Hepatoprotective Activity of Curcumin against Rifampicin-Induced Genotoxicity in Experimental Mice

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Abstract: Tuberculosis is a dangerous disease and its death toll is increasing year by year. Intake of rifampicin, the most common antitubercular drug, leads to fatal genotoxic effects on hepatocytes. We have studied the protective effect of curcumin supplementation against the genotoxic effects induced by administration of rifampicin (80mg/kg B.wt.) for four weeks orally in male mice. Curcumin(175mg/kg B.wt.) were administered orally 2 hours prior to rifampicin administration. We showed that the serum level of 8-hydroxy- 2'-deoxyguanosine (8-OHdG), A marker widely used for oxidative damage to DNA were significantly increased in mice treated with rifampicin also there was an elevation in the level of gene expression of 8-oxoguanine DNA-glycosylase 1(OGG1). administration of rifampicin caused an increase in DNA damage in liver of male mice indicated by the damaged nuclei (comet tail length and % of DNA damage). In addition, The liver tissue of male mice treated with rifampicin showed a decrease in the level of gene expression of anti oxidant enzymes **(**glutathion -s- transferase α2, glutathion -s-transferase mu2 and catalase).The co-treatment with curcumin significantly attenuated rifampicin induced gene toxicity in hepatocytes indicated by the significant decrease in the serum level of 8-OHdG, decrease in damaged nuclei explained by comet assay (tail length and % of DNA damage), also Levels of gene expression of anti oxidant enzymes were also maintained to near normal level by curcumin co-administration. From the results obtained, it can be concluded that curcumin is beneficial against rifampicin-induced hepatic genotoxicity, also can used as a prophylactic measures.

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Keywords: Rifampicin**,** genotoxicity**,** curcumin**,** 8-OHdG**,** comet assay

1. Introduction

Genotoxicity is the genetic damage, due to DNA damaging agents including drugs by different mechanisms. The induction of genetic damage is a critical step in the production of different genetic diseases in future generations, birth defects and contributes to somatic diseases such as cancer in the present generation. Therefore it is necessary to evaluate the genotoxic potential of drugs to evaluate their ability to cause cancer and other defects. Genotoxic potential of drugs is assessed, as part of the safety evaluation process and genotoxicity assays have become an integral component of drug regulatory requirements **(Jena *et al.*,2002) .**

Rifampicin, an antibacterial drug, is highly effective in the treatment of tuberculosis and leprosy. Recently, it has been reported to have neuroprotective effects *in vitro* and *in vivo* models**.(Oida *et al.*,2006).** Rifampicin (RIF) is a semisynthetic derivative of the complex macrocyclic antibiotic rifamycin obtained from a streptomyces organism. It is the first antibiotic of the ansamycin family. The antimicrobial activity of RIF is due to its inhibition of bacterial RNA polymerase (RNAP). RIF binds to conserved amino acids in the active centre of the enzyme and blocks transcription initiation**.(Audrey *et al.,* 2010)**.

Rifampicin, being the first line drug used as antituberculous chemotherapy, is known to be associated with hepatotoxicity**. (Tasduq *et al.,* 2005).** The rate of hepatic damage has been reported to be much higher in developing countries (8- 30%). Oxidative stress produced by RIF causes hepatic injury. **(Sodhi *et al.*,1997).** Majority of normally formed free radicals are removed by the action of reduced glutathione. This causes the initiation of lipid peroxidation (LPO) resulting in tissue injury **(Shanker *et al.*,2005).** Reactive oxygen species play an important role in rifampicin induced liver injury **(Chowdhury *et al.,* 2006).**

The oxidation of DNA bases is produced by reactive oxygen species (ROS), as well as other processes leading to increased oxidation. ROS are generated both from endogenous sources and from the reactions of xenobiotics. The agents producing DNA oxidation in vivo are generally believed to do so as a result of increased production of ROS. **(Kasai, 1997).**

