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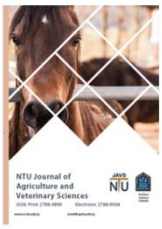

Protective effect of Taraxacum officinale extracts on the liver, heart and kidneys in rabbits exposed to ethylene glycol toxicity

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A B S T R A C T

Ethylene glycol (EG) it is an odorless, colorless, sweet-tasting liquid used for automobile anti-freeze, and is moderately toxic if ingested the leaves of the Taraxacum officinale plant are used as an antioxidant and immune booster. It is found in many countries, including Iraq. The animals were divided into 5 groups, each containing 6 rabbits: the control group (untreated), the group of rabbits treated with ethylene glycol at a dose of 1% ethylene glycol in drinking water for 60 days, in addition to that, groups from three to five) were treated with 1% ethylene. Glycol in drinking water with injections of extracted extracts from Taraxacum officinale leaves (flavonoids, glycosides and alkaloids) at a single daily dose of 50 mg/kg body weight for two months. After the end of the experiment, blood was drawn into tubes devoid of anticoagulants to conduct some tests. Administration of ethylene glycol led to a significant decrease in the levels of measured antioxidants compared to the control group, except for SOD, while the groups treated with extracts isolated from the plant showed a decrease in MDA and a significant increase ($P < 0.05$) in Gpx, CAT, and GSH after treatment with Taraxacum extracts. officinale, while SOD increased significantly ($P <0.05$) compared to the ethylene glycol group. The pathological histopathological changes in the ethylene glycol group include the deposition of oxalates in the lumen of the renal tubules and atrophy of the glomerular tuft, which leads to the expansion of the Bowman's space, necrosis of the epithelium of the renal tubules, congestion of blood cells and infiltration of inflammatory cells in the tissues of the kidneys, liver and heart, while treatment with extracts of Dandelion with ethylene glycol reduced necrosis in epithelial cells and decreased the number of inflammatory cells with less congestion in blood vessels in the tissues of the kidneys, liver and heart compared to the ethylene glycol group.

Introduction

Ethylene glycol (EG) is commonly known as antifreeze. It is a moderately toxic substance, however, an overdose taken orally may lead to death[1].The stages of toxicity occur over three time periods and occur during the first 27 hours of oral administration in humans. The first stage has been documented as central nervous system depression and gastrointestinal disorder, and occurs in the first half hour to 12 hours. As for the next step of taking ethylene glycol, it is accompanied by heavy breathing, rapid breathing, irregular heart beat, and cyanosis, and may be accompanied by heart failure and lung injury. The effect will be in the last stage on the kidneys after a full day of taking ethylene glycol. The last stage is accompanied by back pain and urination. This may be caused by renal tubular necrosis due to the deposition of calcium oxalate crystals. often, at this stage, the cardiopulmonary effects increase clearly, and therefore one of the symptoms of poisoning is also acidosis. The matter may get worse and reach a fourth stage after six days, leading to facial paralysis, deafness, cranial nerve impairment, and other things [2]. Negative impact on a number of systems and organs such as liver, spleen, and blood. This is what a number of studies have indicated when giving ethylene glycol orally in experiments conducted on laboratory animals [3].

Taraxacum officinale, also known as Alhondoba, is a plant belonging to the Asteraceae family. This plant grows in the regions of East Asia, European countries, America, India, and southern Africa[4]. Mesopotamia is also considered one of the countries where the dandelion plant grows, especially in the central and northern regions of our country, Iraq. Figure (1) shows a picture of the plant [5]. The Latin name Taraxacum is derived from the Greek word meaning "to cure diseases"[6].

Figure 1: *Taraxacum officinale*

Bioactive substances found in plants are important in alternative medicine research. Many treatments for many diseases were discovered after discovering the effective compounds in herbs and plants, especially with better knowledge of how to take them and use them to be preventive and therapeutic for diseases[7]. Taraxacum commonly known as Dandelion and its compounds are considered antioxidant and anti-inflammatory, especially the leaves and roots, as they contain a group of biologically active compounds, including vitamins C, D, A, E and choline, and mineral elements such as iron, silicon, magnesium, sodium, potassium, zinc, manganese, copper, and phosphorus, as well as phenolic compounds (flavonoids, glycosides, alkaloids, terpenes, and lactones. In addition to fiber, amino acids, and essential fatty acids make it a rich source of nutrients [8]. Taraxacum officinale, used as medicines against human diseases such as cancer and parasitic infections (malaria). The roots are also used as a medicine for joint pain (rheumatism), neuralgia, diarrhea, hemorrhagic fever, skin rashes, and worms. They are also eaten to increase the mother's milk supply before and after childbirth. The leaves are useful for treating cystitis, type 2 diabetes, and migraines. Cooked leaves are also used topically to treat ulcers [9].

