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Safety profile of suppository *Hamamelis virginiana* leaf extract

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The increased use of herbal remedies combined with their increasing availability in pharmacies, beauty and nutrition shops and supermarkets necessitates the evaluation of their efficacies and safety. The objective of the present study is to evaluate the safety of the rectal administration of the dry leaf extract of witch hazel (*Hamamelis virginiana* L. Family: Hamamelidaceae), one of the traditionally used herbs for treating hemorrhoids. *H. virginiana* dry leaf extract was formulated in suppositories. An acute single dose study on rabbits and a 28 day repeated dose rectal administration of hamamelis extract on rats were preformed. No deaths occurred in either the placebo or the hamamelis treated rabbits. The general condition of all the rabbits was normal without any significant changes in their body weights. The changes in clinical and hematological parameters were non significant ($p > 0.05$). In the 28 day repeated dose study, no deaths occurred and normal body weight gains were recorded in all groups. Before sacrifice, the rats behaved normally with no detected signs of sickness or discomfort. After sacrifice, the inspected organs were normal in morphology and histopathology without any detected weight differences. All treated rats showed normal biochemical and hematological profiles compared to placebo treated animals ($p > 0.05$). The results obtained from the present study indicate that the rectal administration of *H. virginiana* dry leaf extract (up to 300 mg/kg) formulated as suppositories was devoid of systemic toxicity.

Key words: Witch hazel, rectal, safety, hemorrhoids, astringent, suppositories.

INTRODUCTION

Witch hazel (*Hamamelis virginiana* L.; family: Hamamelidaceae) is one of the traditionally used herbs thought to possess anti-inflammatory, antibacterial and antioxidant activities (Korting et al., 1993; Iauk et al., 2003; Thring et al., 2011). It has been reported that hamamelis is capable of inhibiting tumour necrosis factor alpha (TNF) and could have genotoxic and antigenotoxic effects (Habtemariam, 2002; Dauer et al., 2003). Recently, hamamelitannin has been shown to provide protection from cultured human colon cancer cell line (Sanchez-Tena et al., 2012). But most importantly, astringent, venotonic and vasoconstriction activities made witch hazel useful in healing hemorrhoids (MacKay, 2001). *H. virginiana* is used in a variety of forms including the solid crude leaf and bark, fluid extracts, poultice and commonly as witch hazel water. Native Americans applied

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poultices of hamamelis leaves and bark as a remedy for hemorrhoids, wounds, insect bites, painful tumours and ulcers (Duke, 2000). The astringent and hemostatic properties of hamamelis are believed to be associated with the presence of tannins (Okuda, 2005). The hamamelis leaf is reported to contain 3 to 10% tannins (Sagareishvili et al., 1999; ESCOP Monographs, 2003). According to the European Pharmacopoeia, the presence of tannin is expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) that is used as a standardization marker in witch hazel extracts (Ph.Eur (European Pharmacopoeia), 2007).

The external use of witch hazel has become widely reported and has been approved by many regulatory authorities for its astringent properties associated with the tannin content of its leaves and bark. In Europe, for example, topical products containing witch hazel's leaf and bark extract rich in tannin are available in the market. Such products have approval status by the German Commission E for minor injuries of the skin, local inflammation of the skin and mucous membranes and most notably, hemorrhoids and varicose veins (ESCOP Monographs, 2003; Blumenthal et al., 1998). Moreover, hamamelis water (Witch Hazel, USP-31- NF 26, 2008) is available in the United States as an over-the-counter astringent and is currently marketed as an ingredient in many non-prescription drugs for treating hemorrhoids. Nevertheless, due to lack of adequate preclinical and clinical studies to support the claimed safety and effectiveness of these products, witch hazel was only marketed in the United States as a dietary supplement in 1994 under the Dietary Supplement Health and Education Act (DSHEA).

