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Studying the Protective Effect of Gundelia L. Against Induced Liver Diseases in Male Rats

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A B S T R A C T

The plant (*Gundelia tournefortii*) L (GL) was found in the temperate regions of Western Asia. Historically, it has been employed for its therapeutic attributes and biological efficacy in addressing diverse ailments. This study aimed to explore the protective role of GL. against the harmful effects of ethanol. Twenty-five male rats were split into five groups: the control group, the ethanol group, and other groups that received GL. extracts containing flavonoids, alkaloids, and oils. The results demonstrated that treating the rats with ethanol elevated the activity of liver enzymes such as alkaline phosphatase, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, the concentrations of triglycerides, total cholesterol, low-density lipoprotein, very low-density lipoprotein, malondialdehyde, and reducing the concentrations of high-density lipoprotein, total protein, albumin, glutathione, antioxidant enzymes activity like catalase, glutathione peroxidase, superoxide dismutase. Conversely, GL extracts reduced the toxicity of ethanol by decreasing the activity of liver enzymes, the concentration of triglycerides, total cholesterol, low-density lipoprotein, very low-density lipoprotein, and malondialdehyde and elevating the concentrations of total protein, albumin, high-density lipoprotein, and antioxidant enzymes activity in the groups treated with these extracts. These results showed the hepatoprotective effects of GL extracts against ethanol which attributed to their antioxidant capacity.

1. Introduction

Gundelia tournefortii L. commonly known as GL, is a medicinal plant indigenous to the Asian-temperate regions of Western Asia, encompassing nations like Egypt, Cyprus, Jordan, Palestine, Iraq, Azerbaijan, Turkey, and Turkmenistan. Different parts of the plant, including flowers, seeds, leaves, and stems, have been historically consumed as food. Particularly valued in the Middle East are the tender, immature flower buds, which bear a resemblance to artichoke hearts and are traded in local markets (Bagci *et al.,* 2010). In addition to being used as food, this plant is well-known as a medicinal plant (Çoruh *et al.,* 2007). It is used to treat bronchitis, diarrhea, vitiligo, diabetes, heart attacks, chest pain, and stomach pain, hyperlipidemia, inflammation, cancer (Ebrahimi and Sani, 2015). It also has nonparasitic, antiseptic and hepatoprotective properties. These pharmacological effects may be due to antioxidant compounds found in Gundelia tournefortii (Kendir, 2023). Liver disorder is one of the most dangerous health problems in the world, many people die because of the complications of hepatocellular carcinoma, cirrhosis and hepatitis. Drug-induced liver damage is also becoming

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more prevalent as a leading cause of acute hepatitis. Although liver transplantation is common, it meets only a fraction of the world's transplantation needs. Despite these concerning statistics, there is a promising opportunity to improve public health, as most liver disease causes are preventable (Asrani *et al*., 2019).

2. Previous studies

Many studies dealt with this plant and its medical properties as I have already mentioned in the introduction (Ebrahimi and Sani, 2015) (Kendir, 2023).

3. MATERIALS AND METHODS

3.1 *(Gundelia tournefortii) L.* Extraction: The plant was obtained from local markets in Mosul city in March 2023. After removing impurities, the plant's stems were allowed to dry in the shade for three days. Following drying, 500 grams were weighed and ground using a blender in preparation for the extraction process.

1. Isolation of Oils from GL: The method of Khanzadeh et al. was followed for isolating oils from GL. After the plant's crusts were removed, a grinder was used to mill them. Diethyl ether (1:10 ratio w/v) was quickly added to the grounded components, and they were combined for 12 hours in the dark. Using a Buchner funnel and filter paper, the resulting micelle was sorted (Whatman No. 41). A rotary vacuum evaporator, operating at 30ºC, was employed to remove the solvent (Khanzadeh *et al.,* 2012).
2. Isolation of Flavonoids from GL: Flavonoids were extracted by taking the plant after extracting the oil after it had dried from the effects of solvents. Then it was soaked in ethanol for a full day in the dark, then the ethanol was evaporated using a rotary evaporator (Harborne, 2013).
3. Isolation of Alkaloids from GL: The method by (Yubin *et al.,* 2014) was utilized to isolate alkaloids after extraction of oil and Flavonoids from the plant.