Aim of study: It is to evaluate the preventive and therapeutic role of isolated dandelion plant extracts (flavonoids, glycosides, and alkaloids) on resistance to toxicity resulting from ethylene glycol on organ tissues (kidneys, liver, and heart).

Material and Methods

Plant materials: Taraxacum officinale was purchased from the local Mosul market. It was classified according to the books of classification of medicinal plants[10]. It was also documented in the laboratories of the College of Education, Biology Department, at the University of Mosul.

Preparation of extracts: The flavonoids, glycosides and alkaloids of the Taraxacum officinale plant were extracted according to the method described in [11].

Animal used: 30 male local rabbits weighing between 700-800 grams were used. Male rabbits were raised in cages with a special feeding system and given tap water, at a temperature of approximately (25℃) with a cycle (12 hours of light and 12 hours of night).

Experimental design and groups: The rabbits were distributed into five groups (control, EG, EG+ flavonoids, EG+ glycosides, EG+ alkaloids) each containing 6 animals. The first group was a natural control group and was maintained on a regular feed regimen and tap water. The second to fifth group was treated with 1% ethylene glycol in water until the sixtieth day [12]. The animals of group three to five were given treatment (extracts of flavonoids, glycosides, and alkaloids from the Taraxacum officinale plant at a dose of 50 mg/kg of body weight from day 30 to day 60, respectively). The extracts were administered
Two daily orally[13]

Experimental parameters: After the end of the experiment period, 5 ml of blood was drawn from the limbal vein of the ear pinna in the morning from all animals in one day, and the blood was placed in tubes devoid of anticoagulants for the purpose of conducting the required tests.

Determination of lipid peroxidation and antioxidant enzyme in serum. Serum malondialdehyde (MDA) level was estimated using the thiobarpuric acid method [14] and glutathione reductase was estimated using the method [15]. The activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were measured by methods [16], [17] and [18], respectively.

Tissue examined: After killing the rabbits using chloroform, they were placed in a glass jar. A piece of cotton was added to it and washed with chloroform. The jar was closed to make the rabbits breathe in the volatile chloroform so that they would die. After death, they were dissected and the organs (liver, kidneys, and heart) were removed for histological study. They were fixed in 10% formalin for 48–72 hours. They were then cut into appropriate sizes and washed with distilled water. The samples were dried by passing them through a series of ascending concentrations of ethyl alcohol (70, 90, 100) and filtered in xylol. They were embedded in paraffin, densely sectioned (5–6 μm) using a rotary microtome, and then stained with hematoxylin and eosin. The slides were examined under an optical microscope [18].

Statistical analysis

Values were stated as mean ± SD. statistical interpretation was done using one-way analysis of variance (ANOVA) followed by Duncan's test. The level of statistical significance was evaluated at $P < 0.05$. [19]

Results

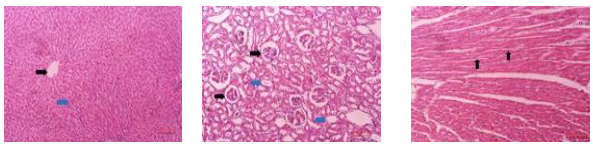
Giving oral ethylene glycol to the second group led to the death of a portion of the rabbits during the experiment. However, administration of Taraxacum officinale extracts (flavonoids, glycosides and alkaloids) simultaneously with ethylene protected the rabbits from the fatal toxic effect of EG, and therefore no deaths were recorded for the rabbits in the third, fourth and fifth groups. Table 1 shows that the mannolehyde concentrations indicated by TBARS are significantly higher in the serum of the EG group compared to the control. The results also indicated a significant increase in the level of GSH in the serum of rabbits. In addition, the effectiveness of SOD, CAT and GPx was clearly lower than that of the control group, while injection of therapeutic extracts separated from Taraxacum officinale led to a significant increase in the defensive effectiveness of the antioxidant compared to before treatment. The results are shown in (Table1) on the last page after References

