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ASSESSING EFFECTS OF *EQUISETUM RAMOSISSIMUM* EXTRACT ON HEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN PREGNANT SPRAGUE-DAWLEY RATS

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Abstract

Background: As a medicinal herb, *Equisetum ramossisemum* has been utilized for centuries as a diuretic and has been recommended for different disorders. The aim of this study was to investigate the maternal toxicity of aerial parts of *Equisetum ramosissimum* extract on pregnant Sprague-Dawley rats.
Methods: Females were mated and the coupling time was recorded at gestation day 0– E0. Four experimental groups I, II, III, and IV, received daily gavage doses of 0 mg, 500 mg, 250 mg, and 125 mg/kg, respectively, of *Equisetum ramosissimum* extract. Pregnant rats were observed for mortality and toxicological effects during daily treatment. On day E20, samples of blood were withdrawn from the retro-orbital sinus under light ether anaesthesia for haematological and clinical chemistry examinations.
Results: Data analyses detected significant differences in biochemical and haematological parameters between the control group and other groups receiving extract.
Conclusion: This study constitutes a first approach to defining adverse effects of using *Equisetum ramosissimum* as a medicinal plant during pregnancy. Daily gavage doses of *Equisetum ramosissimum* extract produced significant differences in biochemical and haematological parameters in pregnant Sprague-Dawley rats as compared to the control group.

Key words: *Equisetum ramosissimum* extract; Pregnant Sprague-Dawley rats; Haematology; Serum

Introduction

There is no visible difference between medicinal plants and those having an adverse effect on animals (Al-Qura'n, 2005). A review of the literature shows that most medicinal plants can be toxic when used in small doses over a long period of time (Al-Qura'n, 2005). It is known that an accumulation of certain active components, even in small doses, can cause metabolic disturbances and toxic symptoms in animals (Al-Qura'n, 2005). Toxicological effects of medicinal plants can be assessed using different parameters, including clinical chemistry and haematology data (Petterino and Argentino-Storino, 2006).
Equisetum ramossisemum is one of the medicinal plants, which is collected, packed and marketed (Syouf and Duwayri, 1996). European workers have known the toxicological effects of different species of *Equisetum* for a long time (Rapp, 1954). Indeed, some countries have recorded *Equisetum ramossisemum* as a toxic plant, causing dizziness and cardiac disturbances (Al-Qura'n, 2005; Forero and Nader, 2011).

As a medicinal herb, *Equisetums* has been utilized for centuries as a diuretic and has been recommended for different disorders, including haemoptysis, hemorrhoids, varicose ulcers tuberculosis, and edema (Phillipson and Melville, 1960; Boulos et al., 2011; Langhammer and Nilsen, 2013). Nowadays, *Equisetums* plants are sold as weight loss supplement in South America (Boulos et al., 2011). However, it has been reported that *Equisetums* can cause fatalities among livestock (Phillipson and Melville, 1960).

Recent studies suggest that a thiaminase may contribute to these fatalities even though toxicity has been attributed to alkaloids (Phillipson and Melville, 1960). Thiaminase I and II enzymatic activities have been detected in *Equisetum ramossisemum* (Meyer, 1989). It is thought that the high thiaminase activity may cause neurodevelopmental toxicity through thiamine depletion (Meyer, 1989; García et al., 2011; Tokarnia et al., 2002). Research has shown that animals exposed to *Equisetums* develop a number of neurological disorders, including Polioencephalomalacia (Amat et al., 2013; Bebbington and Wright, 2007). *Hypovitaminosis in* livestock *has also been associated with* ingesting plants such as *Equisetums* which contain thiaminase (Finnie et al., 2011). In fact, thiaminase in *Equisetums* was shown to be the actual poison when horses rapidly recovered after injection of 50-100mg of thiamin per day (Pohl, 1955).

