Assessement of hepatic mitochondrial complex-I activity, adenosine triphosphate and B cell lymphoma-2 contents:** determination of mitochondrial complex I activity (NADH: ubiquinone oxidoreductase) was carried out based on the method of **Whitfield et al. (1981)**. Liver mitochondrial contents of adenosine triphosphate (ATP) and B cell lymphoma-2 (Bcl-2) were determined using ELISA techniques according to the manufacturers' instructions of each of the kits used.

- **Assessment of hepatic malondialdehyde, reduced glutathione and hydrogen peroxide contents:** liver malondialdehyde (MDA) content was determined according to the method of **Uchiyama and Mihara (1978),** liver content of reduced glutathione (GSH) was determined according to the method of **Beutler et al., (1963)**, and the concentration of hydrogen peroxide (H_2O_2) in the liver was determined according to the method of **Fossati et al (1980) and Aebi (1984)**.

- **Examination of liver tissue by light microscope:** after animal sacrifice, liver sections were immediately fixed in 10% formalin, embedded in paraffin, then 5μm sections were sliced, and stained with hematoxylin and eosin (H&E) for evaluation using light microscopy.

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Values are given as means ± standard error (S.E.) The level of statistical significance was taken at $p<0.05$.

RESULTS

Effect of garlic oil against hepatic damage

APAP (1g/Kg) administration induced a marked and severe hepatic injury in rats as observed by the significant elevation of the serum activities of the enzymes; AST, ALT, ALP, LDH and MDH. Treatment of animals with GO prior to APAP administration resulted in a significant reduction in the serum AST, ALT and ALP activities by 33%, 62% and 49% respectively, while NAC treatment led to a drop in these enzymes to 59%, 52% and 76%, respectively. Similarly, the increase in MDH enzyme activity induced by APAP was also markedly suppressed in the serum of rats treated with either GO or NAC. A sharp drop in elevated serum LDH activity by about 48% was also observed in GO-treated animals when compared to APAP-treated group **(Figures 1 and 2a)**.

Figure **(2b)** showed the effect of APAP on hepatic microsomal CYP2E1 activity. Pretreatment of rats with GO was able to reduce the marked rise in CYP2E1 activity by nearly 66% as compared to APAP-treated animals. NAC, similarly, was effective in reducing microsomal CYP2E1 activity by about 64%.

APAP treatment was also associated by disruption of the redox balance in the liver which was reflected by the marked reduction in hepatic content of GSH, as well as the associated increase in hepatic content of MDA. The results of the current study demonstrated that GO pretreatment led nearly to two-fold increase in liver GSH content while pretreatment with NAC resulted in a dramatic increase in GSH content reaching 272%. Both GO and NAC were effective in reducing the rise in hepatic contents of MDA, such that it reached 54% and 69% for MDA content respectively, while hepatic H_2O_2 was only reduced after NAC pretreatment by about 48%, compared to APAP-treated animals **(Figures 2c, 2d and 3a)**.

Treating the animals with either GO or NAC attenuated the depletion in mitochondrial ATP content and complex-I activity evoked by APAP administration **(Figures 3b and 3c)**. The marked decrease in hepatic mitochondrial Bcl-2 content observed in APAP-treated group was also markedly reversed by pretreatment with either GO or NAC **(Figure 3d)**.

Effect of low dose radiation alone or combined with garlic oil against hepatic damage

Exposure of animals to a single dose of 0.5 Gy prior to APAP administration led to a significant suppression of the marked increase in serum AST, ALT, ALP and MDH and LDH activities by 38%, 19%, 35%, 20% and 25% respectively. Treating the animals with garlic oil in combination with LDR was able to reduce the activities of the serum enzymes ALT and ALP significantly such that it reached 41% and 48%, respectively as compared to irradiated APAP-treated animals. GO, combined with LDR, normalized serum AST activity. The combination of NAC treatment with LDR also showed a significant drop in serum ALT and ALP activities by 50% and 79% respectively. Treatment with NAC, combined with LDR, led to a marked reduction in serum AST activity below normal value. Serum MDH activity, on the other hand, was normalized after treating the animals with LDR combined with either GO or NAC . Furthermore, the combination of LDR, with either GO or NAC was effective in reducing the increase in LDH activity, induced by APAP, by 65% and 56%, respectively when compared to irradiated APAP-treated group **(Figures 1 and 2a)**. In addition, hepatic microsomal CYP2E1 activity was reduced after LDR exposure by nearly 21% compared to APAP-treated rats. On the other hand, GO pretreatment, accompanied by LDR resulted in a dramatic reduction in microsomal CYP2E1 activity reaching 57% while the combination of LDR with NAC led nearly to 44% decrease compared to irradiated APAP-treated animals **(Figure 2b)**.

Prior exposure of animals to LDR showed a significant rise in hepatic content of GSH to 185% and a marked decrease in that of MDA and H_2O_2 to about 65% and 69%, respectively when compared to animals treated only with APAP. The changes in the levels of oxidative stress markers induced by APAP were significantly attenuated by LDR exposure combined with either GO or NAC administration, leading to a significant rise in hepatic content of GSH, and a marked drop in that of MDA and H_2O_2 **(Figure 2c, 2d and 3a)**.