Histopathological findings:

Microscopic examination of tissue sections taken from the control group showed: (A) the liver shows the normal histological structure of the central vein (black arrow) and surrounding liver cells (blue arrow). (B) kidney showing normal histological structure of glomeruli (black arrows) and surrounding tubules (blue arrows) in the cortical part of the kidney. (c) cardiac examination shows normal histological structure of cardiac muscle. The nucleus is elongated oval and the cytoplasm is homogeneous in normal cardiomyocytes (black arrows) (Figure2). the histopathological examination of samples taken from the liver, kidneys and heart of animals treated with ethylene glycol for 60 days (group EG) showed. Firstly, the liver's histological structure shows that the liver has lost its distinctive features as a result of congestion and dilatation of the central vein and significant infiltration of mononuclear inflammatory cells in the portal area. Vacuolar atrophy and coagulative necrosis of hepatocytes. microscopic examination of kidney tissue shows the occurrence of deposition of oxalate crystals in the lumen of the renal tubules to degeneration and necrosis of the epithelial cells lining the renal tubules, as well as tubular dilatation with cystic formation in the renal parenchyma. Further sections revealed infiltration of inflammatory cells in the interstitium, and vascular changes characterized by vascular congestion and inflammation were observed. Histopathological lesions of the glomeruli are characterized by dilatation of the Bowman's capsule space, which appears as a result of atrophy and shrinkage of the glomerular tuft. Microscopic examination of histological heart tissue showed myocardial necrosis of the acinar necrosis type (black arrow) as well as focal spread of inflammatory cells (blue arrow) (Figure 3). While the third group (EG + flavonoids), which was treated with ethylene glycol and flavonoid extract, showed an improvement in the pathological anatomy of the liver, as microscopic examination of the tissue showed the absence of congestion in the central vein and the presence of a small number of mononuclear inflammatory cells in the portal area. The same applies to renal tissue, as the examination showed limited dilatation of the renal tubules, the presence of very few oxalate deposits in the lumen of the renal tubules, the spread of a small number of inflammatory cells, and congestion of blood vessels, and the glomeruli appeared normal compared to the ethylene glycol group. As for the heart, microscopic examination showed a small focal spread of inflammatory cells with normal muscle fibers and without any necrosis (Figure 4). the fourth group (EG + glycoside), which was treated with ethylene glycol and glycoside extract, showed an improvement in the pathological anatomy of the liver, as microscopic examination of the tissue showed the absence of congestion in the central vein and the presence of an average number of mononuclear inflammatory cells in the portal area. As for the kidneys, the histological structure showed congestion of blood vessels in the interstitial tissues, as well as the spread of a very small number of inflammatory cells in the interstitial tissues, and there are still a small number of oxalate deposits in the lumen of the renal tubules. As for the heart, the

histological section shows normal muscle fibers with a small spread of inflammatory cells. Focally (Figure 5). while the results of the histological examination of the fifth group (EG + alkaloids) treated with ethylene glycol and alkaloid extract for 30 days showed an improvement in the histological structure of the liver, as it was noted that there was no congestion in the central vein and the presence of a small number of mononuclear inflammatory cells. The renal tissue also improved, as it was noted that there were small calcium oxalate deposits in the lumen of the renal tubules and the spread of a small number of inflammatory cells. It also led to an improvement in heart tissue, as a small focal proliferation of inflammatory cells was observed with normal muscle fibers and without any necrosis (Figure 6)

Histopathological Changes:



A B C

Figure 2: Microscopic images of liver, kidney and heart tissue (H and E stain ×200). For the control group: (A)the liver shows normal histological structure of the central vein (black arrow) and surrounding hepatocytes (blue arrow). (B) Kidney showing the normal histological structure of the glomeruli (black arrows) and surrounding tubules (blue arrows) in the cortical part of the kidney. (C) heart). Shows the histological structure of normal cardiac muscle. The nucleus is elongated oval and the cytoplasm is homogeneous in normal cardiomyocytes (black arrows).