Although *H. virginiana* has long been used as a traditional medical herb, limited knowledge is available on its safety. Scientific literature still lack reports on its toxicity, especially preclinical systemic toxicity studies. In fact, a study published by Bernard et al. (1972) seems to be the only study of reference in most official hamamelis monographs.

This study reports the oral systemic safety of a hamamelis preparation in rats and mice that were treated repeatedly with 100 mg/kg dose for 3 months (Bernard et al., 1972). On the other hand, few studies have shown clinically that witch hazel water relieves itching, burning and other discomforts when applied to the anorectal area; although according to the report of the European Medicines Agency (Doc. Ref.: EMA/HMPC/114585/2008), it has been judged as safe and effective in concentrations of 10 to 50% when applied up to 6 times daily. However, and in support of a regulatory submission for a new herbal product (Vilgin® Suppositories, Delass Natural Products, Amman, Jordan), published reports on the rectal toxicity of *H. virginiana* dry leaf extract could not be found. The aim of the current investigation is to evaluate the systemic toxicity of *H. virginiana* dry leaf extract formulated in suppositories as an herbal treatment option for hemorrhoids. Both rabbits and rats were utilized to assess the acute and the 28 day repeated dose rectal toxicity of the prepared suppositories.

MATERIALS AND METHODS

Plant

H. virginiana dry leaf extract was purchased from Frutarom Switzerland LTD, Wädenswil, Switzerland. 30% ethanol was used to prepare *H. virginiana* dry leaf extract with an extract ratio of 4:1 and was standardized to a minimum of 10% tannins. This extract was described as a brown powder with astringent taste. Tannic and gallic acids were used as reference markers in a pharmacopeial thin layer chromatography (TLC) test to authenticate the extract and check the quality of the finished product (Ph.Eur (European Pharmacopoeia), 2007). A sample of the extract was deposited in the Department of Pharmaceutical Medicinal Chemistry and Pharmacognosy, University of Petra, Amman, Jordan (voucher number: EX032012).

Formulation and manufacturing of *H. virginiana* dry leaf extract suppositories

In order to prepare a batch of *H. virginiana* dry leaf extract suppositories, an accurately weighed amount of hard fat (Suppocire® NB Pellets, Gattefossé GmbH, Bad Krozingen, Germany) was initially melted with white beeswax (Kahl GmbH & Co., Trittau, Germany) in a 60 L tank. Later, *H. virginiana* dry leaf extract and colloidal anhydrous silica (CAB-O-SIL® M-5P, Cabot GmbH, Schaffhausen, Switzerland) were added to the tank with stirring. The mixture was finally transferred to a suppository filling machine at 40°C (SAAS2 suppository machine, Sarong S.p.A., Reggiolo, Italy). The composition of the prepared suppositories is shown in Table 1.

Animal handling

Eight male and eight female inbred white New Zealand rabbits were housed individually in rabbit cages. Adult male and female Sprague Dawley rats were obtained from Yarmouk University animal house unit (Irbid, Jordan). All animals were housed in University of Petra's Animal Care Unit (Amman, Jordan) and accommodated in a 12 h light/dark cycle and at a temperature of 20 ± 2°C. All animals were acclimatized for at least 5 days prior to experiments with free access to standard diet and drinking water. All animal experiments were performed in compliance with the University of Petra Animal Care Guideline which adapts Federation of European Laboratory Animal Science Association guidelines (FELASA). Study protocols were revised and approved by the Ethical Committee of the Higher Education Counsel of University of Petra, Amman, Jordan.

Dose level selection for the toxicity studies

Calculations of the dose strength of the administered suppositories

Table 1. Manufacturing formula for *Hamamelis virginiana* dry leaf extract suppositories.

