

Protective role of *Urtica dioica* on the pathological alteration induced by ethylene glycol in male rabbits

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ABSTRACT

Ethylene glycol (EG) is a colorless, odorless, sweet-tasting compound that is primarily used as antifreeze that is lethal when swallowed. *Urtica dioica* is used in the many countries as seasoning and as a herbal medicine (antioxidant). Purpose of the research was to determine the protecting function of *U. dioica* against the Ethylene glycol as toxicant in rabbits. Twenty-five rabbits were divided into five groups: (GI, control group) 5 untreated rabbits with ethylene glycol and (GII) 5 rabbits gave 0.75% ethylene glycol in drinking water for 30 days only, while (GIII, GIV and GV) 15 rabbits gave 0.75% ethylene glycol in drinking water and *U. dioica* oral supplement (flavonoids, glycosides and alkaloids) extracts (100mg / kg bw) Two times daily for 30 days. Blood samples (plain tube & EDTA tube) were collected for clinical examination. EG induced a significant reduction (20%) in rabbits BW in G II compared to GI, GIII, GIV and GV. As a result, AST, ALT, ALKP, TBIL, MDA, SOD, urea, creatinine, globulin and monocytes G II were markedly increased (P=0.05); meantime there was a markedly decrease (P=0.05) in whole Protein, albumin, ratio A / G, Gpx, CAT, GSH, BP, Hb, WBC and RBC. However, after treatment with *U. dioica* extracts, there was a significant reduction in AST, ALKP, TBIL, MDA, urea, creatinine and monocytes, while SOD, ALTno was significantly decreased. In the meantime, there has been a significant increase (P=0.05) in total protein, A / G ratio, Gpx, GSH, BP, Hb, WBC and RBC.

Keywords: *Urtica dioica*, Ethylene Glycol, Toxicity, Blood, Liver and Renal Antioxidants, Oxidative stress.

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INTRODUCTION

Urolith kidney stones, also known as renal calculi, are crystalline deposits formed in the urine from minerals in the kidneys for food. Urolithiasis is the term used to characterize the disorder under which urinary stones develop or are found in the urinary system. Calcium oxalate is the main portion of urinary stones in humans. The calcium stones excrete more calcium and oxalate compared to normal people. Kidney stones are more common in males than females with approximately 80 % of male kidney stones (1).

Kidney stone disease is a common disease among the population of industrialized countries and the affects rate is 10-12%. The highest prevalence occurs between 20 and 40 years, Whereas the highest disease prevalence in females later is (3). There are several ways to treat kidney stones and the recurrence rate of the disease is a concern and in most cases the recurrence rate may be more than 50% after 10 years.

Recently, many methods of treating kidney stones are available, including ultrasound lithotomy and Endurological approaches include ureteroscopy and percutaneous extraction. Such severe side effects are therefore correlated with these forms of treatment. Side effects include traumatic from shock waves, severe hematuria, infection and fragmented remaining stone fragments that may form future additional stones. In addition, side effects associated with the percutaneous and endurological methods extravasation of irrigating fluid and ureteral damage (5-10).

A lot of medications are used orally to treat kidney stone formation. Due to side effects and lack of tolerance by patients, its long-term use is however limited. As a result, alternative solutions have emerged including herbal remedies that have been used for hundreds of years to

treat urinary stone disease without obvious adverse side effects.

The effect of some herbal extracts on extracorporeal stones by Changes of ionic urine composition, such as calcium ions and magnesium ions. In addition, there are a variety of saponin-rich herbal extracts that release mucoprotein suspensions and facilitate crystallisation (11).

Material and Method

Plant material: *Urtica dioica* was purchased from the local market. It was classified according to plant classification references related to medicinal plants (10). Also, a voucher a plant specimen was identified and authenticated at the herbariums of the College of Education, University of Mosul.

Preparation of extracts: Preparation of flavonoids, glycosides and alkaloids extracts of *U. dioica* were done according to the method described by (11).

Animal grouping: Male locale rabbit's weightings between 750-850 gm were used; The animals were divided into five groups, each of which included three animals. Group I animals served as normal control and maintained on a regular laboratory diet, water. Group II to V animals were fed 0.75 % ethylene glycol (EG) in water to induce kidney stones by 30 days. (12). Group III to V animals were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the plant of *U. dioica* at a dose of 100 mg/kg body weight from 15th day to 30th day. The extracts were administered two daily by oral route.