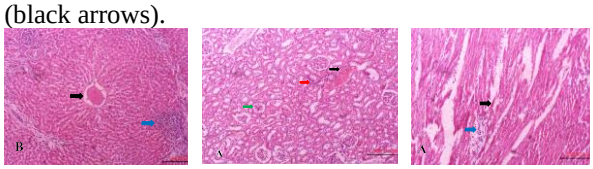


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Figure 3: Microscopic images of liver, kidney and heart tissue (H and E stain ×200). For the group treated with ethylene glycol (EG): (A) The liver histological structure shows congestion and dilatation of the central vein (black arrow) and significant infiltration of mononuclear inflammatory cells in the portal area (blue arrow). (B) Kidney, the histological structure shows congestion of blood vessels in the interstitial tissues (black arrow), as well as changes in the shape of the renal glomeruli (green arrow) and focal spread of inflammatory cells in the interstitial tissues (red arrow). (C) The heart's histological structure shows myocardial necrosis of the acinar necrosis type (black arrow) as well as focal spread of inflammatory cells (blue arrow).

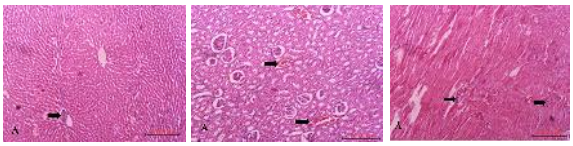

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Figure 4: Microscopic images of liver, kidney and heart tissue (H and E stain ×200). For the third group treated with (flavonoids + EG): (A) Liver. The histological structure shows the absence of congestion in the central vein and the presence of a small number of mononuclear inflammatory cells in the portal area (black arrow). (B) The kidneys, the histological structure shows congestion of blood vessels (black arrows). (C) The heart, the histological section shows normal muscle fibers with a small spread of inflammatory cells in a focal manner (black arrows).

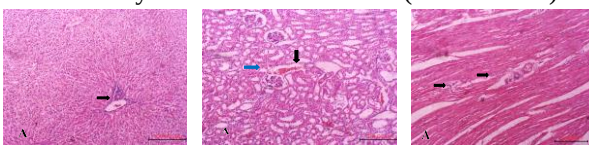

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Figure 5: Microscopic images of liver, kidney and heart tissue (H and E stain ×200). For the fourth group treated with (glucoside + EG) it appears: (A) The liver, the histological structure shows the absence of congestion in the central vein and the presence of a moderate number of mononuclear inflammatory cells in the portal area (black arrow). (B) The kidneys, the histological structure shows the congestion of the blood vessels. In the interstitial tissues (black arrow), there is also a very small spread of inflammatory cells in the interstitial tissues (blue arrow). (C) Heart histological section shows normal muscle fibers with a small proliferation of focal inflammatory cells (black arrows).

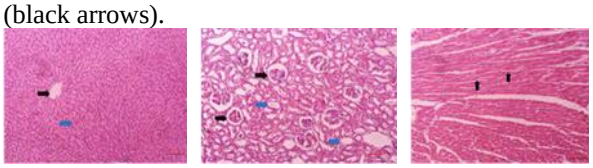

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Figure 6: Microscopic images of liver, kidney and heart tissue (H and E stain ×200). For the fifth group treated with (EG + alkaloid) it appears: (A) The liver, the histological structure shows the absence of congestion in the central vein (blue arrow) and the presence of a small number of mononuclear inflammatory cells (black arrow). (B) The kidneys, the histological structure shows the congestion of the vessels. Blood circulation (black arrows) and the presence of small deposits of oxalate inside the renal tubules (blue arrow). (C) Heart The histological section shows normal muscle fibers with a small spread of inflammatory cells in a focal manner (black arrows).