Ingredient	Quantity			Function
	Mg/suppository	Kg/20,000 suppository	Percentage (w/w)	
Hamamelis virginiana dry leaf extract	200.2	4.004	14	Active ingredient
White bees wax	20.02	0.4	1.4	Melting point adjuster
Colloidal anhydrous silica 200	10.01	0.2	0.7	Suspending and viscosity- increasing agent
Hard fat (Suppocire)	1199.77	23.995	83.9	Base
Total	1.43 g	28.6	100	-

are based on the manufacturer's claimed recommended daily human dose of hamamelis extract for the treatment of hemorrhoids. It was reported as 5 mg *H. virginiana* dry leaf extract/kg of patient for 24 h and reflected treating hemorrhoids patients having 80 kg average weight with two suppositories for 24 h (Vilgin® leaflet, Delass Natural Products Co., Amman, Jordan). However, for the purpose of testing the toxicity of the animals, dose strength multiples (X) were selected to be 4 X, 20 X and 60 X, the recommended daily doses. Therefore, the animal groups were rectally administered 20, 100 and 300 mg/kg doses, respectively. In order to allow reasonable quantities of melted suppositories to be administered rectally to the animals (1 ml/kg of the animals' weight), concentrated suppositories stocks were prepared to have 20, 100 and 300 mg/ml of hamamelis extract. In this study, control groups received placebo formula composed of hard fat, white beeswax and colloidal anhydrous silica.

Single dose acute toxicity of *Hamamelis virginiana* dry leaf extract suppositories in rabbits

Study design

Sixteen rabbits were randomly divided into 4 groups: each group contained 2 males and 2 females and each rabbit was separated in single standard cages to avoid pregnancy during the study. The first group served as a control group where rabbits were treated with placebo, while the other three groups were rectally administered a single dose of 20, 100 and 300 mg/kg extract, respectively. Suppository formulations were melted at approximately 36°C in an appropriate clean glass beaker and a graduated pipette with a sterile plastic tip was used to administer the calculated dose rectally in each rabbit. No leakage of the administered dose was observed in any of the treated rabbits.

Clinical monitoring and tests

All groups were closely monitored for seven hours after dose administration. The monitoring focused on reporting any behavioural changes, side effects, or deaths that might occur during post-dose period. A daily examination was performed for each rabbit for the duration of the study (a period of fourteen days). A local examination of the anorectal region was conducted on days 2, 7 and 14 post administrations. Body weights were recorded twice: at the beginning and end of the study. Blood samples were withdrawn from each rabbit directly from their marginal ear veins at the end of the study. Whole blood samples (transferred into 2 ml ethylenediaminetetraacetic acid (EDTA) tubes) were used for hematological analysis carried out using a hematology system (Model Mythic 18, Orphee Medical Co., Geneva, Switzerland); and serum samples, prepared by centrifugation for 10 min at 2500 rpm (601 × g) using a Hettich EBA 20 centrifuge, Tuttlingen, Germany, were used to perform clinical chemistry tests using an automated clinical chemistry analyzer (Model BioLis 24i, Tokyo Boeki Machinery Ltd., Tokyo, Japan). Later, all animals were sacrificed by cervical dislocation under light ether anaesthesia, and full autopsy including gross pathological examination of the main organs was performed (heart, liver, lung, kidneys, stomach, intestine, pancreas, spleen, sex organs and anorectal area).

28 day repeated dose study of *Hamamelis virginiana* dry leaf extract suppositories in rats

Study design and tests

Twenty Sprague Dawley rats of each sex were used for repeated dose toxicity testing of hamamelis suppositories (n = 5 of each sex for each dose strength). Each group of rats was rectally administered its assigned test preparation (1 ml/kg) daily for 28 days at dose levels of hamamelis extract of 20, 100, or 300 mg/kg. A placebo group received the suppository base (free of extract) also daily for 28 days. Rats were maintained in clean cages and had free access to food and water. All rats were weighed daily while food and water consumptions were assessed weekly. The animals were observed for clinical symptoms daily at 1 h post treatment. At the end of the experimental period (28 days) all rats were sacrificed by cervical dislocation under light ether anaesthesia and blood samples were obtained by cardiac puncture after exploring the thorax. These were used for hematological analysis and for the preparation of serum samples to perform clinical chemistry tests for each rat.