Hematological Assays: EDTA blood samples were used to measure red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), haemoglobin concentration (Hb) and hematocrit percentage (Ht

Protective role of Urtica dioica on the pathological alteration induced by ethylene glycol in male rabbits

percent) using an automated haematology analysis system.

Liver and Kidney function tests: Serum was taken from each blood sample for spectrophotometric gamma-glutamyltransferase (GGT) (13), aspartate transaminase (AST) and alanine transaminase (ALT) (14), alkaline phosphatase (ALP) (15), total bilirubin (TBIL) (16), total protein & albumin (17). The globulin and albumin / globulin ratios, the urea, were calculated. (18) and creatinine (19).

Estimation Lipid peroxidation and antioxidant enzyme in serum.

Estimation of malondialdehyde (MDA) levels using the Thiobarbituric acid reaction method. Thiobarbituric acid (TBARS) in the serum was estimated by the method of (20) & estimating the GSH by the method of (21). Antioxidant enzyme activities (CAT, SOD and GPx), the activities were assayed by the methods of (22,23) and (24) respectively.

Findings

The toxicant EG in (G II) resulted in 20% rabbit (1/5) fatalities rate in rabbits over the study period. However, when extracts of nettles (glycosides, flavonoids and alkaloids) were co-administration with EG, rabbits were fully protected from the acute lethal effects of EG and no fatalities rate of rabbits was recorded in G III, IV and V. Rabbits subject to EG alone (GII) have shown markedly liver, kidney destruction as demonstrated by significant elevation (P=0.05) in serum activity of ALT, AST, ALK PH, TBIL, creatinine, urea, total protein and calculated globulin levels. In the meantime, there was a significant decrease (P=0.05) in albumin and A / G ratio compared to the control GI and the treated group (GIII GIV & GV)

Table1: Alteration in liver and kidney function tests of the ethylene glycol group and treated ethylene glycol and extracts group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
AST (U/L)	30.13±0.41	61.33±0.32*	35.95±1.57	27.95±1.37	28.25±1.47
ALT (U/L)	35.66±1.2	52.19±2.02*	43.22±1.76*	43.32±1.56*	49.62±1.16 *
GGT (U/L)	100±4.3	210±11.5*	154±3.9*	147±4.8*	109±5.6*
ALKP (U/L)	31.81±0.78	67.11±1.13*	35.73±1.91	45.73±1.19	39.13±1.00
TBIL (mg/dL)	87.23±1.51	195.61±2.45*	95.17±4.32	92.57±3.01	121.57±2.12
urea(mg/dl)	18.95±0.805	38.17±1.02*	21.61±0.119	31.55±2.325 *	30.01±1.14*
creatinine(mg/dl)	2.283±0.08	4.133±0.16*	2.13±0.13	4.33±0.20 *	2.113±0.29
Total Protein (g/dL)	10.72±0.03	12.46±0.11*	11.89±0.11	12.11±0.10	10.79±0.11
Albumin (g/dL)	6.35±0.03	5.79±0.55*	7.17±0.61	7.77±0.42	6.47±0.42
Globulin (g/dL)	3.57±0.05	4.44±0.23*	4.54±0.12*	4.44±0.12*	4.21±0.11*
A/G Ratio	1.78±0.04	1.31 ±0.23*	1.57±0.20	1.75±0.12	1.53±0.12*

Blood samples were taken after 30 days of oral administration, number of rabbits each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level

As shown in Table 2, there was a significant increase in the number of monocytes; while there was a reduction in: count of WBCs, RBCs, & platelets, HB percent, and hematocrit value in the blood of the EG group (GII)

nevertheless, treatment of these EG-received rats with crude extract of *Urtica dioica* (GIII, GIV, GV) significantly reversed these findings.

Table 2: Alteration in hematological tests of ethylene glycol group and treated ethylene glycol and extracts group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
Monocytes	0.20 ±0.11	0.49 ±0.17*	0.27±0.02*	0.31±0.12*	0.41±0.01*
White blood cells count (103/uL)	6.01±0.33	2.41±0.11*	5.65±0.20	4.76±0.2	5.79±0.2
Red blood cells count (103/uL)	6.42±1.2	3.34±0.56 *	5.67±1.1	5.11±1.1	6.29±1.3
Blood Platelets (103/uL)	155±15	75±8.3*	118±11	130±14	121±14
Hb concentration (g/dL)	14.1±1.1	5.5±1.11*	12.1±2.2	12.1±2.1	12.1±1.1