Many case studies revealed the potential toxicity of *Equisetums* in humans. For example, the ingestion of a commonly used supplement of *Equisetums* for weight loss caused the death of a healthy female after cardiopulmonary arrest (Boulos *et al*., 2011). The patient developed generalized symptoms of thiamine deficiency (Boulos et al., 2011). Another example of the potential toxicity of *Equisetums* in humans is that of a man who developed hepatotoxicity after drinking *Equisetums* juice for a period of time (Klnçalp et al., 2012). Still, other case studies correlate thiamine deficiency with the use of Equisetums supplements and neurodevelopmental disorders (García et al., 2011). For instance, a mother utilized *Equisetums* supplements a year before giving birth to a girl with autism spectrum disorder (García et al., 2011).

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The chemical composition of essential oils from *Equisetum ramossisemum* (In press) revealed that the oils contain a high content of monoterpenoids and sesquiterpenoids as compared to the oils of other *Equisetum*s (Saxena and Basit, 1982). Various toxicities have been ascribed to oils that contain terpenes (Gordon et al., 1982). Among these are reports of cytotoxic effects on different organs, including liver, lungs, and the central nervous system (Gordon et al., 1982).

Reports describing toxic effects of *Equisetums* in animals are prominent in the literature. However, there is very little information about the toxicity of *Equisetums* in humans (Tago et al., 2010). Most doses recorded for *Equisetums* intake are based on historical use or expert belief (Boulos et al., 2011). Thus, given the limited data concerning prescribed safe and effective doses of *Equisetums*, especially during pregnancy, we were motivated to study the toxic effect of aerial parts of *Equisetum ramossisemum* on female Sprague-Dawley rats.

Material and Methods

Plant preparation and extraction

Equisetum ramossisemum was obtained from a local market and authenticated by Dr. Dawud Al-Eisawi, Professor of Botany, Biology Department, and The University of Jordan. Aerial parts of *Equisetum ramossisemum* (500 g) were grounded and extracted with a total of 10 L of 95% ethanol at room temperature over 5 days. After that, the solution was filtered and concentrated to a semisolid form using a rotary evaporator.

Animal grouping, treatment and data collection

Male and female Sprague-Dawley rats were obtained from the animal house of the Faculty of Medicine, Jordan University of Science and Tecchnology, Irbid, Jordan. Rats were used after one week of quarantine and accommodation in an animal room with constant temperature and humidity, which operated under a12-h light/12-h dark cycle. Rats were fed with a standard rodent diet and tap water *ad libitum*, except for a short fasting period before oral administration of extract doses.

For mating, an adult virgin female rat, at least 11 weeks old and weighing approximately 187 g, was placed with an adult male in the same cage overnight. Observation of vaginal plug was considered as embryonic day 0 (E0). All animal procedures were conducted in accordance with Jordanian Regulations for Animal Experimentation and Care and were approved by the Institutional Animal Care and Use Committee.

Mated females were randomly distributed into four groups. Group I received 0mg as control. Groups II, III, and IV received doses of 500 mg, 250 mg, and 125 mg/kg, respectively.

The doses of *Equisetum ramossisemum* extract were administrated once a day in 2 mL of water solution through gavage. Treatment was from E0 to E19 for all groups. Animals were observed daily for mortality and toxicological effects. On day E20, Samples of blood for haematological and clinical chemistry examinations were withdrawn under light ether anaesthesia from the retro-orbital sinus.

Blood was aliquoted into two different test tubes to generate blood plasma (tube with anticoagulant) and blood serum (tube without anticoagulant). Hematological examinations included the following parameters: Red blood cells count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), red blood cells distribution width (RDW),white blood cells count (WBC), neutrophils (NP), lymphocytes (LC), and monocytes (MC). Serum biochemistry was performed to examine glucose (Glu), urea(U), creatinine (CRN),sodium(Na), potassium (K), calcium (Ca), Chloride(Cl), phosphorus (P), total bilirubin (T-Bil), total protein (TP), albumin (ALB), globulins (G), albumin/ globulins ratio (A/G), total cholesterol (T-Cho),triglycerides (TG), alkaline phosphatase (ALP) , aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT). Analyses for hematology and serum biochemistry were conducted at Shamla Medical Laboratories (Zarka, Jordan) using automatic analyzing machines, Erma PCE-210(N) and *ACE clinical chemistry system* for blood and serum analyses, respectively. Resulting data were fed into Statistical Package for the Social Science (SPSS 20) software and analyzed using descriptive analysis and One-Way Analysis of Variance (ANOVA). A p value <0.05 was considered statistically significant.

