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Research Article

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Evaluation of Hepatoprotective Activity of Neem Extract in Rifampin Induced Acute Hepatic Failure in Rats

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ABSTRACT

The liver plays a role in many body functions such as immune defense and the metabolism of sugar and fat. Rifampin (RIF) is an antibacterial drug prescribed to treat tuberculosis (TB) along with multiple drugs. Other types of infections may also be treated with rifampin. Although the therapeutic effect of RIF has many adverse effects such as hepatoyoxicity. Neem (NM) has more than 140 compounds from various parts that have been isolated and can thus play a role in preventing hepatotoxicity. The research was carried out to examine the protective effect of neem leaves extract (NMLE) on RIF-induced liver damage. Forty male rats had been divided into four groups; Group I) non-treated negative control group, (Group II), which was given RIF (54 mg/kg/day) for thirty days, (groups III and IV intoxicated rats received orally the NMLE in doses of two hundred and fifty and five hundred mg/kg/day respectively, for 30 days. At day 30, blood was collected for biochemical analysis, as well as the liver was also examined histopathologically. The results revealed that the NMLE at the two dosage levels significantly decreased serum levels of liver enzymes and MDA accompanied by significantly increased in activities of GSH, SOD, and showed ant-inflammatory effects as evidence by significantly decreased in TNF-α and IL-1α levels compared to RIF group II. There was also an improvement in histopathological alterations observed in liver tissues of hepatotoxic rats. Therefore, the administrations of NMLE has hepatoprotective effects in hepatotoxic rats via antioxidant and ant-inflammatory pathway.

Key words: *Liver toxicity, Neem, Rifampin, Rats, Cytokines, Histopathological.*

INTRODUCTION

Liver is an important organ in metabolism of drugs. Drug that induce liver failure is a major medical problem [1]. Tuberculosis, an immunodeficiency virus, is one of a major problems worldwide. It is from the deadliest causes among curable infectious diseases, caused in human by various strains of mycobacteria; Mycobacterirum tuberculosis, MTB. It is spread through the air from transmitting the saliva of people who have an active MTB infection that attacks lungs [2, 3]. Rifampicin (RIF), an anti-tuberculosis drugs, is an antibiotic produced by stretomyces mediterrenei that induced hepatocellular dysfunction with early stage of administration [4, 5]. It affects bilirubin excretion and induced hyperbilirubinemia [6], and causes cholestasis, hepatic lesions, centrilobular necrosis and liver injury [6, 7].

Production of ROS is a crucial factor inducing tissues oxidative damage [8]. Herbal medicine has gained attention for maintaining health [9]. It plays a major role in the management of liver' disorders via regeneration of liver cells and prevention the oxidative damage induced by ROS [10].

The features of plants have created world-wide interest about medicinal plants which lead to novel sources of drugs for wide modern applications [11]. They are also widely utilized to control various insect pests [12]. *Azadirachta indica*, known as Neem, is therapeutic plant. It possesses diverse pharmacological properties. Neem leaves extract has anti-inflammatory, antipyretic and antimicrobial activities [13-15]. It showed hyperglycemic and hypolipidemic activities in diabetic rats [16], and gastroprotective properties [17]. Neem leaf comprises several valuable constituents as polyphenolics, flavonoids, glycosides, querctin and terpenoids, which possess gastroprotective [18, 19]. Administration of NMLE protected liver from cisplatin hepato- and nephro-toxicity

[20]. This work aims to assess the protective role of NMLE on RIF-induced hepatotoxicity.

MATERIALS AND METHODS

Plant material

Neem (*A. indica*) (NM) leaves, were bought from local market, Jeddah, KSA.

Drugs and chemicals

Rifampin (RIF) was purchased from a local pharmacy (Jeddah). All chemicals and kits with high analytical grade were bought from Sigma-Aldrich Co., USA.

Rats

Sprague Dawley adult male rats (180± 10 g) were provided from King Fahd Medical Research Center (KFMRS), KAU. They were kept in standard laboratory conditions, fed on a standard AIN-93 diet [21]. They were kept in accordance to the standard guidelines in KFMRS, KAU for the use and care of laboratory animals.

Plant material and extraction

Five hundred grams of the NM fresh leaves were cleaned with distilled water, then soaked in 500 ml ethanol (80%), for 48 h at room temperature. The extract was filtrated, evaporated by a rotary evaporator, then concentrated on water bath (36° C). The extract, greenish paste, stored at (<4°C) until use [22].

Protocol design

Wister male rats (n=40, 180 ± 10 g) were used in the experiment. The rats fed standard formula diet in a steady environment (22 -25 °C temp., 45-55% humidity). Rats were put under the observation for a week before the beginning of the protocol experiment. After the acclimatization period, the rats were randomly distributed to 5 groups (ten each).