Organ weights, gross necropsy and histopathology

Autopsy and gross pathological examinations were performed for each rat, and the major organs, namely liver, spleen, kidneys, submandibular salivary glands (SSG), heart, testis and lungs were removed, examined and accurately weighed. The gastrointestinal

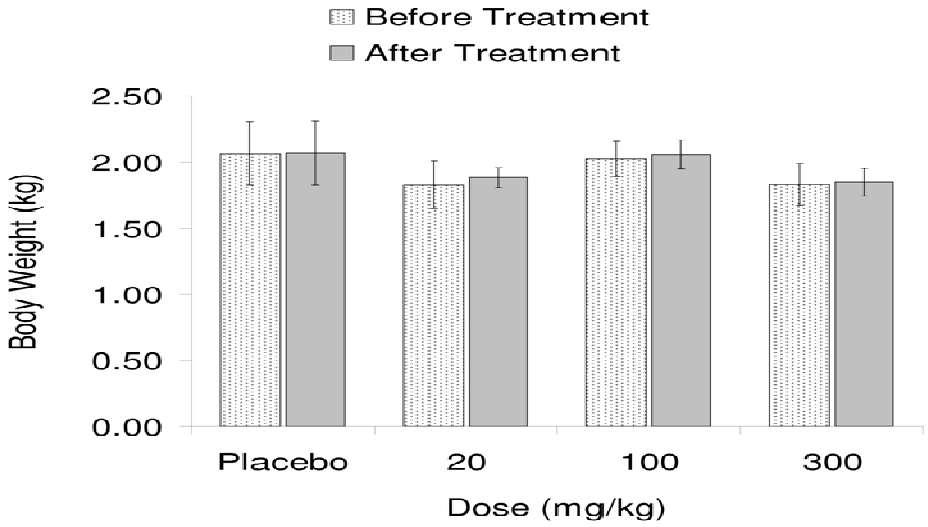

Figure 1. Rabbit body weights measurements before and after 14 days of acute rectal administration of *Hamamelis virginiana* dry leaf extract. Data points are expressed as mean±SEM (n=4).

tract, from oesophagus to anus, was isolated, dissected and inspected visually for any pathological abnormality. Liver, kidney and rectal (dissected as a ring that is 0.5 cm interior of the rectum orifice) biopsies isolated from the placebo treated rats and the high dose (300 mg/kg) hamamelis treated rats (n = 2/sex/group) were fixed in 10% (v/v) formaldehyde (Merck Chemicals, Darmstadt, Germany). The fixed biopsies were sectioned, mounted on slides and stained with hematoxylin and eosin (H&E) for histopathological examination under light microscope.

Statistics

All variables were analyzed by statistical package for social sciences (SPSS) version 17 statistical package (SPSS Inc., Chicago IL, USA). The overall differences between the groups were analyzed using one way analysis of variance (ANOVA). In certain cases, Tukey's post-test was performed following ANOVA to reveal differences between selected groups. For all of statistical compare-sons, P values less than 0.05 were considered as significant.

RESULTS

Preparation of *H. virginiana* dry leaf extract suppository

Brown colour, torpedo-like shape suppositories with an average weight of 1.43 ± 0.01 g were obtained. The analysis of the formulated suppositories indicated positive for the presence of tannic acid and gallic acid, both used as reference markers, using a pharmacopeial TLC test described in the monograph of Hamamelis leaf (Ph.Eur (European Pharmacopoeia), 2007).