ROS plays an important role in mediating cellular responses , 8-OHdG is a byproduct of ROS damage to DNA, which can cause mutation of G:C to A:T if it remains in the DNA at the time of replication , 8-OHdG in urine and blood is a widely accepted marker of oxidative DNA damage and oxidative stress. Normal DNA repair removes 8-OHdG adducts,

which are excreted and measurable in urine, blood and tissues**.(Breton *et al.,* 2007).**

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-3,5-dione, 1) is a naturally occurring phenolic compound isolated as a yellow pigment from turmeric (dry rhizomes of *Curcumin longa*) which is commonly used as a spice and food colorant **(Buescher and Yang, 2000).** This compound has been reported to possess a variety of biological and pharmacological activities, including antioxidative**(Daniel *et al.,* 2004),** anti-inflammatory **(Chainani-Wu, 2003),** anticarcinogenic (**Aqqarwal *et al.*,2003)** and anti-HIV **(Mazumder *et al.*,1995).** The antioxidant mechanisms of curcumin have recently been the focus of interest of free-radical chemists and biologists. Curcumin is known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes, and the inhibition of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation. Most of the antioxidants have either a phenolic functional group or a β-diketone group. Curcumin is an unique antioxidant, which contains a variety of functional groups, including the β-diketo group, carbon–carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents **(Wright,2002).**

2.Material and methods:

I- Expermental animals:

A total 40 Male white Swiss mice aged 9–12 weeks will used in all experiments. The animals will obtained from central animal house of Veterinary Medicine , Zagazig University. Animals housed in stainless steel cages , Maintained under hygienic conditions and received standard diet and water two weeks before starting the experiment to accommodate them to the experimental condition in the laboratory

II- *Tested compounds*

Ciprofloxacin(CPX): purchased from Amoun Pharmaceutical Co., Egypt. used by a dose (130mg/kg B.Wt/day)

Rifampicin: is produced by Novartis Pharma, Cairo. used by a dose (80mg/kg B.Wt/day).

Curcumin: It was obtained in commercial form (Turmeric Extract with BioPerine® Directions). The drug was obtained from Vitacost,USA. used by a dose (175mg/kg B.wt/day**).** Piperine is an adjuvant used to improve the bioavailability of curcumin by interferes with glucuronidation.

III- Animal grouping

The rout of administration of all treatments are orally by using stomach tube, The experimental animals were randomly divided into 4 groups 10 of each as following :

Group A: (N=10), control group received normal saline.

Group B: (N=10), received 80mg/kg B.wt. rifampicin for 4 weeks.

Group C: (N=10), received curcumin(175mg/kg B.wt.) then after 2 hours give rifampicin (80mg/kg B.wt.) for 4 weeks simultaneously.

Group D: (N=10),), received firstly curcumin(175mg/kg B.wt.) for 1week then co- treated with curcumin(175mg/kg B.wt.) + rifampicin (80mg/kg B.wt.) for 4 weeks simultaneously.

Methods:

1) Sampling protocol

- Animals will sacrified using highly sterilized scissors (180°Cfor 6 hours) to avoid RNA degradation by RNases and latex gloves weared to minimize RNase contamination.
- Liver samples will immediately excised, part of it will wrapped in aluminium foil and put immediately in liquid nitrogen container to make snap-freezing of tissue and minimize action of endogenous RNase. this part for RT-PCR.
- Another part of liver samples will Placed in 1 mL cold HBSS containing 20 mM EDTA/10% DMSO. Mince into fine pieces, let settle, remove and mix 5 - 10 µl with 75 µl LMPA. For comet assay
- Serum samples: blood samples were collected without anticoagulant into clean and dry test tubes for obtaining serum samples which were preserved at – 20° C for detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG).