Group I: Control negative(Cont); rats administered orally1 ml/kg (1% CMC) for 30 d.

Group II: Control positive (RIF); rats treated orally with RIF (54 mg/kg/day) for 30 d. [23].

Group III: Rats received orally NME (250 mg/kg/day) [24], and receive RIF 1 h after administration of NMLE for 30 d.

Group IV: Rats received orally NME (500 mg/kg/day) and receive RIF 1 h after administration of NMLE for 30 d

After 30 d of experimental time, all rats for each group sacrificed under light anesthesia with ether. Blood samples were collected by heparinized capillary tubes, kept for couple of h and centrifuged for 15 min at 3000 rpm. Then serum samples stored at −20˚C for subsequent followed analyzes. The liver was removed for histopathological examination.

Determination of serum biomarkers

Serum liver enzymes activities (aspartate aminotransferase (AST), alanine aminotransferase (ALT)) determined as described [25], and alkaline phosphatase (ALP) [26]. Oxidative stress biomarkers (reduced glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA)) tested chemically [27-29], respectively]. Anti-inflammatory cytokines (interleukin– 1α (IL- 1α) and tumor necrosis factor-α (TNF- α)) were tested [30, 31, respectively].

Histopathological examination

The liver tissue samples from all groups were prepared and stained with hematoxylin-eosin by routine procedures [32].

Statistic

The analysis of the results was done by SPSS ver. 24, one-way ANOVA. Values were showed as mean ± SDM. The results considered significance at $P \leq 0.05$ [33].

RESULTS

Administration of RIF orally to rats caused significant ($P < 0.05$) decreases in BWG %, FI and FER (24.18 ± 0.43,18.88 ± 0.089 and 0.013 ± 0.009, respectively) when compared to Cont group (64.75 ± 0.61, 31.22 ± 0.32 and 0.021 ± 0.004, respectively). Oral administration of NMLE to rats inflicted with hepatoxicity induced significant ($P < 0.05$) increases in all biological parameters as compared with RIF group (Table 1).

Table 1. Hepatoprotective effect of Neem leaves extract (NMLE) on biological evaluation in hepatotoxic-rats.

Groups	BWG %	FI (g/rat/day)	FER
Cont	64.75 ± 0.61a	31.22 ± 0.32 a	0.021 ± 0.004 [a]

RIF	24.18 ± 0.43 b	18.88 ± 0.089 b	0.013 ± 0.009 b
NMLE (250 mg/kg)+RIF	59.38 ± 1.15 a	28.56 ± 0.65 a	0.021 ±0.001a
NMLE (500 mg/kg)+RIF	61.53 ± 1.26 a	29.76 ± 0.97 a	0.021 ± 0.005 a

- Values are presented as mean ± SDE.
- In the same column different superscript letters considered significantly different (P< 0.05).

From data recorded in Table (2) it might be recognized that intoxicated rats by RIF (54 mg/kg) caused significant (P<0.05) increases the serum liver enzyme activities (AST, ALT and ALP: 141.19±2.13, 130.36±0.26 and 143.22±2.43, respectively) as compared to Cont group (53.56±1.65, 48.21± 0.53 and 58.92±2.15, respectively). Administration of NMLE (250 and 500 mg/kg) to intoxicated with a RIF exhibited significant decreases (P<0.05) in all the elevated liver markers as compared with RIF group.

The NMLE effect on MDA, GSH and SOD in hepatotoxic rats is shown in Table (3). In a group of hepatotoxicity, MDA increased significantly (25.37±1. 32) compared to Cont rats (13.34±1.54), while there were significant decreases in GSH and SOD (3.87±0.09 and 23.46 ± 1.45, respectively) compared to Cont rats (13.34±1.54, 6.27±.03 and 57.34 ±1.95, respectively). The pretreatment with NMLE at the two dosage levels resulted in significant increases in MDA level and significant decreases in GSH and SOD activities compared to RIF hepatotoxic rats. Pretreatment with NMLE at a dose of 500 mg/kg/ day showed strong efficacy in MDA, GSH and SOD compared to pre-treated group a dose of 250 mg/kg.

Anti-inflammatory cytokines IL-1β and TNF-α serum levels are significantly (P < 0.05) increased in rats intoxicated by RIF (54 mg/kg) compared with their values in the Cont group. Administration of NMLE extract in doses of 250 and 500 mg/kg/ day results in a significant decrease in serum IL-1β and TNF-α compared to hepatotoxic group. Administration of NMLE extract in a dose of 500 mg/kg results in non-significant serum IL-1β and TNF-α compared to Cont group. The high dose (500mg/kg) of NMLE extract is the most effective compared to the NMLE low dose group as presented in Table (4).

