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Goji Berry (*Lycium barbarum L.*) Extract Alleviate Acute Hepatotoxicity Induced by Methotrexate in Rats

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ABSTRACT

Methotrexate (Metho) is widely used in the treatment of autoimmune and malignancy diseases. Goji berry (G. berries), Lycium barbarum L., fruit extract has immune-modulating, antioxidant and antitumor effects. This study assessed whether G. berries fruits' extract alleviated the hepatotoxicity induced by Metho in rats. Forty rats were randomly segregated into four groups (G); GI (Cont), GII (Metho); rats on the 21th day injected interperitoneal (ip) with Metho (20 mg/kg), GIII (G. berries 200 mg/kg +Metho) and GIV (G. berries 400 mg/kg + Metho). Rats in GIII and GIV given G. berries 200 and 400 mg/kg orally once a day and on the 21th day rats were injected with Metho i.p as in GII. Blood and hepatic tissues samples were collected after 5 days of Metho injection. Serum liver enzyme activities, interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were measured. Liver oxidative stress indices (superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and nitric oxide (NO)) levels were measured in the homogenate liver tissues. Liver sections were examined under microscope to detect the changes in liver tissues. The results showed that there were significant (p< 0.001) increases in serum liver enzyme activities and anti-inflammatory cytokine (IL-1β and TNF-α) levels, as well as hepatic oxidative stress, as marked by significant (p< 0.001) increases in hepatic MDA and NO levels, with significant (p< 0.001) decreases in SOD and CAT activities in Metho group compared with Cont group. Examined hepatic sections showed hydropic degeneration of hepatocytes, congestion of hepatoportal blood vessels, portal infiltration with mononuclear cells. Administration of G. berries extract (200 mg/kg) and (400 mg/kg) revealed significant (p< 0.001) reduction in serum liver enzyme activities and anti-inflammatory cytokines levels, and elevating the hepatic antioxidant status compared with Metho injected rats, as well as overcoming the tissue changes, the majority of the cells tend to be normal especially in the pretreated group with G. berries 400 mg/kg + Metho. In conclusion, the results revealed that G. berries has acute hepatoprotective effect; this could be explained by its ability to restore the antioxidant status and suppress inflammatory responses.

Keywords: *Goji berry fruits extract- Methotrexate- Hepatotoxicity-Rats- Antioxidant- Anti- inflammatory*.

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INTRODUCTION

The liver is a vital organ that functions principally for the maintenance of the body's metabolic homeostasis [1]. Liver disorders have far-reaching consequences since it is the first organ to encounter a wide variety of toxic, metabolic, neoplastic and microbial harms. Acute and chronic exposure to toxicants detrimentally alter the major functions of the liver [2]. It is the greatest common damage site in laboratory animals administered with chemicals and drugs [3]. Although chemicals are delivered to the liver for metabolism and excretion, this can frequently lead to liver injury [4], when performing its functions of biotransformation of xenobiotic, endogenous compounds, including hormones carbohydrate metabolism and storage synthesis

of blood proteins (albumin and lipoproteins), urea formation, fat metabolism and bile formation [5].

About a quarter of cases of fulminant hepatic failure are thought to be associated with drug use [4]. More than 900 drugs were involved in liver injury [6], and this is a reason why a drug should be withdrawn from the market. Conventional or synthetic drugs used in liver disease treatment are inadequate and can sometimes have severe side effects [7]. This is the reason that many people in the world have turned to complementary and alternative medicines [8]. Including those in developed countries. Many traditional remedies use herbal medicines to treat liver diseases [9].

The Metho structural is similar to folic acid; it is one of the anti-metabolite class of chemotherapeutic agents used in cancer treatment [10]. It is used in a wide range as an anti-rheumatic agent as well as inflammatory diseases [11]. The main reason for discontinuing Metho therapy is toxicity [12]. Hepatotoxicity is one of the adverse effects of Metho therapy [13]. The Metho related hepatotoxicity appears to be a result of the interaction of many issues; length of treatment, dosing schedule, type of disease, presence of genetic and molecular apoptotic factors and patients risk factors [14].

The use of plants as traditional medicine and pharmacopoeia drugs has been in existence from past time [15]. Majority of the global population depend on plants due to their medicinal benefits [16]. Currently, the use of medicinal plants is increasing depending on its affordability, availability, promising efficacy comparable to the often-high cost, and inaccessibility, as well as adverse effects associated with standard synthetic drugs [17].