Results

Serum Biochemistry

To study the effect of *Equisetum ramosissimum* extract on serum biochemistry among pregnant Sprague-Dawley rats, we utilized One-Way ANOVA analyses. Results (Table 1) revealed that there are statistically significant differences in four serum factors TP, G, ALB, and GLU detected between Group I and Group II.

For TP, F (3, 32) = 8.643, p = 0.000, the difference was not only between Groups I (M = 68.11, SD= 4.53) and II (M = 53.57, SD= 5.69) but also between Groups I and III (M = 58.58, SD= 5.16). This relates to the results for G, F (3, 32) = 8.266, p= 0.000 where Group I (M = 32.55, SD= 2.55) differed significantly from each of Group II (M = 25.75, SD= 1.98) and Group III (M= 27.44, SD= 2.54).

For ALB, F (3, 32) = 6.379, p = 0.002, the difference was not only between Group I (M= 35.58, SD= 2.45) and Group II (M= 25.57, SD= 9.07) but also between Group II and IV (M= 35.54, SD=5.59).

Finally, ANOVA analyses disclosed a significant difference between Group I (M= 5.12, SD = 1.47) and the other groups, with a higher mean value for Group I in GLU, F (3, 32) = 8.854, p = 0.000 values.

Blood Hematology

One-Way ANOVA analyses were conducted to determine whether there were hematological differences among four groups of pregnant Sprague-Dawley rats receiving daily doses of *Equisetum ramosissimum* extract. Results (Table 2) indicate that

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there are statistically significant differences in three blood parameters: PLT, WBC, and MC. One-Way ANOVA analyses indicated that there is a significant difference in PLT values, F (3, 32) = 11.927, p= 0.000. Post-hoc analysis revealed that the difference was between Group I (M= 648.00, SD= 86.18) and Group II (M= 479.11, SD= 143.24) as well as between Group II and the other groups (III and IV) with lower mean values for Group II. Similarly, for WBC, F (3, 32) = 3.153, p = 0.038, the difference was between Group I (M= 6.51, SD= 1.48) and Group II (M= 4.14, SD= 1.24).

Table 1: Serum Biochemistry Results for Pregnant Sprague-Dawley Rats Given *Equisetum ramosissimum* Extract, (n=9)

Parameters	Units	GI(0mg/kg)	GII(500mg/kg)	GIII(250mg/kg)	GIV(125mg/kg)
Glu	mmol/l	5.12±1.47	2.83±0.80[a]	2.55±1.12[a]	3.26±1.16[a]
U	mmol/l	8.94±1.61	10.58±2.38	8.54±1.07	8.75±3.20
CRN	µmol/l	85.06±9.14	89.18±22.19	59.91±9.55	79.06±16.09
G	g/l	32.55±2.55	25.75±1.98[a]	27.44±2.54[a]	30.03±4.65
ALB	g/l	35.58±2.45	25.57±9.07[a]	31.14±2.68	35.54±5.59
A/G	ratio	1.08±0.06	1.10±0.05	1.13±0.05	1.17±0.04
TP	g/l	68.11±4.53	53.57±5.69[a]	58.58±5.16[a]	65.68±10.20
TG	mmol/l	0.82±0.27	2.23±2.36	2.40±2.65	1.34±0.92
T-Cho	mmol/l	1.5±0.21	1.31±0.19	1.36±0.48	1.24±0.47
T-Bil	µmol/l	5.05±.1.38	9.33±6.86	10.52±5.92	7.50±4.05
Na	mmol/l	136.0±2.34	134.66±3.77	143.88±11.16	138.22±2.48
K	mmol/l	3.90±0.30	3.96±0.38	4.21±0.36	4.81±3.10
Cl	mmol/l	108.55±2.06	108.66±4.06	112.22±8.10	109.22±2.86
Ca	mmol/l	2.45±0.18	2.35±0.17	2.26±0.20	2.41±0.20
P	mg/dl	6.17±0.37	6.84±0.97	6.12±0.88	6.70±2.01
AST	IU/L	99.11±12.89	114.66±17.14	129.77±44.06	74.88±25.54
ALT	IU/L	33.33±7.41	43.22±6.15	31.55±19.22	28.77±12.07
GGT	IU/L	10.33±1.22	14.11±5.01	9.0±5.50	11.0±2.00
ALP	IU/L	173.66±95.61	103.44±33.01	79.66±21.85	132.55±86.87

Note. Values are mean ± SD. [a]Significantly different from the control group (p<0.05).