Data shown in the present work demonstrated that LDR exposure led to an effective increase in hepatic mitochondrial complex-I activity and ATP content to 357% and 162%, respectively compared to APAP-treated animals **(Figure 3b and 3c)**. Hepatic mitochondrial content of Bcl-2 was also markedly raised to about 239% by pre-exposure of APAP-treated rats to LDR. The

hepatic mitochondrial complex-I activity as well as ATP content were nearly normalized after treating the animals with either GO or NAC, in combination with LDR. Moreover, the exposure of rats to LDR, associated with either GO or NAC pretreatment, was able to raise the liver mitochondrial content of Bcl-2 to 185% and 178% respectively, as compared to irradiated APAP-treated rats **(Figure 3d)**.
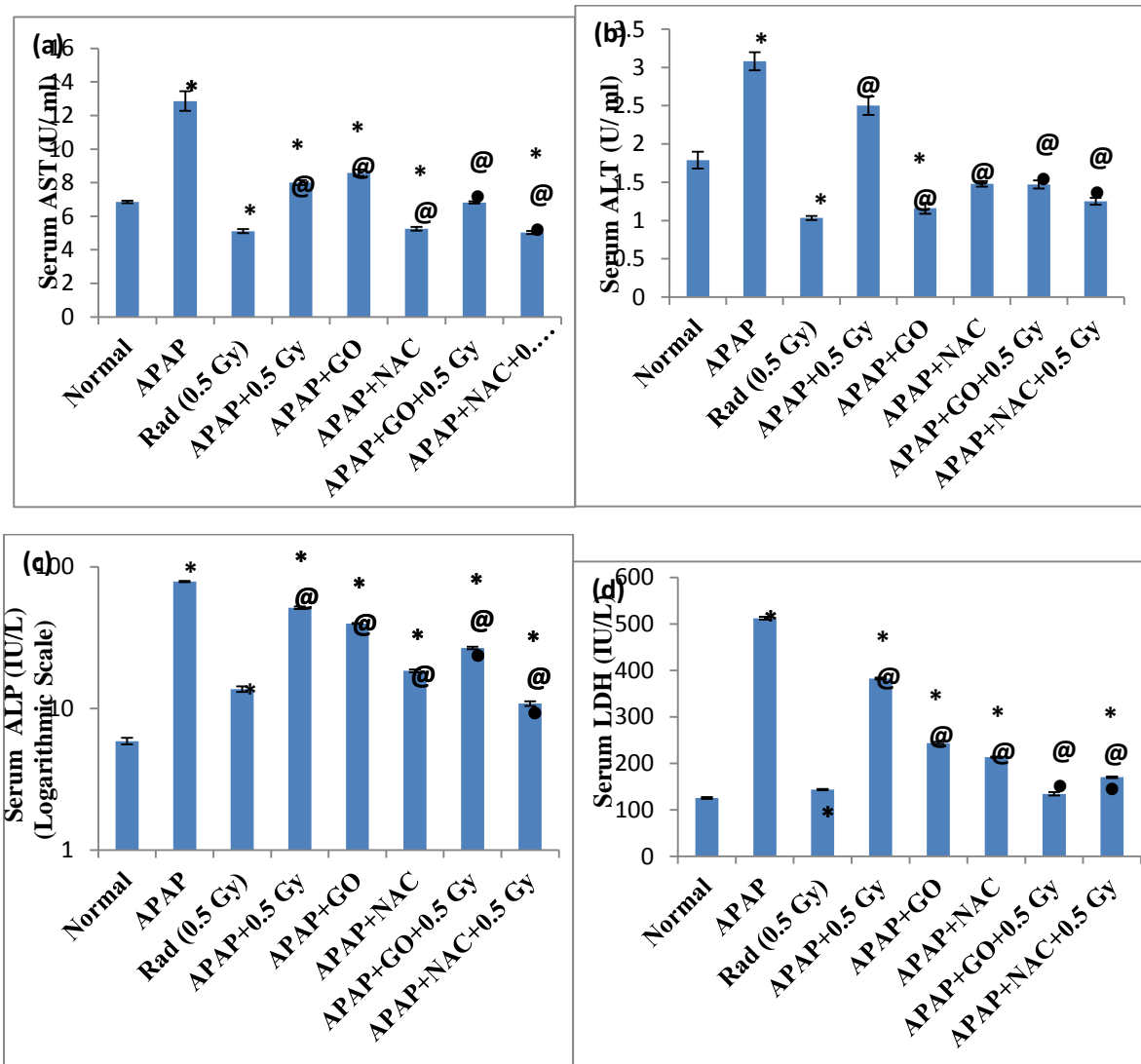

Figure (1). Effect of low dose gamma-irradiation (0.5 Gy) alone or combined with oral garlic oil (GO, 100 mg/kg/day) treatment for 14 days or N-acetyl cysteine (NAC, 390 mg/kg) single intraperitoneal dose treatment on changes of serum aspartate transaminase (AST) (a), alanine transaminase (ALT) (b), alkaline phosphatase (ALP) (c) and lactate dehydrogenase (LDH) (d) activities of animals subjected to oral paracetamol (APAP) injection (1000 mg/kg) 2 hours after radiation exposure (Values are given as means ± Standard Error (SE), n=8).