Single dose acute toxicity of *Hamamelis virginiana* dry leaf extract suppositories in rabbits

In the acute study on rabbits, no deaths occurred in either the placebo or the hamamelis treated groups. The general condition of all rabbits was observed to be normal. Average body weights of the rabbits before and after 14 days of acute rectal administration of hamamelis suppositories did not differ significantly ($p > 0.05$) in all tested groups (Figure 1). At the end of the 14 days observation period, liver and kidney functions of all rabbits were not significantly changed (Table 2). The increase in serum urea content seen in hamamelis treated rabbits seemed not to have a dose-related effect ($p = 0.063$). In addition, the rectal administration of hamamelis extract in rabbits did not induce treatment-related effects on the hematological parameters evaluated (Table 3). None of the checked organs showed any gross anatomical abnormalities in this acute study.

28 day repeated dose study of *Hamamelis virginiana* dry leaf extract suppositories in rats

In the repeated dose study, same dose levels (20, 100 and 300 mg/kg) were selected in the acute study of hamamelis extract; they were administered to Sprague Dawley rats rectally for 28 days and compared to a placebo control group (without extract). No deaths occurred and normal body weight gains were observed in males and females of all dose groups (Figure 2). Normal

Table 2. Clinical tests of rabbits after 14 days of rectal administration of different doses *Hamamelis virginiana* dry leaf extract.

Parameter	Treatment			
	Placebo	20 mg/kg	100 mg/kg	300 mg/kg
GOT (IU/L)	36.8±6.6	48±8	37.2±6	51.4±23
GPT (IU/L)	75.8±3.4	65.8±7.5	94.3±15.4	64.7±10.8
GGT (IU/L)	7.6±1.8	10.3±2	10.7±2.5	7.3±1.5
ALP (IU/L)	142±25	193±78	193±36	114±48
Urea (mg/dL)	54.8±4.5	53.8±7.2	82.3±8	69.5±11.6
Total Proteins (g/dl)	69.7±4.7	65.1±1.4	69.6±3.2	63±12.3
Creatinine (mg/dl)	0.4±0.1	0.4±0.1	0.6±0.2	0.5±0.1
Na (mmol/L)	146±1.8	144±0.8	146±3.2	150±12
K (mmol/L)	4.9±0.7	4.2±0.3	4.7±0.5	5.5±1
Cl (mmol/L)	113±3	111±1	113±3	115±6

Data points are expressed as mean±SEM (n=4). GOT: glutamic-oxaloacetic transaminase; GPT: glutamate pyruvate transaminase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase.

Table 3. Hematological parameters of rabbits after 14 days of rectal administration of different doses *Hamamelis virginiana* dry leaf extract.

Parameter	Treatment			
	Placebo	20 mg/kg	100 mg/kg	300 mg/kg
RBC ($x10^{12}$/L)	6.3±0.5	7.3±0.5	5.5±0.8	6.5±1.1
Hb (g/dl)	11.9±0.3	13.5±0.9	12.9±0.5	12.3±2
PCV (%)	39.8±1.6	44.7±2.7	38.8±1.7	42.5±7.3
MCV (fL)	65.6±2.2	60.9±0.9	75.3±12.9	65.9±1.6
MCHC (g/dl)	30±0.6	30.3±0.6	33.3±1.8	29.1±0.3
WBC ($\times10^{6}$/L)	8.5±2.5	8.7±0.9	6.4±0.8	11.6±3
Neutrophils (%)	61±6.1	58±6	47±3.3	52±6.8
Lymphocytes (%)	28.5±5.4	31.5±4.9	40.5±4.4	39.3±6.7
Monocytes (%)	10.5±1.3	10.5±1.8	12.5±1.9	8.7±0.7

Data points are expressed as mean±SEM (n=4). RBC: red blood cells; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MHCH: mean corpuscular hemoglobin concentration; WBC: white blood cells.