3.2 Experimental Animals:

Experiments were conducted using male albino rats weighing between 200 and 220 grams. The rats were accommodated in standard cages with unrestricted access to laboratory pellet diet and water. The ambient temperature was kept within the range of 25 to 30 °C, and a 12-hour light/dark cycle was maintained in the room.

3.3 Experimental Design:

The study aimed to investigate the preventive effects of G L. extracts against ethanol induced hepatotoxicity in rats. A total of twenty-five animals were used in the experiment. The rats were split into five groups, each consisting of five rats:

Group 1 (control group): Rats were fed a regular synthetic diet.

Group 2: Rats were given ethanol orally for six weeks with 40% v/v/kg/day b.w.

Groups 3 to 5: Rats were given ethanol orally for six weeks with 40%v/v kg/day b.w. then they were given GL extracts containing the dose (150 mg/kg b.w) orally for six weeks on a daily basis

3.4 Biochemical assay:

The lipid profile, consisting of triglycerides (TG) were measured according to (Corso *et al.*, 2016) , total cholesterol (TC) (Laufs *et al.,* 2020), high-density lipoprotein cholesterol (HDL-c) (Matsushima-Nagata *et al.,* 2021) and low-density lipoprotein cholesterol (LDL-c) (Martınez-Morillo *et al.,* 2021), very low-density lipoprotein cholesterol (VLDL-c) according to the equation (TG/5) . while the albumin was measured according to (Doumas *et al.,* 1971) total protein (Tietz, 1999) liver enzymes including Gamma-

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glutamyltransferase (GGT) (Reitman and Frankel, 1957) alanine transaminase (ALT), aspartate transaminase (AST) were measured using (Adeyemi *et al.,* 2015), alkaline phosphatase (ALP) (Szasz, 1974) , To assess antioxidant enzymes activity and oxidant parameters, the following methods were used: Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) activity were estimated following the protocol described by (Wheeler *et al*.1990). The levels of malondialdehyde (MDA) were estimated using the method by (Halliwell and Chirico, 1993). Glutathione (GSH) concentrations were estimated according to the method described by (Rahman et al. 2006).

3.5 Statistical analysis:

Statistical analysis of the results was carried out to determine the differences between the groups before, after treating with ethanol and after treating with GL extracts using the Duncan test for all variables and at the probability of ($P \leq 0.05$) using the statistical program SPSS, version 18 (Hinton, 2004) .

4. Results and discussion

4.1 lipid profile:

As shown in Table 1, the concentrations of Triglycerides, Cholesterol, LDL-c, and VLDL-c in the ethanol group were increased significantly than in the control group, while the HDL-c levels were decreased. These significant changes in lipoprotein levels have a direct relation with the functions of the liver. Ethanol harmed the liver by increasing triglycerides, LDL-c, and cholesterol while lowering HDL-c levels (Lu et *al.,* 2004). The findings presented in table 1 indicate that levels of Triglycerides, Cholesterol, LDL-c, and VLDL-c in the groups treated with GL extracts (alkaloids, flavonoids, and oil) exhibited a significant decrease compared to the ethanol group. Moreover, the concentrations of HDL-c in these groups were notably higher compared to the ethanol group. This suggests that GL extracts possess the capacity to lower lipid levels. Our findings align with Gholami-Ahangaran *et al*., 2021 who observed a reduction in lipids (cholesterol and triglyceride) levels in chickens due to the influence of the *Asteraceae* family (which includes GL) on lipid metabolism. This could be attributed to the potential role of these extracts in enhancing lipid elimination and impeding gastrointestinal absorption. Additionally, the impact of toxins on hepatocytes is mitigated by the digestive tract's capacity for detoxification (Markowiak *et al.,* 2019). Numerous epidemiological studies have shown that eating a lot of natural food rich in antioxidants increases the capacity of the body's antioxidants and reduces the chance to developing several diseases (Lu, 2004).There are several studies concerning the effects of artichokes on lipid metabolism. Rouzmehr *et al*. found that adding 200 grams of dried artichokes per ton to food can reduce blood cholesterol levels and abdominal fat (Rouzmehr *et al*. 2014). Similarly, Edwards *et al*. reported that incorporating 6% of dried artichoke leaves into a meal leads to a decrease in abdominal fat. Furthermore, the *Asteraceae* family appears to lower plasma cholesterol. The active compounds in these extracts raise bile secretion and lower cholesterol biosynthesis via 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Edwards *et al.* 2015).