Table 2. Hepatoprotective effect of Neem leaves extract (NMLE) on serum liver enzymes AST, ALT and ALP in hepatotoxic rats.

Groups	AST (U/L)	ALT(U/L)	ALP (U/L)
Cont	53.56±1.65 c	48.21± 0.53 c	58.92±2.15 c
RIF	141.19±2.13 a	130.36±0.26 a	143.22±2.43a
NMLE (250 mg/kg)+RIF	67.53±2.67 b	75.37±2.85 b	81.32±1.45a
NMLE (500 mg/kg)+RIF	56 .20±3.43 c	54.64±0.62 c	62.59±2.21 b

- Values are presented as mean ± SDE.
- In the same column different superscript letters considered significantly different (P< 0.05).

Table 3. Hepatoprotective effect of Neem leaves extract (NMLE) on the levels of MDA, GSH and SOD in hepatotoxic rats

Groups	MDA (nmol/g protein)	GSH (µg/mg protein)	SOD (U/mg)
Cont	13.34±1. 54 c	6.27±.0.03a	57.34 ± 1.95 a
RIF	25.37±1. 32 a	3.87±0.09 c	23. 46 ± 1.45 c
NMLE (250 mg/kg)+RIF	19.36±1.32 b	5.07±0.05 b	64.23±1.74 b
NMLE (500 mg/kg)+RIF	14 .30±1.12 c	6.14±0.08 a	59.03±1.12 a

- Values are presented as mean ± SDE.
- In the same column different superscript letters considered significantly different (P< 0.05).

Table 4. Hepatoprotective effect of Neem leaves extract (NMLE) on serum IL-1 and TNF-α level in hepatotoxic rats.

Groups	IL-1 (pg/ml)	TNF-α (pg/ml)
Cont	23.82 ± 0.24 c	12.03 ± 1.26 c
RIF	58.41± 0.21 a	27. 53 ± 0.74 a
NMLE (250 mg/kg)+RIF	33.26±1.30 b	19.49± 0.06 b
NMLE (500 mg/kg)+RIF	24 .76±1.63 c	14.84±0.03 c

- Values are presented as mean ± SDE.
- In the same column different superscript letters considered significantly different (P< 0.05).

Liver tissue of Cont rats showed normal histological structure of hepatic lobule with normal hepatic sinusoids

and hepatocytes (Fig.1.A). Administration of RIF to rats induced inflammatory cell infiltration, hepatic necrosis and hepatocytes apoptosis (Fig.1.B). Liver tissue of rats given NMLE (250mg/kg) + RIF showed slight congestion of hepatic sinusoids (Fig.1.C). In group received NMLE (500 mg/kg) + RIF revealed apparently normal architecture of hepatic tissue (Fig.1.D).

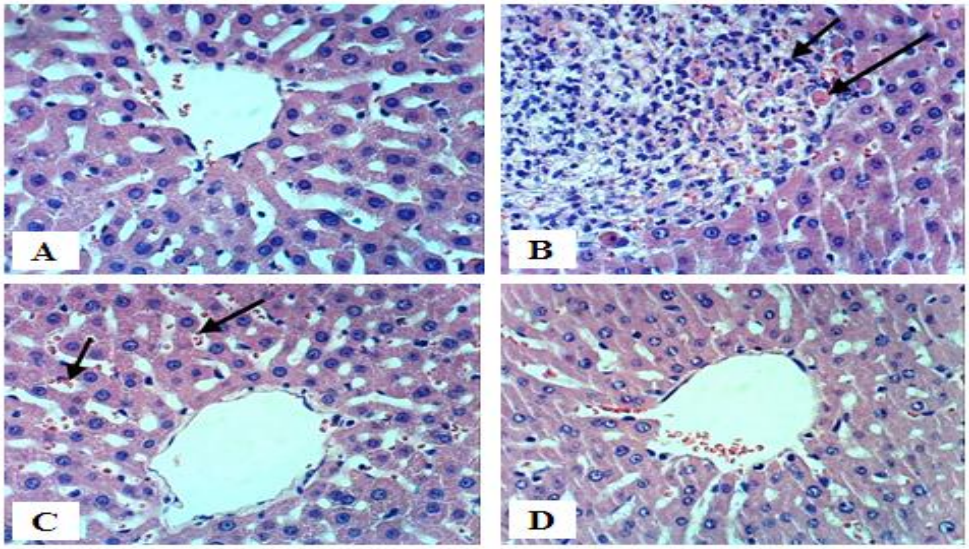

Figure 1: Hepatoprotective effect of Neem leaves extract (NMLE) on liver sections in hepatotoxic rats (H&E stain x 400). Liver section of Cont rats (-ve) showed normal histological structure of hepatic lobule [A]. In RIF intoxicated rats' liver section showed inflammatory cell infiltration, focal hepatic necrosis and apoptosis of hepatocytes [B]. Liver section of pretreated intoxicated rats with NMLE (250mg/kg) showed slight congestion of hepatic sinusoids [C]. Liver section of pretreated intoxicated rats with NMLE [500 mg/kg] showed normal hepatic lobule.