food and water consumption was observed. Before sacrifice, all treated rats behaved normally with no detected signs of sickness or discomfort. After sacrifice, all inspected organs appeared normal in morphology without any detected differences in their weights when different dose levels were compared with placebo groups of both males and females (Table 4). No hamamelis-related histopathological findings were observed in the liver and kidneys of treated rats, and rectum samples showed normal submucosal glands and vasculature with normal muscularis propria (Figure 3). Furthermore, all treated rats showed normal liver and kidney functions without significant changes in serum lipids and protein profiles as illustrated in Table 5. Fluctuated glucose readings due to the use of non-fasting rats were noticed in and between tested groups, nevertheless, in a non significant treatment-related trend as revealed by Tukey's post hoc comparisons ($p > 0.05$). Finally, all hemato-logical parameters evaluated after the 28 days of rectal administration of *H. virginiana* dry leaf extract were in normal ranges ($p > 0.05$) (Table 6).

DISCUSSION

The increased use of herbal remedies combined with

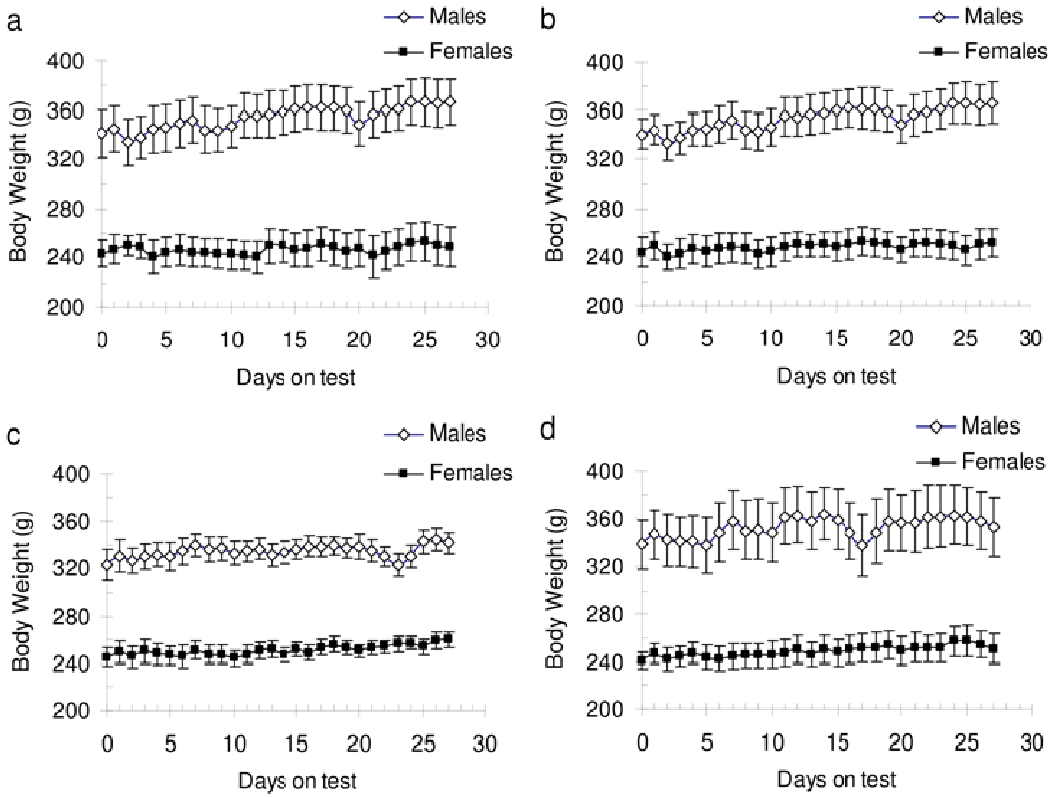

Figure 2. Body weights of rats treated rectally with different doses of *Hamamelis virginiana* dry leaf extract for 28 days: (a) Placebo, (b) 20 mg/kg, (c) 100 mg/kg and (d) 300 mg/kg. Each data point represent the mean±SEM (n=5).

Table 4. Organ weights of males and females rats post 28 days of repeated rectal administration of different doses of *Hamamelis virginiana* dry leaf extract.