This creates needed for supplementation of natural antioxidants within target food, which offers antioxidant properties. Berry fruits like strawberry, blackberry, cranberry, and goji berry are recognized for their diverse phenolic compounds, which offer high antioxidative properties as well as various other health benefits. Goji berry (*Lycium barbarum*) is one of the well-reported berries rich in diverse groups of phytochemicals [18-20]. The present study was undertaken to evaluate the effect of G. berries on hepatotoxicity induced by Metho drug.

MATERIAL AND METHODS

Goji berries (G. berries) Lycium *barbarum* L. fruits were purchased from Abazeer organic store, Jeddah, KSA. Metho (25 mg/1ml) was purchased from local pharmacy, KSA. Other chemicals with high analytical quality were purchased from Sigma.

G. berries fruits extraction

The dried G. berry fruits was powdered in an electrical blender. 100 g powder was soaked in 1 L hydroalcoholic mixture (ethanol 700 ml and water 300 ml) by cold maceration at room temperature for 48 h and the filtrate was collected. The collected extract was evaporated under reduced pressure at <35 °C, then lyophilized using a Gamma 2-20 freeze dryer, Germany for 48 h, at -20 °C [21]. The G. berries extract was stored at 4 °C until further use. The percentage of extract was 7.45% /100 g dried powder G. berries.

Animals protocol

Forty male rats (200 -220 g) were taken from animal unit of KFMSC, KAU, KSA. Rats after acclimatization period of 1 wk., under the standard lab conditions, were randomly segregated to four groups, ten each. GI (Cont); rats orally were given saline till end of the experiment (26 days). GII (Metho) rats orally were given saline, then rats on the 21th day injected ip. with a single dose of Metho (20 mg/kg) [22]. GIII (G. berries 200 mg/kg +Metho) rats were given G. berries (200 mg/kg) orally once a day [23], and on the 21th day injected with Metho ip. GIV (G. berries 400 mg/kg+ Metho) rats were given G. berries (400 mg/kg) orally once a day, and on the 21th day rats were injected with Metho i.p as in GII. On the 26th day, after 5 days of Metho ip. injection, blood samples were collected, serum was separated then stored at -80 °C till biochemical analysis. Liver samples were collected and prepared for biochemical and histopathological studies.

Biochemical hepatotoxicity indices

Serum liver function activities of alanine and aspartate aminotransferases (AST and ALT), and alkaline phosphatase (ALP) were assessed as described in protocol provided in ELSA kits obtained from MyBioSource, USA.

Serum pro-inflammatory cytokines

Serum interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were measured using ELISA kits from Abbexa, Cambridge, UK.

Liver oxidative stress indices

Enzymatic antioxidant superoxide dismutase (SOD), catalase (CAT) activities and non-enzymatic antioxidant malondialdehyde (MDA) and nitric oxide (NO) levels were measured in liver tissues homogenate as in ELISA kits' instructions. The used kits obtained from MyBioSource, USA.

Liver histopathological studies

Liver sections were stained after prepared with hematoxylin and eosin, then examined under a microscope to detect the changes in liver tissues.

Statistic

Results analysis by ANOVA one-way analysis of variance, and values are means ± SEM for 10 rats/ group. $p< 0.05$ considered significant.

RESULTS

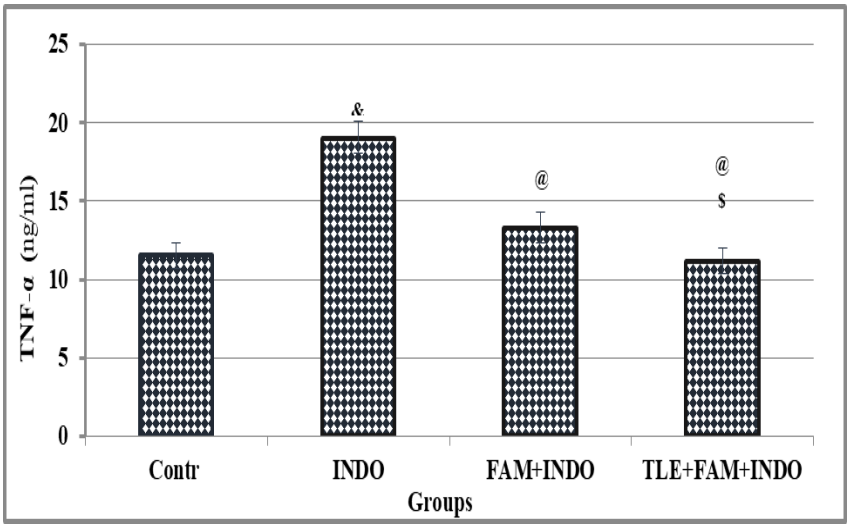
The results showed that there was significant ($p< 0.001$) increase in liver enzyme activities in Metho group compared with Cont group. Administration of G. berries extract (200 mg/kg) and (400 mg/kg) induced significant ($p< 0.001$) decrease in ALT, AST and ALP enzyme activities compared with Metho injected rats. It was interesting to find a significant ($p< 0.05$) change between G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho, thus indicating that the improved was dose dependent Table 1.