2)- Estimation of serum 8-hydroxy- 2'-deoxyguanosine (8-OHdG)by ELISA kits : It was estimated according to the method described by **Tomoko *et al*. (2000)**

3)- Comet assay:

- The single cell gel electrophoresis / comet assay protocol:- according to**(Singh *et al.,* 1988):**

4) Molecular determinations:

- Determination of (Glutathione -s-transferase α2, Glutathione -s-transferase mu 2, catalase, Cytochrome P450 2E1, 8-oxoguanine DNA glycosylase 1) gene expression: Using a semi-quantitative RT-PCR according to **(Meadus, 2003).**

A- RNA extraction: Total RNA was extracted with E.Z.N.A.spin column RNA extraction kit (Qiagen biotech).

B- Synthesis of cDNA : The Synthesis of cDNA was occurred by using (Qiagen long rang) RT-PCR kits (20). Mat. No. 1042845.

C- PCR amplification.

The PCR amplification was occur using Long-Range 2 step RT-PCR kits from (Qiagen long rang).

Table (A):The thermal cycler conditions used during RT-PCR

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
Glutathione s-transferase α2	95/5 min	94/30 sec	57/30 sec	72/1 min	72/7 min	35
Glutathione s-transferase mu 2	94/5 min	94/30 sec	60/45 sec	72/1 min	72/7 min	30
Cytochrome P450 2E1	95/5 min	91/60 sec	48/ 60 sec	70/1 min	72/10min	29
catalase	95/5 min	94/30 sec	60/30 sec	72/1 min	72/10min	35
8-oxoguanine DNA glycosylase 1	95/5 min	94/30 sec	57/30 sec	72/1 min	72/7 min	35
GAPDH	95/5 min	94/30 sec	60/30 sec	72/1 min	72/7 min	30

Table (B):primers used in determination of the expression of the pervious genes. (Yuichi et al.,2007).

Gene	Forward primer	Reverse primer
Glutathione s-transferase α2	ACATGAAGGAGAGAGCCCTGAT	GCAGTCTTGGCTTCTCTTTGGT
Glutathione s-transferase mu 2	TTCCCAATCTGCCCTACTTGA	TCTCCACACAGGTTGTGCTTTC
catalase	GCTGAGAAGCCTAAGAACGCAAT	CCCTTCGCAGCCATGTG
8-oxoguanine DNA glycosylase 1	GCCAACAAAGAACTGGGAAACT	CAGCATAAGGTCCCCACAGATT
Cytochrome P450 2E1	5′ CGGTTCTTGGCATCACCGT3′	5′GCAGGTGCACAGCCAATCA3′
GAPDH	CCCGTAGACAAAATGGTGAAGG	GCCAAAGTTGTCATGGATGACC

PCR products were separated on a 1.5% Eithidum bromide treated agarose gel electrophoresis in Tris acetate EDTA buffer with 0.5µg/ml ethdium bromide. The gel examined by UV transilluminator. The electrophoretic picture was taken by digital camera 12 mega pixels and quantified with image J software.

5)Statistical analysis:

The obtained data were analyzed and graphically represented using the statistical package for social science (**SPSS**, 18.0 software, 2011) for obtaining means and standard error. The data were analyzed using one way **ANOVA** to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

3. Results:

Serum level of 8 hydroxy 2` deoxy guanosine (8OH2dG)

Table (1): Means ± SE of serum level of 8 hydroxy 2` deoxy guanosine (8OH2dG) (ng/ml) of the liver tissue of male mice.

Groups	Group A (Control)	Group B Treated with rifampicin	Group C Treated with rifampicin and curcumin	Group D Treated with curcumin a week prior rifampicin and curcumin
Means ± SE	11.075 ± 0.295^{c}	30.20 ± 1.180^{a}	20.56 ± 0.648^{b}	9.925 ± 0.205^{c}

*** Means within the same row carrying different superscripts are very highly significant at (**P** ≤0.001).

Data presented in table (1) show that there was a very high significant increase of serum level of 8 hydroxy 2` deoxy guanosine (8OH2dG) in group B when compared to the control group, while the level of (8OH2dG) show drastic decrease in(group C) and the decrease in the expression level become nearly around the control group

Comet assay

Table (4): Means ± SE of Tail length and % of Tail DNA of comet assay of the liver tissue of male mice.