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Table 1. Effect of ethanol and G L. extracts on the lipid profile levels in the rats

Biochemical Parameters	Control group (Mean ± SD)	Ethanol group (Mean ± SD)	group of ethanol + alkaloids (mean±SD)	group of ethanol + flavonoid (mean ± SD)	group of ethanol+ oil (mean± SD)
TG (mmol/l)	1.72±0.62 a	3.88±0.13 c	2.41±0.25 b	1.8±0.32 a	2.26±0.22 b
Cho (mmol/l)	3.66 ±1.14 a	6.36±1.22 c	4.82±1.11 b	3.85±1.24 a	4.65±0.98 b
HDL-c (mmol/l)	1.36±0.21 b	0.68±0.32 a	1.21±0.31 b	1.30±0.19 a	1.26±0.23 b
LDL-c (mmol/l)	2.22±0.86 a	5.84±1.12 c	3.88±0.78 b	2.65±0.46 a	3.65±0.64 b
VLDL-c (mmol/l)	0.34±0.22 a	0.856±0.11 c	0.54±0.23 b	0.38±0.11 a	0.48±0.32 b

*The different litters horizontally mean there is a significant difference.

4. 2 liver enzymes:

As indicated in Table 2, the activity of liver enzymes (ALT, AST, GGT, ALP) was significantly higher in the ethanol group than those in the control group and other groups treated with G L. extract compounds (alkaloids, flavonoids, oil). The increased activity of ALT, AST, GGT, and ALP is due to oxidative stress caused by ethanol (Osna *et al.,* 2017). This finding corroborates with Meharie *et al*. who noted increased liver function parameters due to toxicities (Meharie *et al.,* 2020). Conversely, the results also indicated that liver enzymes activity decreased in rats serum which treated with GL. extract compounds (flavonoids, alkaloids, and oil). Among the GL. extract compounds, the lowest liver enzyme activity was observed in male rats treated with flavonoids. These results were consistent with the hepatoprotective effect of natural plants against hepatotoxicity (Meharie *et al.,* 2020). The damage in the liver cell membrane causes the release of these enzymes into the blood stream (Gholami-Ahangaran *et al.,* 2021). The evaluation of liver enzyme levels might be supported by the hypothesis that the accumulation of lipids in hepatocytes can lead to hepatocyte destruction and an increase in hepatic enzyme levels in the plasma (Osna *et al.,* 2017). Therefore, a decrease in liver enzyme activity may indicate the absence of liver damage (Gholami-Ahangaran *et al.,* 2021).

Table 2. Effect of Ethanol and GL. extracts on enzymes in rats

Liver enzyme	Control group (Mean ± SD)	Ethanol group (Mean ± SD)	group of ethanol + alkaloids (mean ± SD)	group of ethanol + flavonoid (mean ± SD)	group of ethanol + oil (mean ± SD
ALT (U/l)	28.89±1.21 **a**	49.56±1.31**c**	38.49±1.71 **b**	33.19±1.11 **a**	36.35±1.21 **b**
AST (U/l)	28.64±1.26 a	59.29±1.39 c	43.79±1.59 **b**	34.72±1.72 **a**	39.69±1.49 **b**
GGT(U/l)	43±2.6 **a**	96±2.22 **c**	64±1.22 **b**	49±2.31 **a**	58±1.24 **b**
ALP (U/l)	36.26±1.81 a	43.67±1.76 **b**	41.11±1.52 **b**	38.27±1.62 **a**	40.87±1.42 **a**

- The different litters horizontally mean there is a significant difference.

4.4 Proteins:

The results presented in Table 3 indicate that the concentration of total protein and albumin were significantly lower in male rats having ethanol than the control group while there was slightly higher in globulin. The decreased levels of total protein and albumin are due to liver cell necrosis because of the oxidative stress caused by ethanol (Osna *et al.,* 2017) on the other hand the groups treated with GL. extract compounds (alkaloids, flavonoids, oil) had significantly higher total protein and albumin levels than the ethanol group. Furthermore, the results revealed that the highest concentrations of total protein and albumin were observed in male rats treated with flavonoids, compared

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to other GL. extract compounds (alkaloids, oil). These findings align with Gholami-Ahangaran's research, which suggests that the increase in total protein levels may be attributed to the effect of these extracts on the gastrointestinal tract's secretory function, leading to enhanced digesting food and absorption and consequently raising total protein levels in the plasma (Gholami-Ahangaran *et al.,* 2016).The variations observed in albumin levels after treatment with GL. extracts may be attributed to the antioxidant capacity of these extracts (Bati *et al.,* 2023) .