Parameter	Treatment			
	Placebo	**20 mg/kg**	**100 mg/kg**	**300 mg/kg**
Males organ weights (g)				
Liver	11.7±0.8	11.6±0.8	10.4±0.1	11.7±0.5
Spleen	0.79±0.02	0.75±0.07	0.65±0.07	0.9±0.13
Kidneys	2.4±0.17	2.2±0.18	2.2±0.07	2.2±0.12
Heart	1.2±0.1	1.1±0.1	1.1±0.05	1.1±0.06
Lung	3.3±0.31	2.9±0.25	2.8±0.11	2.8±0.4
SSG	0.5±0.07	0.55±0.04	0.58±0.04	0.55±0.04
Testis	2.8±0.38	2.6±0.16	2.8±0.4	2.9±0.13
Females organ weights (g)				
Liver	10.4±0.9	9.7±0.5	9.6±0.3	9.7±0.6
Spleen	0.64±0.11	0.81±0.14	0.64±0.06	0.65±0.08
Kidneys	1.8±0.14	1.8±0.11	1.9±0.07	1.8±0.05
Heart	0.82±0.05	0.9±0.06	0.9±0.05	0.9±0.06
Lung	2.7±0.5	3.1±0.6	2.3±0.4	2.6±0.3
SSG	0.5±0.05	0.4±0.05	0.5±0.02	0.6±0.07

Data points are expressed as mean±SEM (n=5). SSG: Submandibular salivary glands.