Asterisks designate significant difference from normal (vehicle-treated) group (*), APAP-treated group ($^@$) and respective irradiated APAP-treated group ($^\bullet$) at p<0.05.
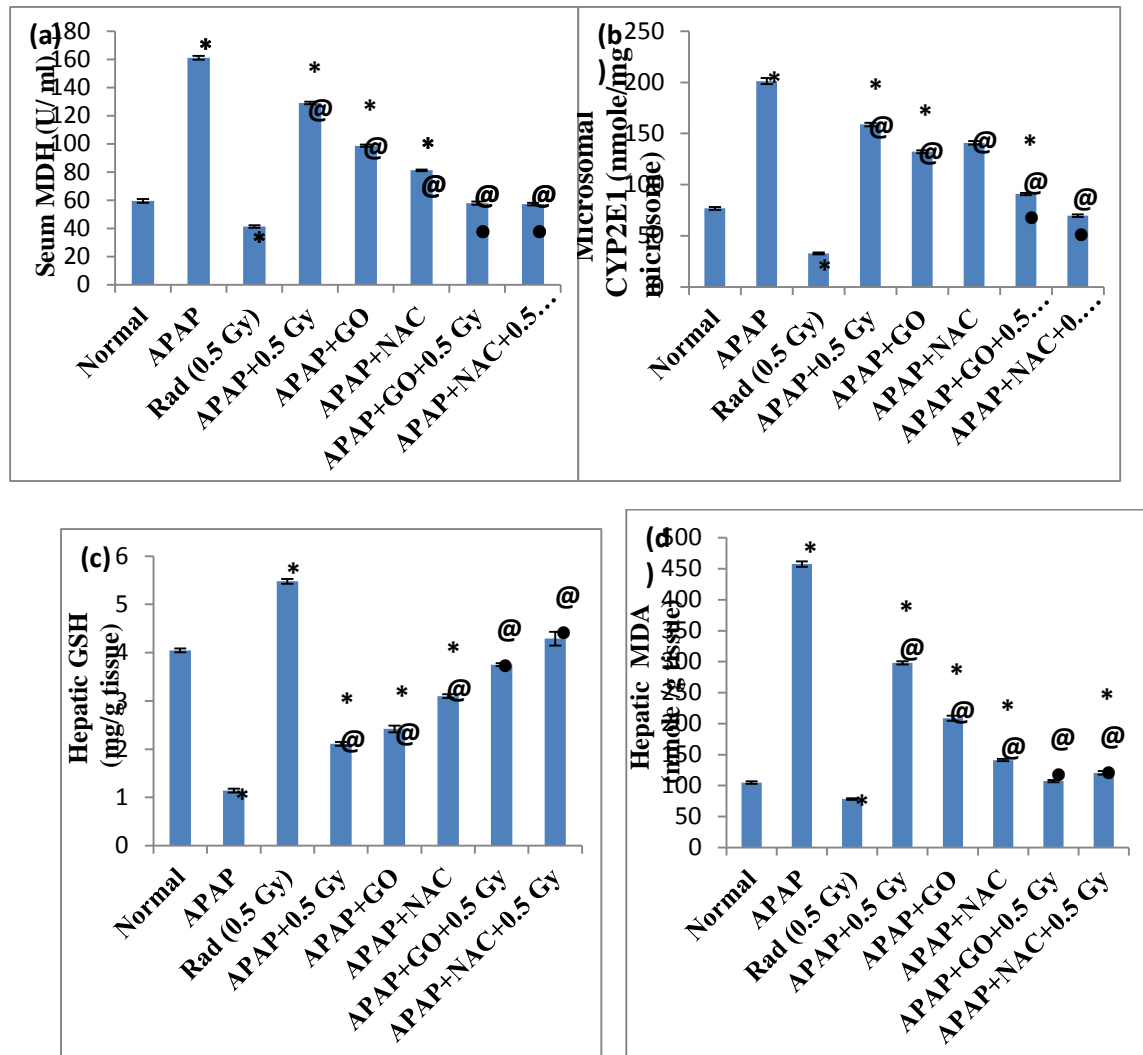
Figure (2). Effect of low dose gamma-irradiation (0.5 Gy) alone or combined with oral garlic oil (GO, 100 mg/kg/day) treatment for 14 days or N-acetyl cysteine (NAC, 390 mg/kg) single intraperitoneal dose treatment on changes of serum malate dehydrogenase (MDH) (a), hepatic microsomal cytochrome P2E1 (CYP2E1) (b) activities as well as the hepatic glutathione (GSH) (c) and malondialdehyde (MDA) (d) of animals subjected to oral paracetamol (APAP) injection (1000 mg/kg) 2 hours after radiation exposure (Values are given as means ± Standard Error (SE), n=8).