Groups	Group A (Control)	Group B Treated with rifampicin	Group C Treated with rifampicin and curcumin	Group D Treated with curcumin a week prior rifampicin and curcumin
Tail length	2.79±0.469^{c}	7. 53± 0.241^{a}	4.60 ± 0.296^{b}	5.00 ± 0.246^{b}
% of Tail DNA	2.70±0.178^{c}	7.25± 0.567^{a}	4.87 ± 0.353^{b}	3.57± 0.290^{b}

*** Means within the same row carrying different superscripts are very highly significant at (**P** ≤0.001).

Regarding the oxidative DNA damage caused by rifampicin , the present study was undertaken to test the *in vivo* genotoxic potential of rifampicin in mice using the single cell gel electrophoresis (comet assay). Our result revealed that administration of rifampicin caused an increase in DNA oxidative damage in liver of male mice indicated by the damaged nuclei (comet tail length and % of DNA damage). rifampicin caused very high significant increase in comet tail length and % of DNA damage compared with the control group. While co-treated groups with rifampicin and curcumin revealed a considerable ameliorative effect in comet tail length and % of DNA damage in group C and D compared with control one.

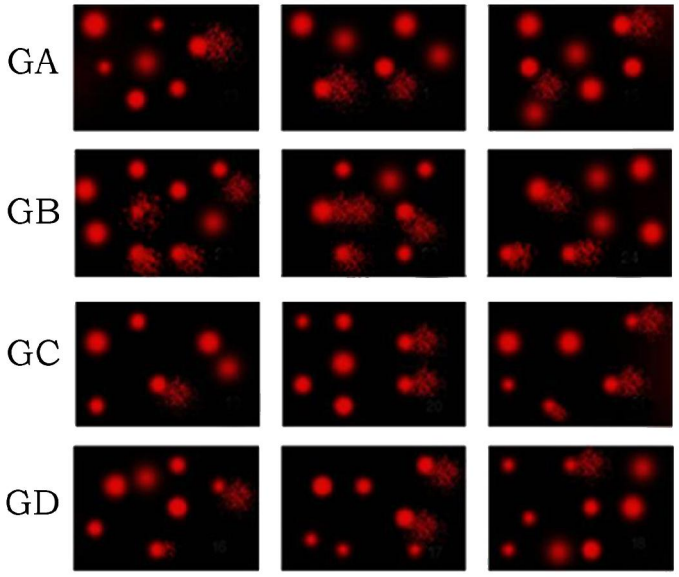
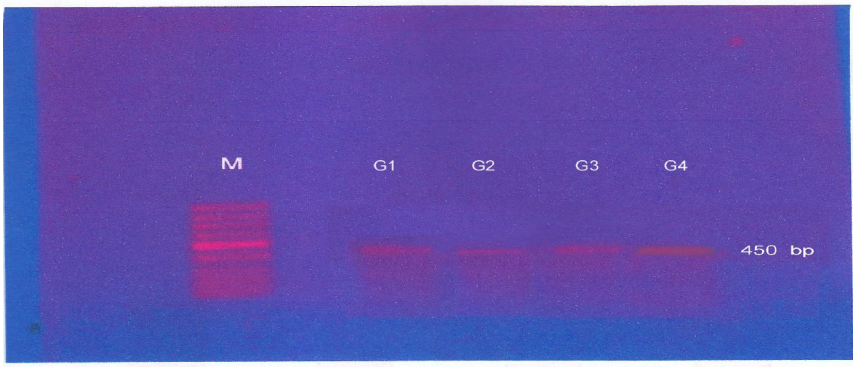


Fig. (1): photomicrograph with florescent microscope showing the effect of administration of curcumin(175 mg/kg b.wt with RIF(80 mg/kg b.wt) for four weeks on The oxidative DNA damage (comet assay) of the liver tissue of male mice.