Discussion

The study was design to determine the effect of active compounds extracted from Taraxacum officinale (flavonoids, glycosides, and alkaloids) on preventing the toxicity of EG administered orally and its risk of life-threatening side effects. It is controversial that the information obtained from the results of the study succeeded in preventing the fatal effect of EG toxicity and protecting liver, heart and kidney tissues from the devastating effects resulting from EG intake. this is consistent with what some researchers have found when using Oil gum resin in mice suffering from ethylene glycol toxicity, due to its demonstrated protective effect on the tissues of kidney, liver and heart. [20]. The results of our current study were consistent with Younis et. al. [21] who reported that the level of SOD, CAT, GPX, and GSH was reduced. the level of MDA increased in rabbits treated with EG compared to the control group, and after treatment with extracts (flavonoids, glycosides, alkaloids), the activity of SOD, CAT, GPX, and GSH was restored, and MDA levels decreased in the treated groups compared to the EG group. Another study stated that taking EG leads to an increase in the production of reactive oxygen species (ROS), which play an important role in exacerbating the disease and increasing its toxic effect. Therefore, the materials used as a treatment and isolated from the Taraxacum officinale plant can reduce the production of reactive oxygen species (ROS), slowing or stopping the infection. Ingestion of ethylene glycol may also lead to the appearance of severe infections in animals, which leads to the production of additional quantities (ROS), especially H_2O_2, and ultimately increases the damage to cells. The reason for the increase in antioxidants and the apparent improvement of tissues after administration of the extracts may be attributed to the action of these extracts in reducing acute inflammatory conditions [22]. In another similar recent study, some Iranian researchers found the same truth in rabbits, where they concluded that giving an excessive dose of ethylene glycol contributed to the production of some toxic agents that have strong destructive effects on the nervous tissue, Kidney and liver. This harmful effect disappeared in rabbits treated with Extracts isolated from the seeds of the plant Prunus mahaleb L., which are rich in alkaloids, flavonoids, glycosides and other phenolic compounds. The toxic effect of EG on these tissues was reduced [23].as in our results. Eating ethylene glycol leads to the formation of crystals that are deposited in various organs of the body, such as the liver and kidneys [24], and it can also occur in the heart, as happened in the tissues of groups of rabbits that consumed ethylene only, as it showed a clear effect on those tissues under study. In rabbits, which received Taraxacum officinale extracts, there was no obvious histological change and this is consistent with the study conducted by Karawaya et.al.[25]. Due to the protective effect of Taraxacum officinale extracts by preserving liver, heart and kidney tissues [25]. The phenomenon of congestion, oozing, or bleeding did not occur in the groups treated simultaneously with Taraxacum officinale extracts. Compared with total ethylene glycol only, this reflects the protective effects of Taraxacum officinale extracts against EG-induced cell and tissue injury in rabbits. Which was confirmed by the histopathological results of the animals. While similar results were found by Ismail et.al.[26]. who found liver and kidney damage induced by ethylene glycol toxicity and the protective effect of Annona extracts against kidney damage. As indicated by researchers Hassan & Ahmed [27]. When studying the preventive role of the aqueous extract of the roots of the cichorium intybus l. in plant, a number of biochemical variables in the blood serum and tissues of rabbits infected with experimental kidney stones (ethylene glycol), where the use of the extracts led to improving the condition of kidney tissue and contributed to the removal of inflammation resulting from the formation of kidney stones by Ethylene glycol and associated bacterial infections led to an improvement in the shape of the glomerulus and urinary tubules and led to the disappearance of calcium oxalate crystals. This improvement indicates the great therapeutic role of the extracts isolated from the plant**.**

Conclusion:

The results of the current study showed that dandelion plant extracts (flavonoids, glycosides, and alkaloids) have an improving effect on pathological histological changes in the kidneys, liver, and heart, and biochemical changes induced by ethylene glycol**.**

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Table 1: Serum lipid peroxidation and Antioxidants enzyme of EG (glycol group) and treatment group as compared with control.

Parameters	Control	EG.	EG. with flavonoid	EG. with glycoside	EG. with alkaloids
MDA (µmol/L)	0.52±0.02b	0.79±0.02a	0.65±0.03ab	0.87±0.13a	0.45±0.02bc
GSH (µmol/L)	63±3.21a	37±2.1b	68±3.1a	69±3.3a	61±2.1a
CAT (U/ML)	0.18±0.01a	0.10±0.01b	0.112±0.02b	0.12±0.01ab	0.116±0.02ab
Gpx(U/ML)	6.02±0.21a	3.01±0.32c	5.45±0.42b	5.43±0.49b	5.98±0.49a
SOD	2.11±0.1a	1.33±0.12cd	1.87±0.12ab	1.41±0.13c	1.56±0.12b

Different letters horizontally indicate significant differences ($P < 0.05$) between treatments.