Asterisks designate significant difference from normal (vehicle-treated) group (*), APAP-treated group (@) and respective irradiated APAP-treated group (•) at p<0.05.
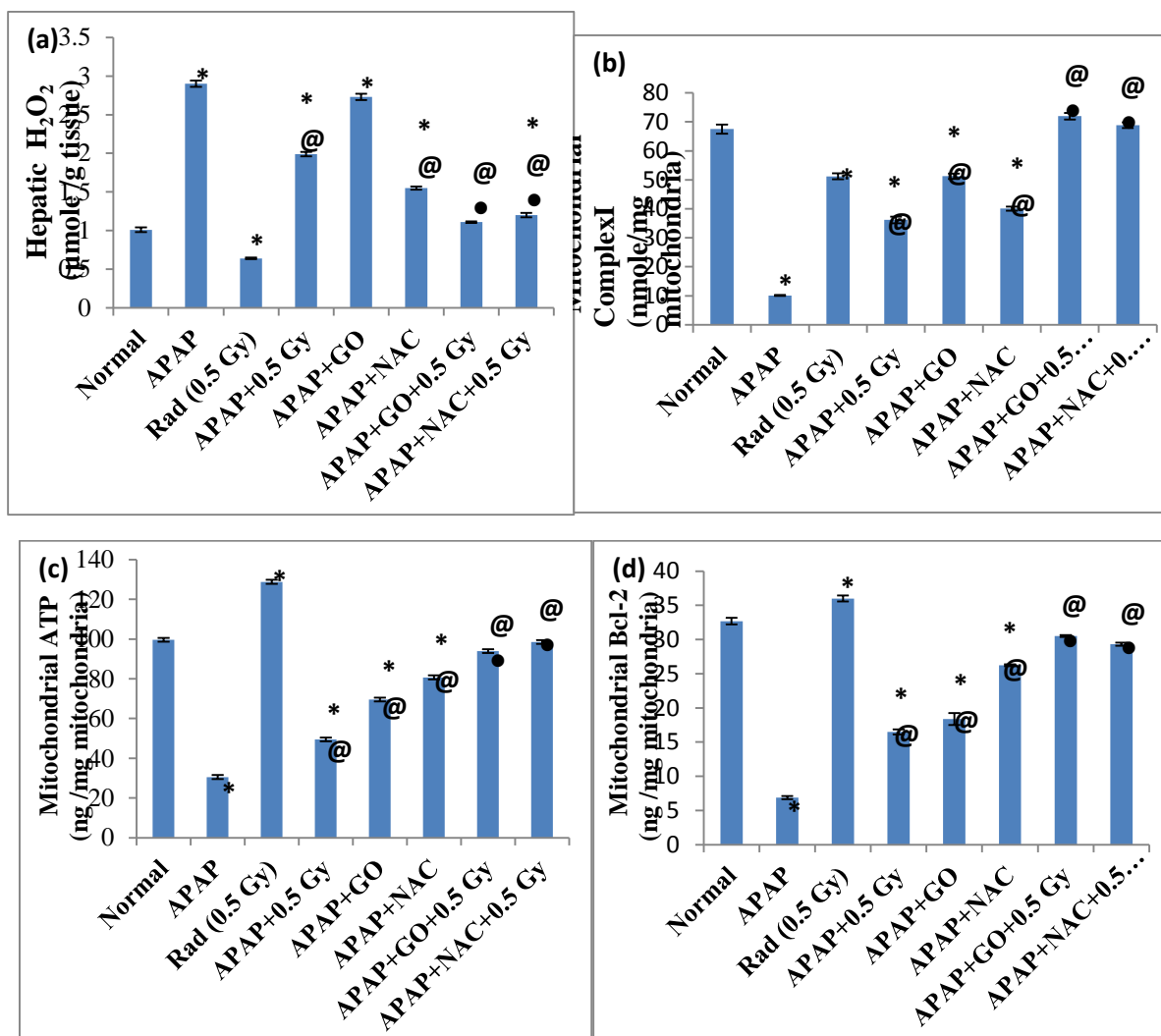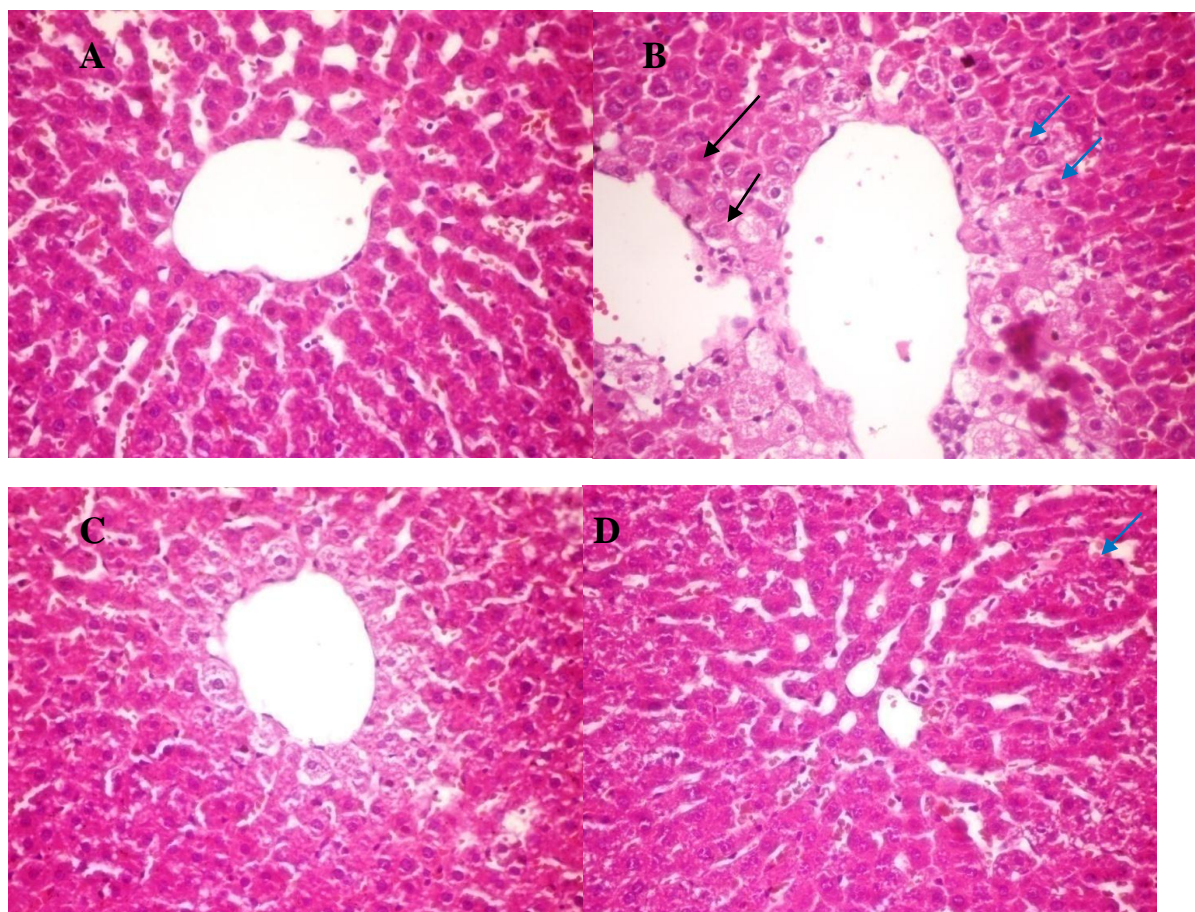
Figure (4). Light microscopy of liver sections of: normal rat (A), paracetamol (APAP)-treated rat (B), rat exposed to low dose γ-irradiation (C), rat treated with low dose γ-irradiation prior to APAP administration (D) (Haemotoxylin and eosin, original magnification X400).