Protective role of *Urtica dioica* on the pathological alteration induced by ethylene glycol in male rabbits

Hematocrit value (%)	40.7±2.2	15.5±1.3 *	32.21±1.1	30.23±2.1	28.71±1.1
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Blood samples were taken after 30 days of oral administration in EDTA tube, number of rabbits each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level

Table 3 Demonstrates that the rates of lipid peroxidation indicated by TBARS were markedly higher in the EG serum group compared to normal subjects. Also, the

result showed a significantly elevate in GSH level in the serum of rabbits. Further, the activity of SOD, CAT and GPx were significantly lower than the normal subjects were treatment of EG group with *Urtica dioica* significantly elevated the antioxidant defense activity compared with that before treatment.

Table 3: Changes in serum lipid peroxidation and enzymatic antioxidants of ethylene glycol group and treated ethylene glycol and extracts group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
MDA (µmol/L)	0.42±0.02	0.89±0.02*	0.55±0.03	0.67±0.03*	0.48±0.02
GSH (µmol/L)	75±3.21	47±2.1*	78±3.2	61±3.9	65±2.3
CAT (U/ML)	0.17±0.01	0.110±0.01*	0.137±0.02*	0.129±0.02	0.146±0.02
GPx (U/ML)	5.82±0.21	3.11±0.32*	5.65±0.12	5.63±0.49	5.88±0.39
SOD	2.22±0.2	1.43±0.2*	1.97±0.12	2.00±0.13*	1.76±0.11*

Blood samples were taken after 30 days of oral administration, number of rabbits each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level

Discussion

We designed the study to investigate the influence of the Nettles extracts (flavonoids, glycosides and alkalis) on EG development on Dose intoxication and related life-threatening. Intriguingly, our data have shown that the concomitant utilization of the Nettles had a role without the acute lethal effect of EG toxicity and protected rabbits and kidneys from the damaging effects of toxic EG overdose.

Various biochemical parameters recorded a significant increase in serum rabbits of the second group of ethylene glycol pressurized on the normal group and the treatment of Nettles extracts, in this study, assigned to evaluate the toxicant effects of EG exposed on rabbits Livers, kidneys and possible protective roles of nettles. EG utilization resulted in a weight loss and several rabbits were killed.

The effect of Nettles antioxidants against induced hepatic chemical toxicity has been approved with a previous study (25). Abnormalities in EG liver function parameters have been identified in the current study and other studies have shown a steady rise in ALT, AST, GGT and protein concentrations. In line with the following results: Gunathilake, et al.2014 (26) Who found ginger a great role as an antioxidant and prevention of tissue toxicity. Among the results we reached in this study were hematological results, while there was a noticeable decrease in the number of white blood cells, red blood cells, and platelets in rabbit blood by increasing the overdose of ethylene glycol. This was also related with considerable reductions in overall hemoglobin and hematocrit, which were also associated with a rise in the number of monocytes. It was in agreement with Starek, et al. 2012. (27)

Nevertheless, the treatment of these rabbits with nettle extracts significantly reduces these hematotoxic effects of ethylene glycols as Samira demonstrated, 2013 (28). In accordance with the findings, a study of the same truth was recorded in mice and researchers found that a lot of

chemicals such as liberating ethylene glycol in an overdose of Some of the toxic components that have strong DNA destruction effects on the bone marrow DNA and thus lower all the components of blood as well as The reactive oxygen species (ROS) play a crucial role in the development of the disease (29).

Materials that can reduce ROS production, such as nettle, may slow down or stop it development of the disease. The same was also recorded in a recent Iranian study in mice, and researchers found that taking an ethylene glycol overdose resulted in the production of any of the toxic agents that have strong destruction effects on the kidneys. Interestingly, this harmful effect has disappeared in rats treated *Allium Jesdianum* (30).

In agreement with these results: Huang et al. 2002 (31); oxidative harmful as reflected by increased amounts of oxidative marker damage due to higher MDA and decreased activity of antioxidant enzymes such as SOD, GPx, CAT and GSH in serum and also deterioration of renal function as noticed in rabbits due to calculi. While treatment with nettle extracts due to the low level of MDA and increased the function of antioxidant enzymes and the level of GSH suggests that it is protected against oxidative stress and causes tissue damage.

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Protective role of *Urtica dioica* on the pathological alteration induced by ethylene glycol in male rabbits

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