With respect to MC, F (3, 32) = 33.910, p = 0.000, the difference was between Group I (M=1.22, SD= 0.44) and Group II (M= 7.00, SD= 2.23) as well as between Group II and other groups (III and IV) with higher mean values for Group II.

Table 2: Hematological Data for Pregnant Sprague-Dawley Rats Given *Equisetum ramosissimum* Extract, (n=9)

Parameters	Units	GI(0 mg/kg)	GII(500 mg/kg)	GIII(250 mg/kg)	GIV(125 mg/kg)
RBC	$x10^6/\mu l$	7.12±0.33	6.49±0.79	7.02±0.79	7.31±0.84
Hb	g/dl	14.27±0.65	12.76±2.20	14.24±1.54	14.47±1.39
Ht	%	41.58±1.61	37.12±5.82	41.24±4.85	42.77±4.68
MCV	fl	58.40±1.15	56.97±3.31	58.71±2.48	58.60±2.56
MCH	pg	20.02±0.46	19.56±1.61	20.30±0.66	19.86±0.60
MCHC	g/dl	34.32±0.43	34.31±0.99	34.56±0.86	33.90±1.14
PLT	$x10^3/\mu l$	648.0±86.18	479.11±143.24[a]	766.66±79.12	664.66±93.02
RDW	%	15.88±0.63	15.95±0.68	16.22±0.97	16.23±1.14
WBC	$x10^3/\mu l$	6.51±1.48	4.14±1.24[a]	4.78±1.70	6.17±2.78
NP	%	28.22±6.74	33.44±5.89	29.55±6.10	35.55±13.62
LC	%	70.44±7.31	59.33±7.64	68.77±6.61	62.66±13.27
MC	%	1.22±0.44	7.00±2.23[a]	1.77±0.97	1.77±1.30

Note. Values are mean ± SD. [a]Significantly different from the control group (p<0.05).

Discussion

A wide spread belief is that plants are naturally safe, not real drugs, and are beneficial for human health (Colombo et al., 2009). However, an increasing number of medicinal plant poisonings has been registered since the mid-90s (Colombo et al., 2009). We investigated one such plant, *Equisetum ramossisemum*, because of its wide spread use, especially among pregnant mammals (Langhammer and Nilsen, 2013). No studies, to our knowledge, have questioned the effects of *Equisetums* on a pregnant mammal. To this end, we have utilized *Equisetum ramossisemum* extract to study hematological and serum biochemical changes in pregnant Sprague-Dawley rats.

Data obtained in this study for the control group are consistent with results from many studies utilizing pregnant rats (Honda et al., 2008; De Rijk et al., 2002; Urasoko et al., 2009; LaBorde et al., 1999; Lynch et al., 2011; Syahida et al., 2012; Liberati et al., 2004; Ahokas et al., 1984; Han et al., 2010).

Results also show a significant reduction of Glu levels in pregnant rats after the oral administration of Equisetum ramossisemum extract. Studies utilizing rats and other animal models have reported the same effect of Equisetums extracts on glucose levels (Revilla et al., 2002; Cetto et al., 2000; Soleimani et al., 2000). In fact, it was demonstrated that a water extract of the aerial parts of Equisetum myriochaetum created a hypoglycemic effect in type 2 diabetic patients (Revilla et al., 2002). In addition,

plasma glucose levels in diabetic rats were lowered after oral administration of *Equisetum myriochaetum* extract (Cetto et al., 2000). Other *Equisetum* species have also produced significant antidiabetic activity in rats (Soleimani et al., 2000).