A: shows normal histological structure of hepatic lobule (rat was treated with saline solution containing gum acacia (10 mg/ml)), **B:** shows focal area of vacuolated hepatocytes with pyknosis of their nuclei, inflammatory cells infiltration and apoptosis of hepatocytes (rat was treated orally with APAP (1 g/kg)) **C:** shows normal histological structure of hepatic lobule (rat was exposed to 0.5 Gy γ-radiation) and **D:** shows slight dilatation of hepatic sinusoids and few apoptotic bodies (rat was exposed to 0.5 Gy γ-radiation 2 hours prior to the induction of hepatotoxicity by oral administration of APAP (1 g/kg)). Apoptotic bodies are shown by blue arrows
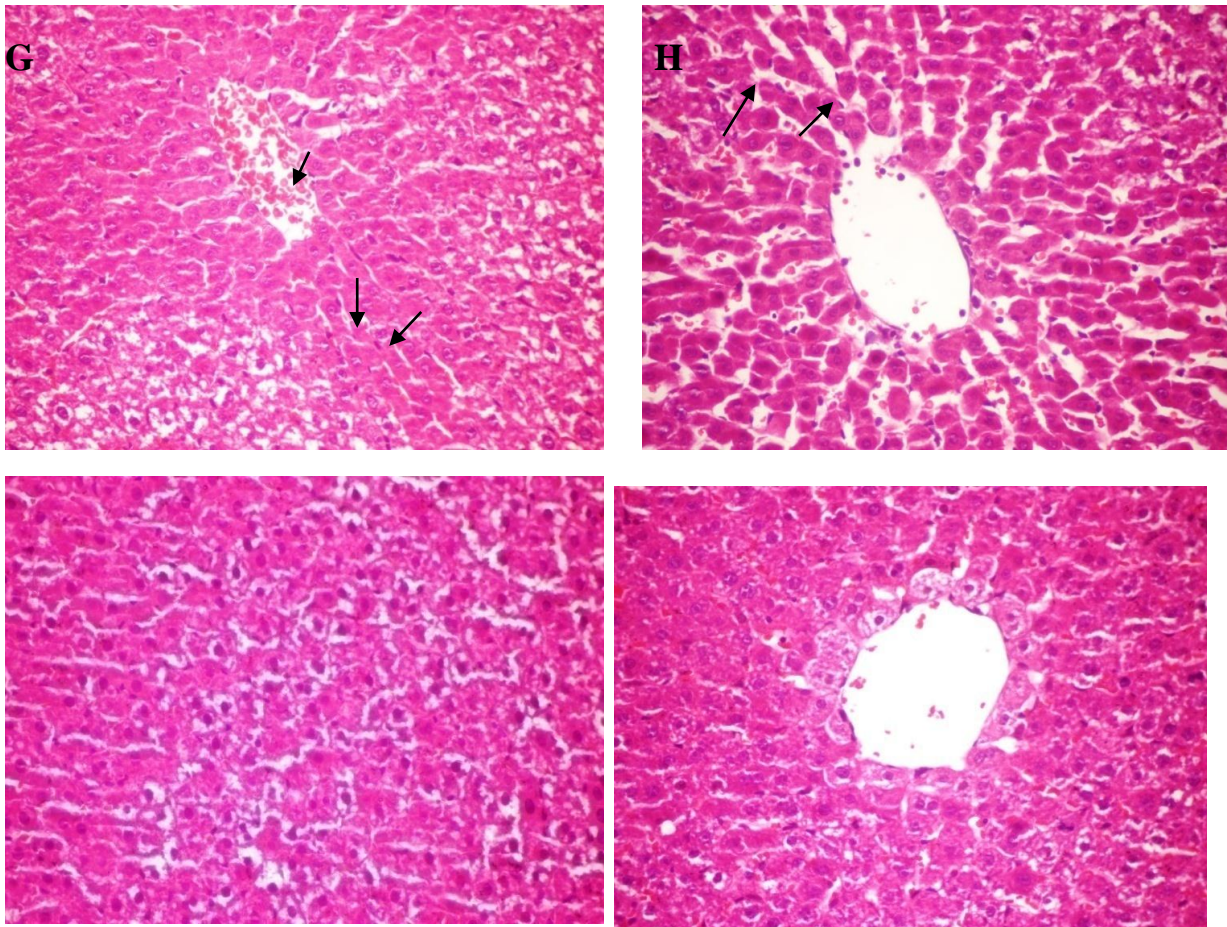
Figure (5). Light microscopy of liver sections of: rat treated with garlic oil (GO) prior to paracetamol (APAP) administration (E), rat treated with N-acetyl cysteine (NAC) prior to APAP administration (F), Irradiated GO-treated rat (G), and Irradiated NAC-treated rat (H) (Haemotoxylin and eosin, X400).