GA: control group, **GB**: RIF treated group, **GC**: RIF + curcumin, **GD**: curcumin then RIF + curcumin

Genes Expressions

1- glutathion –s- transferase α2

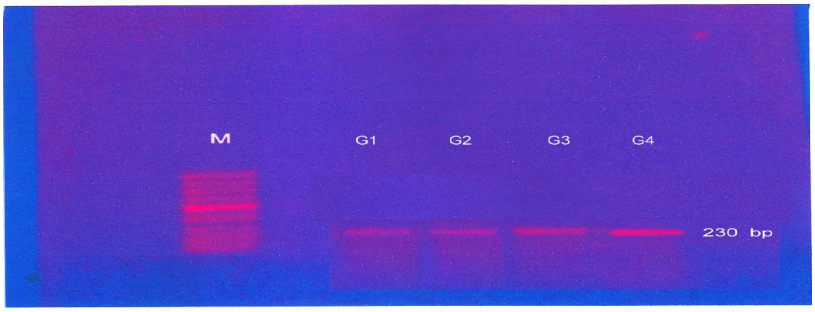


Figure(2):The electrophotograph of glutathion –s- transferase α2 (450bp) mRNA expression in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder, **1: Group A:** Male mice (Control).
2: Group B: Male mice treated with rifampicin,
3: Group C: Male mice co-treated with rifampicin and curcumin .
4: Group D: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

- The liver tissue of male mice show a decrease in the level of gene expression of glutathion s transferase α2 in the group(B) when compared with control one, while the level increased in group (C) than GB and there are drastic increase in the expression level in GD .

2- **glutathion –s- transferase mu2**

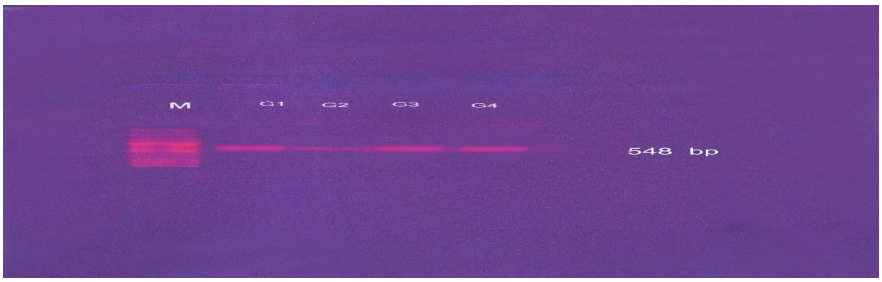


Figure(3):The electrophotograph of glutathion s transferase mu2 (230bp) mRNA expression in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder. **1: Group A:** Male mice (Control).
2: Group B: Male mice treated with rifampicin.
3: Group C: Male mice co-treated with rifampicin and curcumin .
4: Group D: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

- The liver tissue of male mice showed a decrease in the level of gene expression of glutathion s transferase mu2 in the group(B) when compared with control one, while the level increased in group (C) than GB but not reach to the control group, and there was a drastic increase in the expression level in GD become more than the expression level in the control group.

3- **Catalase**

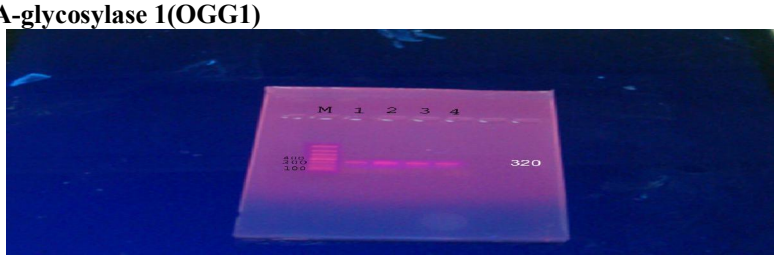


Figure(4):The electrophotograph of catalase (548bp) mRNA expression in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder. **1: Group A:** Male mice (Control).
2: Group B: Male mice treated with rifampicin.
3: Group C: Male mice co-treated with rifampicin and curcumin .
4: Group D: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

- The liver tissue of male mice show a decrease in the level of gene expression of catalase in the group (B) when compared with control one, while the level increased in (GC) and (GD)than(GB).