Table 3. Effect of ethanol and G L. extracts on the levels of proteins in rats

Proteins	Control group (Mean ± SD)	Ethanol group (Mean ± SD)	group of ethanol+ alkaloids (mean ± SD)	group of ethanol + flavonoid (mean ± SD)	group of ethanol + oil (mean ± SD
Total Protein (g/dL)	6.28±0. 42 b	5.43±0.62 a	6.01±0.38 b	6.18±0.28 b	5.88±0.43 b
Albumin (g/dL)	4.26±0.29 **b**	3.27±0.23 **a**	3.72±0.38 **b,a**	3.91±0.32 **b**	3.87±0.42 **b,a**
Globulin (g/dL)	2.02±0.32 a	2.16±0.21 a	2.29±0.32 a	2.27±0.22 a	2.01±0.4 a

*The different letters horizontally mean there is a significant difference at ($p \leq 0.05$)

4.5 Oxidative stress:

As depicted in table 4 the levels of MDA were significantly higher in the group of male rats treated with ethanol than control group. While there was a significant decrease in the activity of antioxidants enzymes (GSH, CAT, GPX, and SOD) in the ethanol group than in the control group. The decrease in antioxidant enzymes activity may be due to oxidative stress caused by ethanol (Osna *et al.,* 2017). While there was a significant reducing in MDA levels and a significant increase in antioxidant enzyme activity in groups treated with GL. extraction compounds (alkaloids, flavonoid oil). The lowest level of MDA was observed in the rat male treated with flavonoids compared to other GL. extracts (alkaloids, oil). The high levels of MDA in the ethanol group may be due to the harmful effect of ethanol on the liver (Osna *et al.,* 2017) .The decreasing levels of MDA after treating with GL. extracts are due to the content of antioxidants with a positive effect on lipid peroxidation (Meharie *et al.,*2020). The activity of antioxidants enzymes (GSH, CAT, GPX, and SOD) was lower in the ethanol group in comparison with the control group and other GL. extraction compounds (alkaloids, flavonoids, oil) treated groups. The highest levels of antioxidants enzymes were observed in the flavonoid treated group among the GL. extraction compounds treated groups. The rise in antioxidant enzyme activity observed in our study post-administration of GL extracts can be attributed to the ability of the active compounds in the *Asteraceae* family to scavenge free radicals (Gholami-Ahangaran *et al.,* 2016)..Total polyphenols and flavonoids in the examined extracts may be connected with antioxidant activities (Abu-Lafi *et al.,* 2018).

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Table 4. Effect of ethanol and GL. extracts on oxidants and antioxidants levels in male rats

Oxidants and antioxidants	Control group (Mean ± SD)	group of ethanol (Mean ± SD)	group of ethanol + alkaloids (mean ± SD)	group of ethanol + flavonoid (mean ± SD)	group of ethanol + oil (mean ± SD
MDA (μM/L)	1.96±0.08 a	3.92±0.12 b	2.65±0.09 a	2.21±0.1a	2.76±0.13 a
GSH (μM /L)	3.86±0.63 c	0.9±0.15 a	1.99±0.19 b	3.21±0.24 c	2.37±0.17b
CAT (kU/L)	10.2±1.04 c	6.07±1.03 a	7.15±0.02 b	9.17±0.05 c	7.64±0.03 b
GpX (U/mg protein)	6.02±0.37 b	3.35±0.38 a	4.5±0.27 a,b	5.95±0.40 b	5.25±0.21 b
SOD (U/mg protein)	108.73±4.24 c	40.19±2.12 a	82.52±3.7 b	96.67±6.3 c	86.49±4.2 b

*The different litters horizontally mean there is a significant difference

5. Conclusions

1. GL extracts demonstrate hepatoprotective effects against ethanol through the reduction of high liver enzyme levels.
2. The antioxidant capacity of GL extracts is demonstrated by an elevation of antioxidant enzymes activity and a decline in the levels of MDA, indicating reduced oxidative stress.
3. GL extracts exhibit lipid-lowering effects by decreasing triglyceride and cholesterol levels while increasing HDL-c levels.

6. Recommendations

Further research on the effect of GL extracts on other types of disease such as Arteriosclerosis.

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Conflict of interests

The author declares that there are no conflicts of interest.

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