E: shows hydropic degeneration of focal hepatocytes, **F:** shows perivascular inflammatory cells infiltration and hydropic degeneration of hepatocytes, **G:** shows hydropic degeneration of focal hepatocytes and few scattered apoptotic bodies, **H:** shows no histological changes.

DISCUSSION

The current study showed that paracetamol hepatotoxicity induced a marked rise in liver marker enzymes, a finding that is in harmony with previous studies that reported a significant rise in the activities of the serum enzymes; ALP, AST, ALT, and LDH after APAP overdose administration to mice **[Lahon and Das, 2011; Lee et al., 2012]**. Furthermore, serum MDH and AST were reported to reach a peak at 24 hours following paracetamol administration to rats **[Zieve *et al.,* 1985]**. The increase in the activity of transaminases and ALP is an indication of cellular leakage and loss of functional integrity of the hepatic cell membranes **[Poole and Leslie, 1989].**

A disruption of the redox balance in the liver was observed in the present work after APAP administration. This finding is comparable to that reported in the study of **Basu et al. (2012)** where the levels of lipid peroxidation products were increased and the level of GSH was significantly decreased in liver of rats intoxicated with a single dose of paracetmol. The increase in hepatic MDA content induced by paracetamol suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism **[Kim et al., 2010].** The depletion of hepatic GSH could be explained by the ability of high doses of APAP to induce covalent bonding of its toxic metabolite N-acetyl p-benzoquinoneimine (NAPQI), to sulfydryl groups of proteins. This causes exhaustion of reduced glutathione in the liver, resulting in lipid peroxidation and cell necrosis **[Burke et al., 2006]**.

Ghosh et al. (2010) also showed a marked rise in hepatic H_2O_2 levels, due to APAP intoxication, that correlated with a low hepatic GSH level. It has been suggested that APAP treatment increased reactive oxygen species production in hepatocytes as well as NO production in liver [**Ghosh et al., 2010].** Oxidative stress-derived reactive oxygen species generation has been suggested to be important in initiating free radical reactions (lipid peroxidation, protein oxidation, etc.) that damage hepatocytes and promote liver injury during APAP-induced hepatotoxicity **[Jaeschke et al., 2003].**

In the current study, the rise in microsomal CYP2E1 activity evoked by APAP is in accordance with the results of **Kim et al (2007)** where expression of CYP2E1 proteins in rat liver was elevated significantly following paracetamol administration. The results of **Knockaert et al (2011)** indicated that when acetaminophen was used as CYP2E1 substrate, the localization of CYP2E1within mitochondria was sufficient to induce reactive oxygen species overproduction, depletion of glutathione, increased mitochondrial dysfunction and cytotoxicity.

The depletion in hepatic mitochondrial ATP evoked by APAP administration herein is compatible with the results reported by **Masubuchi et al (2005)** where APAP caused depletion of mitochondrial ATP content in liver of male CD-1 mice. APAP overdose was reported to trigger mitochondrial dysfunction as indicated by inhibition of mitochondrial respiration **[Burcham and Harman, 1991]**. Since the mitochondrial effects of APAP in vivo can be directly reproduced by NAPQI in isolated mitochondria **[Ramsay et al., 1989; Burcham and Harman, 1991]**, covalent binding to mitochondrial proteins may be to a significant degree responsible for the initial mitochondrial dysfunction.

Results of the current work revealed a significant impairment of hepatic mitochondrial complex-I activity after APAP administration. This finding is in harmony with that reported by **Burcham and Harman (1991)**; where the toxic metabolite of acetaminophen, NAPQI, was found to inhibit NADH dehydrogenase (respiratory complex I). It has recently been shown that the compromised activities of mitochondrial respiratory complexes I and IV are associated with increased ROS production under oxidative stress conditions **[Galati et al., 2009]**, thus, the respiratory complex-I could have been impaired indirectly by the increased oxidative stress induced by APAP administration. Moreover, increased glutathionylation of proteins, including respiratory complexes, particularly complex-I, under oxidative stress conditions have been reported to inhibit their normal electron flow from complex-I donor, NADH, resulting in site specific increase in the production of ROS **[Taylor et al., 2003; Hoffman and Brookes, 2009]**.

Apoptosis is controlled in part by the Bcl-2 family of regulatory proteins (Bcl-2, Bcl-x, Bax, and others). Bcl-2 can prevent or delay apoptosis **[Reed, 1997].** The decrease in hepatic Bcl-2

content observed in the current work in APAP-treated group is comparable with the results reported by **Sharma et al (2012)**; where APAP exposure in rats increased the Bax level and decreased the Bcl-2 in mitochondria of liver, leading to cytochrome-c release, activation of procaspase-3 and DNA fragmentation. The reduction in Bcl-2 content observed in the present study in APAP-treated group, might be at least in part, attributed to the reactive oxygen species generated by GSH-depleted mitochondria, that can activate c-Jun *N*-terminal kinase (JNK) **[Hanawa et al., 2008]**; a member of the mitogen-activated protein kinase (MAPK) superfamily. JNK in turn activates members of the Bcl-2 family **[Latchoumycandane et al. 2007]** and promotes bax translocation to mitochondria by directly phosphorylating bax and thus consequently promoting apoptosis **[Kim et al., 2006]**. Apoptotic cell death was also confirmed in the present work by the microscopical examination of liver sections of APAP-treated rats that showed apoptotic bodies within hepatocytes.