DISCUSSION

Rifampin is one of the major anti-TB drugs prescribed in the treatment of mycobacterial infections. However, it has adverse effect, where it is a leading cause of acute liver failure, which is mainly related to oxidative stress [34, 35]. Neem is versatile medicinal herbs having many biological activities [36]. Its phytochemical constituents have anti-inflammatory, antimicrobial, antioxidant and diverse pharmacological activities [15, 16]. This study aimed to assess the potential protective role of NMLE against RIF-induced acute liver injury in rats.

In this work, administration of RIF caused significant decrease in biological evaluation compared to the negative control group. This effect of RIF explained via its negative impact engendered through oxidative stress [35, 37]. While the non-significant changes in biological evaluation were observed in the groups administrated NMLE (250 and 500 mg/kg) + RIF compared with Cont group, which reflects the inhibitory effect of NMLE against RIF. The same findings were reported by Nwobodo et al. [38] who found that there was a significant increase of body weight in the group pretreated with *A. indica* and paracetamol compared with once treated only with paracetamol, thus could explained via the inhibitory effect of *A. indica* on the oxidative stress induced by paracetamol.

Liver enzymes transaminases and alkaline phosphatase activities show the liver state [39]. RIF group revealed significant increases in the liver enzyme activities comparing with negative control. This is considered a sign of hepatic intoxication and damage [40, 40]. This could be explained via the hepatocellular damaged by RIF induced releasing of enzymes located in the cytosol into the blood stream [42]. In addition, RIF produced cytochrome P450 enzyme lead to elevated production of toxic metabolites from acetyl hydrazine [43]. Hepatoprotective activity of NMLE (250 and 500 mg/kg)+ RIF have been proved via the enhanced liver enzyme activities compared to the RIF group. This results are in agreement with Kausik et al. [36] and Adeshina et al. [44]. Thus, it could be explained by inhibition peroxidation effect by NMLE, which maintains liver's cell membrane integrity [17, 45].

RIF significantly induced oxidative stress and inflammation via significant increase the levels of MDA, as well as IL-1β and TNF-α, with significant deplete in the antioxidant enzymes GSH and SOD activities compared to Cont rats. The observed results agree with Yuhas et al. [46] and Mlambo and Sigola [47] who reported that RIF

increases the cytokines production IL-1β and TNF-α by increasing iNOS mRNA transcription and iNOS protein expression. Anti-TB drugs induced hepatotoxicity via oxidative stress through stimulation of lipid peroxidation as a source of cells membrane destruction and damage, and diminution of cellular defense antioxidant mechanisms which metabolizes toxic compounds to non-toxic compounds [42, 48-50].

Pretreatment with NMLE has anti-inflammatory effect and restored the antioxidant status in a dose dependent manner. This could be due to the active components of NMLE such as flavonoids, nimbin, 6-desacetylnimbinene, quercetin, nimbandiol, 17-hydroxy azadiradione, ascorbic acid, nimbolide, n-hexacosanol, 7-sdesacetyl-7- benzoylazadiradione,7-sdesacetyl-7-benzoylgedunin, and nimbiol [51, 52], that have anti-inflammatory, immunomodulating and antioxidant properties, thereby preventing the oxidative stress and inflammation imposed by RIF, which are the most important mechanisms in hepatotoxicity of this drug [14, 53, 54].

The RIF induced focal hepatic necrosis, inflammatory cell infiltration and apoptosis of hepatocytes. RIF induced liver necrosis [55], thus established in elevated levels of AST and ALT, which used to assess hepatocellular damage and leading to liver cell necrosis [56]. Pretreatment with NMLE almost normalize liver histological architecture. This finding is found in several studies [57-59], thus could be attributed to the phytochemical constituents in NMLE which have hepatoprotective activity [14].

It could be concluded that NMLE attenuates the hepatic damage induced by RIF, it prevents all biological and biochemical parameters, as well as histopathological alterations via antioxidant and anti-inflammatory pathways.

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