Table 1: Effect of G. berries extract on liver enzymes activity (ALT, AST and ALP) in hepattooxic rats induced by Metho

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Cont	41.66 ± 2.55	131.71 ± 7.96	111.97±5.58
Metho	91.53 ± 4.46 [a]	222.67 ± 10.53 [a]	197.16 ± 8.06 [a]
G. berries (200 mg/kg) **+Metho**	58.79 ± 3.71 [b]	170.26 ± 5.95 [b]	141.14 ± 2.72 [b]
G. berries (400 mg/kg) **+Metho**	48.39± 3.49 [b,c]	147.15 ± 6.76 [b,c]	124.65 ± 4.81 [b,c]

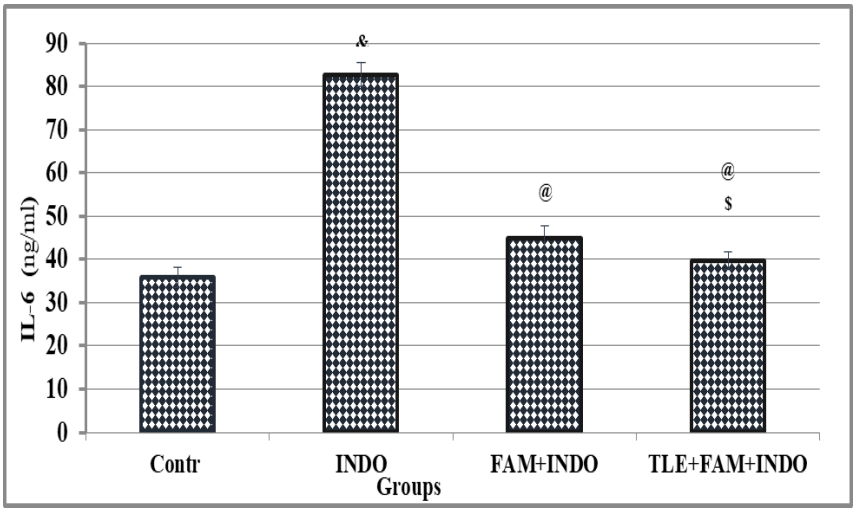
Values are mean ± SME (10 rats/group). [a] significant versus Cont group, [b] significant versus Metho group, [c] significant versus G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho.

Serum anti-inflammatory IL-1β and TNF-α levels in heapatoxic rats induced by Metho are shown in Figure (1) and Figure (2). There were significant ($p< 0.001$) increases in IL-1β and TNF-α levels in Metho group compared with Cont group. Administration of G. berries extract (200 mg/kg) and (400 mg/kg) induced significant ($p< 0.001$) decreases in IL-1β and TNF-α levels compared with Metho group. Significant ($p< 0.05$) difference was observed between G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho, thus indicating that the improved was dose dependent.



Values are mean ± SME (10 rats/group). [&] significant versus Cont group, [@] significant versus Metho group, [$] significant versus *G. berries* (200 mg/kg) +Metho and *G. berries* (400 mg/kg) +Metho.

Figure 1: Effect of *G. berries* on serum TNF-α level in hepatotoxic rats induced by Metho



Values are mean ± SME (10 rats/group). & significant versus Cont group, @ significant versus Metho group, $ significant versus *G. berries* (200 mg/kg) +Metho and *G. berries* (400 mg/kg) +Metho.

Figure 2: Effect of G. berries on serum IL-1β level in hepatotoxic rats induced by Metho

Hepatic oxidative stress biomarkers are shown in Table 2 and Table 3. Injection of Metho induced hepatic oxidative stress as indicated by significant (p< 0.001) decreases in SOD and CAT activities, with significant (p<0.001) increases in MDA and NO levels compared with Cont group. Administration of G. berries extract (200 mg/kg) and (400 mg/kg) induced significant (p< 0.001) improvement in antioxidant status in the liver as evidenced by significant (p< 0.001) increases in SOD and CAT activities, with significant (p< 0.001) decreases in MDA and NO levels compared with Metho group. Even though the high dose of G. berries (400 mg/kg) was more effective than low dose (200 mg/kg), there were no significant difference between G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho groups in all tested liver enzymatic and non-enzymatic antioxidants levels.