In the present study, animals pretreated with garlic oil showed a significant reduction in serum liver enzymes as compared to the group treated with APAP. This finding is in accordance with the study of **Hassan et al. (2009)** where supplementation of sodium nitrite (NaNO$_2$)-intoxicated rats with garlic oil ameliorated the nitrite adverse effects as evidenced by a significant decrease of the serum activities of AST, ALT and ALP enzymes. Combination of garlic oil with NaNO$_2$ was also able to reduce the increase in TBARS concentration and restored the reduced GSH content as well as the catalase activity. On the same pattern, administration of garlic oil caused a remarkable amelioration of APAP-induced liver injury in Wistar rats, as revealed by the drop in serum levels of the liver enzymes AST, ALT and ALP with significant improvement in liver cell injury **[Onaolapo and Onaolapo, 2012]**. This hepatoprotective action of garlic oil could be attributed to garlic flavor constituent; diallyl sulfide (DAS); which has been reported to protect Fischer rats from APAP-induced hepatotoxicity when administered 3 hours before APAP **[Hu et al., 1996]**. Diallyl trisulfide (DATS), another constituent of GO, also markedly suppressed the increase in plasma LDH and AST activities induced by CCl$_4$ in acute liver injury model in rats. DATS may be one of the important factors in garlic oil that protects our body against the injury caused by radical molecules **[Fukao *et al.,* 2004]**.

The results of the present study demonstrated the enhanced liver antioxidant status; due to pretreatment with garlic oil that ameliorated lipid peroxidation. It caused restoration of reduced liver GSH content, and prevented the increase in the hepatic content of MDA. These results are in line with a research work by **Zhang et al. (2012)** where garlic oil was reported to counteract N-nitrosodiethylamine-induced oxidative stress in rats as illustrated by the restoration of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST) levels, and the reduction of the MDA levels in liver of Wistar rats. Hence it may be possible that the mechanism of hepatoprotection of GO is due to its ability to reduce or prevent lipid peroxidation.

In the current work, GO has been able to reduce the liver microsomal CYP2E1 activity when administered to animals prior to APAP treatment. This observation is comparable to that reported in the study of **Zeng *et al.* (2009);** where treatment of male Kun-Ming mice with GO (100 mg/kg body weight) for 1 day or 60 consecutive days, dramatically inhibited the activities of the hepatic CYP2E1, CYP1A2 and CYP3A enzymes, and the protein levels of hepatic CYP2E1 and 1A2. This action could be explained by cytochrome P450 (CYP) enzyme-mediated oxidation of DAS at the sulfur atom that produces diallyl sulfoxide (DASO) and

diallyl sulfone (DASO2), sequentially **[Brady et al. 1991]**. The oxidation of the terminal double bonds of DASO2 by CYP2E1 is the key event leading to the autocatalytic destruction of the enzyme, observed by **Brady et al. (1991)**.

The results of the present study showed that garlic oil was able to restore the depletion in hepatic mitochondrial ATP content and complex-I activity, evoked by APAP administration. On the same line, diallyl trisulfide (DATS) has been reported to dramatically attenuate acute ethanol-induced liver injury and mitochondrial dysfunction, as observed by the amelioration of mitochondrial permeability transition (MPT), membrane potential and mitochondrial ATP level **[Zeng et al., 2008].**

Regarding the effect of garlic on mitochondrial complex-I activity, allicin, one of the important thiosulfinates in garlic, has been shown to preserve the function of mitochondrial electron transport chain (ETC), by increasing the activities of the respiratory chain complexes I-IV, in spinal cord of ischemia-reperfusion challenged rabbits both at 4 and 24 h after reperfusion **[Zhu et al., 2012]**. This action could be attributed to the antioxidant activity of allicin **[Okada et al., 2006]** and its ability to inhibit the production of ROS and the release of mitochondrial cytochrome c **[Zhu et al., 2012]**.

Pre-treatment of animals with garlic oil was able to suppress the marked increase in hepatic mitochondrial Bcl-2 content observed in APAP-treated group. This finding is in accordance with the study of **Flora et al. (2009)** which reported that concomitant administration of garlic extract prevented arsenic-induced hepatic apoptosis in mice as revealed by reversing both the down-regulation of Bcl-2 and the upregulation of Bax gene expression, observed following arsenic treatment. This anti-apoptotic effect of garlic could be explained by its ability to counteract the increase in ROS and the subsequent depolarization of the mitochondrial membrane, as it has been reported that ROS increase and loss of mitochondrial membrane permeabilization (MMP) could lead to apoptosis **[Santra et al., 2007]**. The anti-apoptotic effect of garlic oil was also confirmed by microscopical examination in the current study.