No imbalances in serum electrolytes such as Na, Cl, Ca, P, and K were observed (Table 1) although it is known that some *Equisetums* increase diuresis without a change in electrolytes (Combest et al., 2005). Moreover, our data for values of Na, Cl, Ca, P, and K in serum are consistent with data resulting from female rats treated with *Equisetum arvense* (Tago et al., 2010). For Na and K values, this finding is consistent with a reported human case study (Klnçalp et al., 2012).

The values for triglycerides, cholesterol, creatinine and urea, show no significant differences (Table 1). This result is also consistent with results obtained for a man subjected to 500ml/day of boiled *Equisetum arvense* juice for two weeks (Klnçalp et al., 2012). Furthermore, our data for triglycerides, cholesterol, creatinine, urea, A/G, and ALP concentrations show no significant differences, a finding consistent with results reported for female rats treated with *Equisetum arvense* (Tago et al., 2010).

We found that the levels of ALP in animals treated with the highest dose of extract were lower as compared to the control group. These levels of ALP were comparable to those recorded for two studies of *Equisetums* toxicity in rats ((Tago et al., 2010; Baracho et al., 2009).

Liver test parameters, including AST, GGT, ALT and T-Bil show an obvious tendency to increase in the group treated with the highest dose of *Equisetum ramossisemum* extract, which may indicate that the extract caused some change in the liver (Klnçalp et al., 2012). Hepatic effects produced by *Equisetum ramossisemum* in rats seem to be related to the dosage. Acute hepatoxicity was not observed in our study, which utilized doses of *Equisetum ramossisemum* extract at 500, 250, and 125 mg/kg. However, a study has shown that high doses of *Equisetum arvense* in rats produced significant hepatic damage (Baracho et al., 2009). In contrast, another study found that regular doses of *Equisetum arvense* have a hepatoprotective effect in rats (Baracho et al., 2009).

A significant decrease was detected in TP, ALB and G (Table 1). These data may indicate hypoproteinemia, which is a common finding in liver damage (Larrey, 2001). The significant decrease in values for three haematological factors, PLT, WBC, and MC (Table 2) may indicate that *Equisetum ramosissimum* extract has an effect on the haemopoetic system of the rat, when administered orally at the doses used in this study. In a study to evaluate anti-inflammatory effects of *Equisetum arvense* extract in mice, findings demonstrated that the extract has anti-inflammatory property (Do Monte et al., 2004). Another study questioning the effect of *Equisetums* mixture on rheumatoid arthritis patients revealed that the mixture down regulates preinflammatory factor, tumor necrosis factor alpha (TNF-α), levels in patient serums (Jiang et al., 2014). An additional study, using human peripheral lymphocytes, has shown that treatment with *Equisetum arvense* reduced production of TNF-α (Gründemann et al., 2014).

Given our current results, we hypothesize that the effects of *Equisetum ramossisemum* extract on pregnant Sprague-Dawley rats support a role for anti TNF-α. Studies in rats and in humans provide solid documentations that *Equisetums* extract treatments reduce production of the preinflammatory factor TNF-α (Jiang et al., 2014; Gründemann et al., 2014).

Interestingly, minor elevations of transaminases such as AST and ALT (Table 1) that we observed in rats have also been documented in patients recovering from anti-TNF therapies (Coffin et al., 2011). Additionally, leucopenia and thrombocytopenia have been reported as side effects in humans receiving TNF_ blocking therapy (Pathare et al., 2006). Leucopenia and thrombocytopenia are also documented in this study (Table 2).

Excitingly, results documented in humans after intravenous anti TNF-α treatment showed that there is an increase in plasma triglyceride levels with no difference in total cholesterol levels (Cauza et al., 2006). Still, other studies utilizing rodents also reported increased levels in triglycerides after intravenous anti TNF-α treatment (Cauza et al., 2006), similar to elevations in TG levels we detected in all rats treated with the extract (Table 1).

In summary, our hypothesis that *Equisetum ramossisemum* extract treatments may reduce production of TNF-α will be tested in animals to determine if the extract plays a role in regulating TNF-α.

Conclusion

Data analyses indicate that daily gavage doses of *Equisetum ramosissimum* extract produced significant differences in biochemical and haematological parameters in pregnant Sprague-Dawley rats as compared to the control group.

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