4- 8-oxoguanine DNA-glycosylase 1(OGG1)

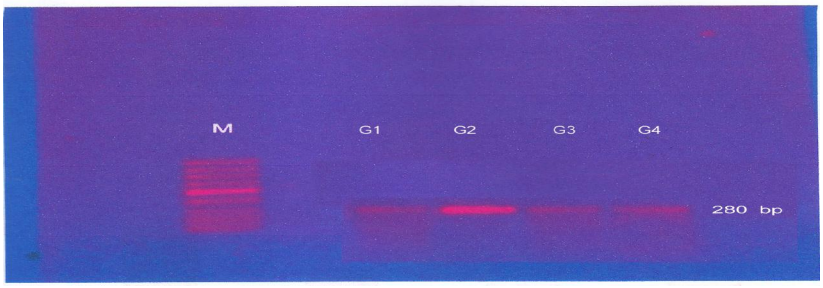


Figure(5):The electrophotograph of 8-oxoguanine DNA-glycosylase 1 (320bp) mRNA expression in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder. **1: Group A:** Male mice (Control)
2: Group B: Male mice treated with rifampicin.
3: Group C: Male mice co-treated with rifampicin and curcumin.
4: Group D: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

- The liver tissue of male mice showed an elevation in the level of gene expression of 8-oxoguanine DNA-glycosylase 1(OGG1) in the group(B) when compared with control one, while the level decreased in group (C) than GB and there are drastic decrease in the expression level in GD.

5- Cytochrome P450IIE1

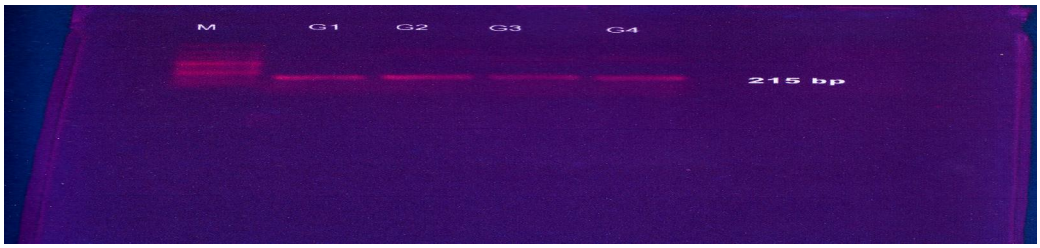


Figure(6):The electrophotograph of cytochrome P450IIE1(280bp) mRNA expression in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder. **1: Group A:** Male mice (Control)
2: Group B: Male mice treated with rifampicin.
3: Group C: Male mice co-treated with rifampicin and curcumin.
4: Group D: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

- The liver tissue of male mice showed a demarcated increase in the level of gene expression of cytochrome P450IIE1in the group(B) when compared with control one, While in groups co-treated with rifampicin and curcumin (GC and GD) the level of expression is more or less similar to control group.

6- GAPDH:



Figure(9):The electrophotograph of Glyceraldhyde 3 phosphate dehydrogenase mRNA (GAPDH) expression(215bp) in the liver tissue of male mice.

M: 100bp-1000bp DNA-ladder, **Group 1:** Male mice (Control)
Group 2: Male mice treated with rifampicin.
Group 3: Male mice co-treated with rifampicin and curcumin .
Group 4: Male mice treated with curcumin one week prior rifampicin and curcumin co-treatment.