Table 2: Effect of G. berries extract on liver enzymatic antioxidant (SOD and CAT) activities in hepatotoxic rats induced by Metho

Groups	SOD (μ mol/g tissue)	CAT (μ mol/g tissue)
Cont	27.92 ± 2.87	40.27 ± 1.79
Metho	14.03 ± 0.98[a]	15.98 ± 1.57[a]
G. berries (200 mg/kg) **+Metho**	23.01 ± 1.75 [b]	35.01 ± 2.34 [b]
G. berries (400 mg/kg) **+Metho**	26.82 ± 1.74 [b]	39.98 ± 1.29 [b]

Values are mean ± SME (10 rats/group). [a] significant versus Cont group, b significant versus Metho group, C significant versus G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho.

Table 3: Effect of *G. berries* extract on liver non-enzymatic antioxidant (MDA and NO) levels in hepatotoxic rats induced by Metho

Groups	MDA (μ mol/g tissue)	NO (μ mol/g tissue)
Cont	34.27 ± 2.36	11.93 ± 0.82
Metho	67.98 ± 3.33 [a]	27.49 ± 1.17 [a]
G. berries (200 mg/kg) **+Metho**	36. 36 ± 1.68 [b]	14.25 ±1.08 [b]
G. berries (400 mg/kg) **+Metho**	35.07 ±1.99 [b]	12.17 ± 1.56 [b]

Values are mean ± SME (10 rats/group). [a] significant versus Cont group, b significant versus Metho group, C significant versus G. berries (200 mg/kg) +Metho and G. berries (400 mg/kg) +Metho.

Histopathological results

Liver section of Cont group showed normal structure of hepatic tissues (Figure 3.A). Hepatic section of Metho injected group showing hydropic degeneration of hepatocytes, congestion of hepatoportal blood vessels, portal infiltration with mononuclear cells (Figure 3.B and Figure

3.C). The section of G. berries (200 mg/kg) +Metho group showing slight thickening in the wall of hepatoportal blood vessels, while other section showing normal structure of hepatic tissues (Figure 3.D and Figure 3.E). While the hepatic section of G. berries (400 mg/kg) +Metho group showed normal hepatic structure (Figure 3.F).

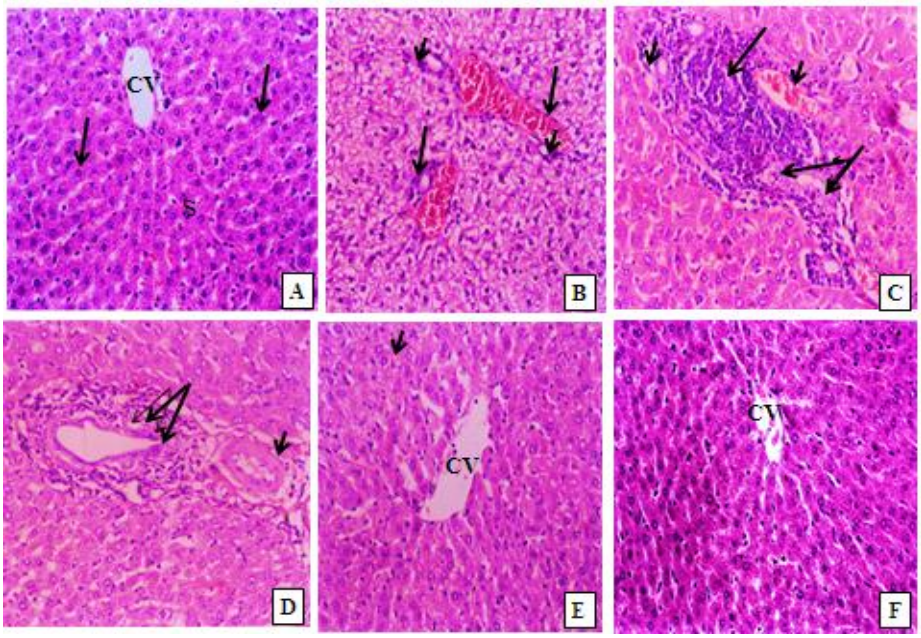


Figure 3: Effect of G. berries extract on hepatic tissue histopathological changes against Metho-induced acute hepatic damage in rats (H&E staining X 200). Photo [A] hepatic section of Cont group showed normal appearance of central vein with radiating cords of hepatocytes, which separated by blood sinusoids (BS), and the hepatocytes are polygonal acidophilic with vesicular nucleus (arrows). Photo [B] hepatic section of Metho injected group showing hydropic degeneration of hepatocytes as well as congestion of hepatoportal blood vessels (arrows). Photo [C] hepatic section of Metho injected group showing portal infiltration with mononuclear cells as well as appearance of newly formed bile ductules (arrows). Photo [D] hepatic section of G. berries (200 mg/kg) +Metho group showing slight thickening in the wall of hepatoportal blood vessels with appearance of newly formed bile ductules (arrows), while other section in the same group showing normal structure of tissues Photo [E]. Photo [F] hepatic section of G. berries (400 mg/kg) +Metho group showing nearly normal hepatic structure.