Exposure of rats to a single low dose γ-radiation (LDR) of 0.5 Gy, prior to APAP administration, resulted in a marked suppression of the increase in serum AST, ALT, ALP and MDH and LDH activities induced by APAP. These findings are in harmony with those reported by **Yamaoka et al. (1998)** where a single prior 0.5 Gy whole body X-ray irradiation of rats significantly suppressed the increase in blood activities of hepatocellular enzymes such as LDH, ALT and AST and increased superoxide dismutase (SOD) activities induced by a single intra-abdominal injection of ferric nitrilotriacetate (Fe^{3+})-NTA). Similar results were demonstrated in the study of **Kojima et al. (2000)** where pre-irradiation with a single low dose gamma rays (0.5 Gy) was able to suppress the marked increase in serum ALT activity and MDA content, in mouse liver, observed 24 hours post treatment with APAP. Furthermore, **Lee and Ducoff (1989)** reported that low irradiation doses (up to 0.5 Gy) can induce resistance of cells to oxygen toxicity, which might be the cause of the improvement of the liver enzymes activities observed following irradiation.

The restoration of the hepatic GSH content and the associated reduction in MDA and H_2O_2 contents recorded in the liver of pre-irradiated animals, is compatible with the previous results showing that 0.5 Gy X-irradiation of mice resulted in attenuation of the oxidative stress produced by CCl_4 in their livers **[Nomura and Yamaoka, 1999; Yamaoka *et al.,* 2004].** A proposed mechanism for these effects of radiation is through the enhancement of antioxidant

enzymes activities resulting from the induction of their synthesis following low dose of irradiation [**Kojima *et al.,* 1998; Yamaoka *et al.,* 1998**].

The present study showed the reduction in hepatic microsomal CYP2E1 activity due to pre-exposure of APAP-treated animals to LDR. This observation is in accordance with that shown in the study of **Yukawa *et al.* (1999)** where radiation-induced damage to the liver microsomal drug metabolizing enzyme activity, was suppressed by pre-irradiation of rats with 5 cGy, mainly by protecting cytochrome P-450. Those authors suggested that low doses of radiation act by increasing the cytosolic radical scavenging ability of rat hepatocytes which resulted in protection of microsomal membrane function which is easily damaged by radiation-induced free radicals. Furthermore, low doses of continuous gamma-radiation caused a reliable decrease of CYP2E1expression on protein mRNA levels in mice liver [**Maksymchuk et al., 2008**], which was supposed to be associated with the peroxidation process and the development of oxidative stress.

In the current work, significant increases in liver mitochondrial complex-I activity and ATP content were observed in pre-irradiated APAP-treated rats as compared to the APAP-treated animals. These apparent increases are comparable with other studies using different irradiation dose levels [**Gong et al., 1998; Sattler et al., 2010**]. In the former study; the level of mitochondrial NADH dehydrogenase mRNA was increased one hour after exposure of human glioblastoma cell line to low dose IR (0.05 Gy X-rays), with elevated expression persisting for at least 24 h. In the study of **Sattler et al. (2010)**; when tumor xenografts derived from human head and neck squamous cell carcinoma were irradiated with 30 fractions within 6 weeks, ATP level was increased in tumor cells. Another study revealed that whole body acute γ-irradiation of developing and adult rats with low doses was able to inhibit ADP and AMP hydrolysis in purified synaptic plasma membrane [**Stanojević *et al.*, 2009**].

Furthermore, pre-irradiated rats showed a significant reduction in apoptosis as revealed by the increase in mitochondrial Bcl-2 level and the microscopical examination. The significant increase in mitochondrial Bcl-2 level was in harmony with the observation of **Bogdándi *et al.* (2010)** where low dose irradiation at 0.01- 0.5 Gy dose levels were reported to decrease apoptosis in mice spleenocytes. The rise of mitochondrial Bcl-2 level in the pre-irradiated-APAP group may be attributed to amelioration of GSH depletion and mitochondrial dysfunction in liver. This could be attributed to the increase in Bcl-2 gene expression in the anterior segments of porcine eyes following irradiation; where irradiation exerted a profound preservative anti-apoptotic effect on these cells [**Akeo *et al.,* 2006**].

Implication to Research and Practice

The current results adds more emphasis to other previous reports showing the involvement of multiple mechanisms in APAP-induced hepatotoxicity. The present study also sheds more light on the diversity of targets involved in the hepatoprotective activities of garlic constituents as well as low dose γ-irradiation, and consequently opens the way for further scientific research and possible practical application.

CONCLUSION

The present findings showed that the combination of GO, LDR produced considerable comparable effects to either treatment alone. This remarkable synergistic protection against

APAP-induced hepatotoxicity, might be attributed partly to the suppressive effect of both GO constituents and LDR on lipid peroxidation by free radical scavenging properties or by restoration of glutathione content and cytochrome P450 2E1 enzyme in the liver. Moreover, the anti-apoptotic actions of both treatments, due to the promotion of the anti-apoptotic regulatory protein Bcl-2, may also account for the detected protection against mitochondrial dysfunction and the subsequent depletion of mitochondrial ATP. It could thus be concluded that the hepatoprotective activity of GO and LDR and their anti-oxidant and anti-apoptotic actions could be of beneficial value in the protective management of excessive hepatic damage induced by APAP hepatotoxicity.

Future Research

Further researches are required to investigate other mechanisms involved in APAP-induced hepatotoxicity, as well as different apoptosis-targeted herbal medicines. This will open a new field in the protective management as well as the treatment of hepatotoxicity induced by APAP overdose using relatively safer and more cost effective products, compared to conventional compounds.

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