4. Discussion

Rifampicin, being the first line drug used as antituberculous chemotherapy, is known to be associated with hepatotoxicity**. (Tasduq *et al.*,2005).** Rifampicin mediated oxidative damage is generally attributed to the formation of the highly reactive oxygen species, which act as stimulators of lipid peroxidation and source for destruction and damage to the cell membrane **(Georgieva *et al.*, 2004).**

The effect of rifampicin on DNA in the present study was confirmed by determination of serum level of 8 hydroxy 2` deoxy guanosine (8OH2dG) and the comet assay protocol on the liver tissue of male mice**.**

8-hydroxydeoxyguanosine is a nucleotide form that results from oxidative DNA damage, which causes mutation in vitro and in vivo. So the occurrence of 8-OHdG (8-hydroxy-2'-deoxyguanosine) has been used to study damaging effects on DNA of ROS. Results of many studies **(kassie *et al.*, 2001 and Yamauchi *et al.*, 2004).** conducted thus far indicate that 8-OHdG is a sensitive biomarker to asses the extent of DNA damage and repair in both clinical and occupational settings, Our results show that there was a very high significant increase of serum level of 8 hydroxy 2` deoxy guanosine (8OH2dG) in rifampicin treated group when compared to the control group, while the level of (8OH2dG) show a drastic decrease in rifampicin co-treated group and the decrease in seum level become nearly around the control group in curcumin pre treated group.

due to the ability of ROS to induce DNA damage via causing DNA-protein cross links and modifications of base residues such as introduction of a hydroxyl group (-OH) into the C-8 position of guanosine and guanine residues forming 8-OHdG and of DNA oxidation **(Valko *et al*., 2006 and Iqbal *et al.*, 2009).**

The SCGE, also known as comet assay, is one of the recent methods established in order to detect different types of DNA damage. The comet assay has been established as a simple, rapid, cheap, flexible and, most importantly, sensitive method to detect DNA damage, which is also able to detect DNA damage in individual cells **(Tice *et al.*, 2008).**The comet assay test is considerd the most reliable and superior to other short term mutagenicity assays **(Gollapudi and McFadden ,1995).** So we select the test for studying genotoxicity of ciprofloxacin than other genotoxicity assays (e.g. micronuclei, mutations,structural chromosomal abrrations) and the technique used to detect the DNA damage for the previous mentioned comet technique advantages. Our result revealed that administration of rifampicin caused an increase in DNA oxidative damage in liver of male mice indicated by the damaged nuclei (comet tail length and % of DNA damage). rifampicin caused very high significant increase in comet tail length and % of DNA damage compared with the control group. While co-treated groups with rifampicin and curcumin revealed a considerable ameliorative effect in comet tail length and % of DNA damage in group C and D compared with rifampicin treated group.

Aly and Donya, 2002 concluded that RIF is a somatic and germ cell mutagen. It induced a dose dependent increase in the percentage of chromosome aberrations in mouse bone marrow after treatment with single and repeated therapeutic doses of RIF**.** The drug increased sister-chromatid exchanges in mouse bone marrow cells and induced chromosome abnormalities in mouse spermatocytes.

Awodele *et al.*, 2010 suggested that rifampicin has a damaging effects on the DNA which evaluated by using micronucleus assay and this damage may be induced by free radicals generated by this drug

Regarding to the oxidative stress related to rifampicin,The liver tissue of male mice in our experiment showed a decrease in the level of gene expression of anti oxidant enzymes **(**glutathion s transferase α2, glutathion s-transferase mu2 and

catalase)in the rifampicin treated group when compared with control one, while the level increased in rifampicin co-treated group than rifampicin treated group but not reach to the control group, and there was a drastic increase in the expression level in curcumin pre treated group, become more than the expression level in the control group.