DISCUSSION

The liver is a vital organ that functions principally for the maintenance of the body's metabolic homeostasis [24]. Liver disorders have far-reaching consequences since it is the first organ to encounter a wide variety of toxic, metabolic, neoplastic and microbial insults [25]. Acute and chronic exposure to toxicants detrimentally alter the major functions of the liver [26]. Since it is the major drug metabolizing and detoxifying organ in the body, it is hence subject to potential damage from the enormous array of pharmaceutical and environmental chemicals, which may lead to life threatening conditions [27].

Medicinal plants have provided numerous plant-derived therapeutic agents for the treatment of ailment since ancient times [28]. This study investigated the hepatoprotective effect of G. berries extract against Metho induced hepatotoxicity by estimating the liver enzymes, MDA, NO, SOD, CAT, IL-1β and TNF-α. The liver histology of the male rats were examined.

The degree of liver injury is widely evaluated by measuring its markers including serum AST and ALT, ALT being a more specific enzyme for liver dysfunction [29]. Increased levels of these serum liver markers were observed upon treatment with Metho. In the present, study the results of biochemical analysis correlates with the histopathological report suggesting liver injury. Treatment with Metho caused ballooning degeneration and vacuolation of hepatocytes around the central veins suggesting its potency in inducing hepatotoxicity in experimental animals compared with Cont group. Previous stud-

ies have documented similar histopathological changes following treatment with Metho suggesting its potency in inducing hepatotoxicity in experimental animals [30, 31]. The liver markers were observed upon treatment with Metho. that may be due to the direct effect of it especially its polyglutamated forms, which inhibit DNA synthesis in all cells including normal liver cells and has been proposed to trigger hepatic cells damage and degeneration [32].

However, decreased liver enzymes level and the improvement in liver tissue were observed in rats pre-treated with G. berries when compared with the Metho treated rats. This shows evidence of alleviation of the injury caused by the hepatotoxicant Metho. Previous studies have shown similar hepatoprotection of the G. berries following injuries caused by drug and chemical. Extract of G. berries is rich in phytochemicals such as saponins and flavonoids, which are well-known plant principles responsible for the hepatoprotection [33, 34]. The tissue preservation observed in the present study may be due to a single or combined effect of the phytochemicals present in the plant material.

In the present research the treatment with Metho induced oxidative stress and inflammation *via* significant increase in serum IL-1β and TNF-α as well as hepatic content of MDA and NO with significant depleted in the content of liver tissues antioxidant enzymes CAT and SOD. These results were in the same line with Khafaga and El-Sayed [35] who reported that Metho increases the cytokines production IL-1β and TNF-α by increasing iNOS mRNA transcription and iNOS protein expression. The Metho drug 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 [36].

The present study reported that in the case of pretreatment with G. berries, there is improvement in the measurements of oxidative stress and increased antioxidant enzymes level have been detected. The study's results support previous studies that demonstrated the G. berries ability in reducing and protecting oxidative stress caused from various medicines [37].

In addition, earlier researches reported that the hepatoprotective effect of G. berries against Metho induced oxidative stress, may be due to the presence of groups of phytochemicals in *G. berries* [19]. The study results displayed that G. berries significantly reduced serum levels of IL-1β and TNF-α. Previous studies have recognized the anti-inflammatory effect of G. berries to its high contents of polyphenols, flavonoids and antioxidants [37, 38].

In conclusion, the current study results confirm that G. berries has been effective in preventing hepatotoxicity induced by Metho drug, which are proved by biochemical analysis and histological examination of the liver tissues. As a possible mechanism G. berries consists of assorted phenolic compounds, which offer high antioxidative properties, which could possess antioxidant activity, inhibit lipid peroxidation, scavenge oxidative free radicals and then improve acute hepatotoxicity. More researches are needed to separate the active constituents that play an important role in hepatoprotection as well as to develop new medicines from G. berries to treat hepatotoxicity initiated by chemical /drug.

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