A successful model of hepatotoxicity was produced by giving 50 mg/kg/day each of INH and RMP in two weeks. Liver showed type II hepatocellular changes (microvesicular fat deposition) with mild portal triaditis. The glutathione and related thiols were significantly decreased in both blood and liver tissues with combination of INH and RMP treatment. Superoxide dismutase, glutathione peroxidase, catalase and glutathione-S-transferases were decreased in the treated group. The altered profile of antioxidant enzymes with increased lipid peroxidation indicated the enhanced oxidative-stress in combination of INH and RMP treatment. Thus the hepatotoxicity of INH and RMP is appeared to be mediated through oxidative-stress. **(Sodhi *et al.*, 1997).**

It was found that the administration of antidotal therapy with curcumin greatly improved the gene expression of antioxidant enzymes , this may be due to the antioxidant effect of Curcumin, The antioxidant mechanisms of curcumin have recently been the focus of interest of free-radical chemists and biologists. Curcumin is known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes, and the inhibition of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation. Most of the antioxidants have either a phenolic functional group or a B-diketone group. Curcumin is an unique antioxidant, which contains a variety of functional groups, including the B-diketo group, carbon–carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents **(Wright,2002).**

(**Ricky *et al.*, 2001)** suggest that Curcumin prevents colon cancer in rodent models. It inhibits lipid peroxidation and cyclooxygenase-2 (COX-2) expression and induces glutathione -s-transferase (GST) enzymes. they tested the hypothesis that 14 days of dietary curcumin (2%) affects biomarkers relevant to cancer chemoprevention in the rat. The curcumin increased hepatic GST by 16% when compared with controls.

Our result showed that the liver tissue of male mice has an elevation in the level of gene expression of 8-oxoguanine DNA-glycosylase 1(OGG1) in the rifampicin treated group when compared with control one, while the level decreased in groups co-treated with curcumin (and there are a clear decrease in the expression level in curcumin pre treated group.

Regarding to DNA oxidation, when persistent during replication of DNA, can lead to mutations, Owing to the significance of DNA oxidative damage for the integrity of DNA, extensive DNA repair pathways have evolved **(Lindahl and Wood, 1999).** Even for the 8-oxo-dG lesion redundancy exists The human repair enzyme 8-oxo-G-DNA glycosylase (OGG)-1 gene has recently been cloned, **(Hazra *et al.*, 1998).**

Our result showed that the liver tissue of male mice showed a demarcated increase in the level of gene expression of cytochrome P450IIE1in the rifampicin treated group when compared with control one, While in groups co-treated with rifampicin and curcumin (GC and GD) the level of expression is more or less similar to control group.

Anti-tuberculosis drugs act as inducers of hepatic cytochrome P450 enzymes. For example, rifampicin is a potent inducer of CYP2D6 and CYP3A4, and isoniazid induces CYP2E1 in human **(Vuilleumier *et al.*, 2006).** The induction of CYT P450 enzymes is known to take part in increased drug disposition and development of multi-drug resistance. Xenobiotics, including anti-tuberculosis drugs, undergo biotransformation in the liver catalyzed by microsomal enzyme systems. The major isozyme of cytochrom P450 enzymes in bioactivation is CYT2E1, which is also involved in hepatic toxicity of carbon tetrachloride, ethanol and acetaminophen. Inhibition of this isozyme by specific inhibitors or herbal drugs has been shown to be hepatoprotective **(Bradford *et al.*,2005).**

Conclusions

From previous investigations and the evidence of oxidative stress of rifampicin , antitubercular antibiotic, it can be concluded that it induce liver injury and oxidative DNA damage. These harmful effects greatly imply the beneficial role of our study to ameliorate these harmful effects. The use of curcumin demonstrate it is beneficial effects against rifampicin induced liver genotoxicity and curcumin ameliorate the deleterious effects of oxidative stress .and it may be not only curcumin is beneficial against rifampicin-induced hepatic genotoxicity, but also as prophylactic measure

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