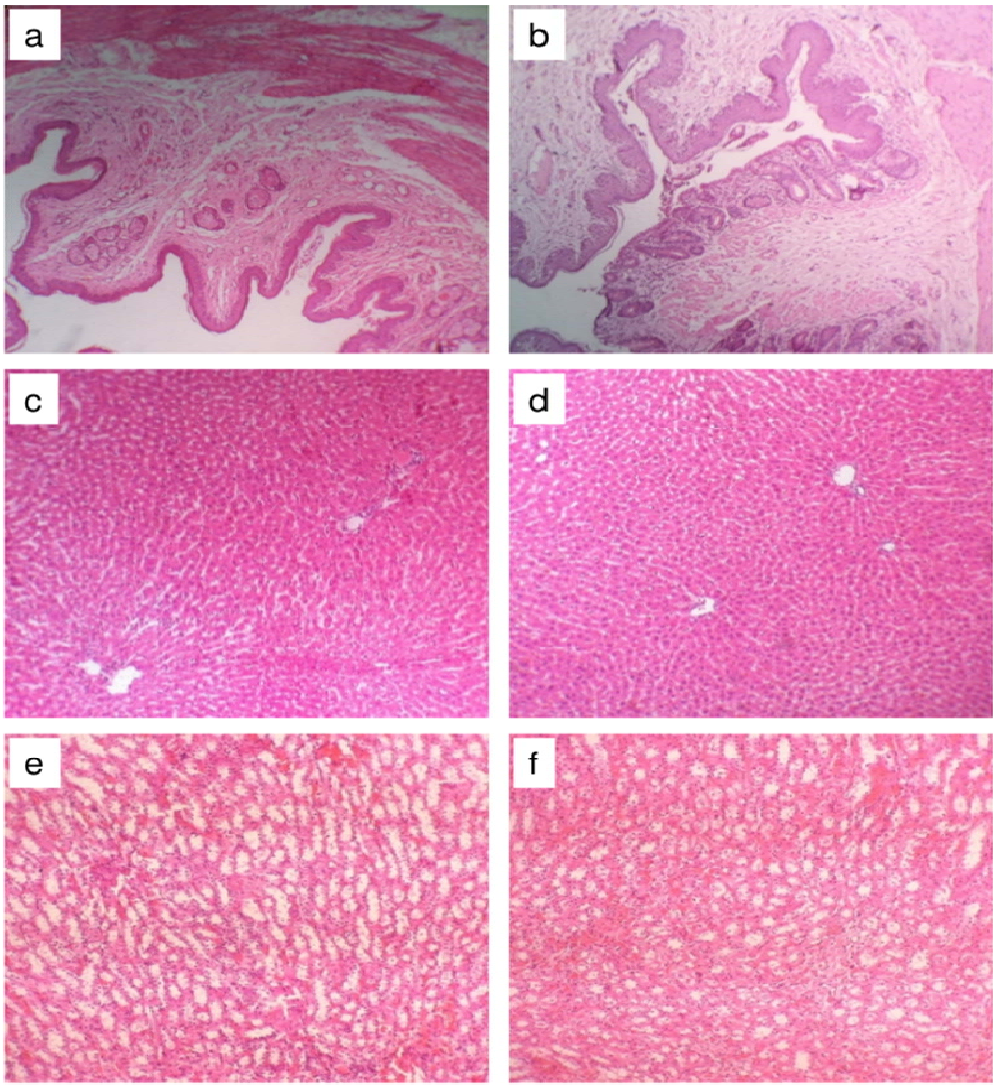

Figure 3. Representative microscopic images (100x magnification) of H&E stained biopsies of the rectum (a, b), liver (c, d), and kidney (e, f) of male rats treated rectally for 28 days with placebo suppository preparation (a, c, e) and 300 mg/kg dose of *Hamamelis virginiana* dry leaf extract suppositories (b, d, f).

their increasing availability in pharmacies, beauty and nutrition shops and supermarkets necessitates the evaluation of their efficacies and safety (Shaw, 1998; Jordan et al., 2010). Several herbs have been investigated as potentially safe and effective therapies in treating hemorrhoids amongst them are *Aesculus hippocastanum* “Horse Chestnut” (Pittler and Ernst, 1998), *Ruscus aculeatus* “Butcher’s Broom” (Cappelli et al., 1988), *Centella asiatica* “Gotu Kola” (Brinkhaus et al., 2000) and *H. virginiana* “Witch Hazel” (Barnes et al., 2007). Because of its anti-inflammatory activity and due to the fact that it improves microcirculation, capillary flow and vascular tone, *H. virginiana* may prevent complications of varicose veins and hemorrhoids (Duke, 2000; Barnes et al., 2007).

The main reported constituents of *H. virginiana* dried leaf extract include: tannins (a mixture of catechins, gallotannins, as well as cyanidin and delphinidin type proanthocyanidins), polyphenolic acids (caffeic and gallic acids), flavonoids (such as kaempferol, quercetin, quercitrin, and isoquercitrin) and volatile oil (consisting mainly of aliphatic alcohols, carbonyl compounds, aliphatic esters and traces of safrole) (Bradley, 2006).

Commercially, witch hazel is used in preparations to

Table 5. Clinical chemistry parameters of Males and Females rats post 28 days of repeated rectal administration of different doses of *Hamamelis virginiana* dry leaf extract.

Parameter		Treatment			
		Placebo	20 mg/kg	100 mg/kg	300 mg/kg
Males					
Liver function tests	GOT (IU/L)	291±27	266±19	242±36	249±20
	GPT (IU/L)	107±12	121±13	102±18	105±15
	GGT (IU/L)	2.3±0.9	1.8±0.6	2.6±0.5	2.5±0.5
	ALP (IU/L)	287±42	245±23	353±58	368±16
Kidney function tests	Urea (mg/dl)	42±0.7	46±2.1	43.5±4	41.1±1.6
	Glucose (mg/dl)	67.1±19	105±26	106±38	76.8±23
	Creatinine (mg/dl)	0.59±0.03	0.64±0.02	0.58±0.06	0.57±0.4
	Na (mmol/L)	160±2	163±0.7	163±2.3	158±0.4
	K (mmol/L)	10.4±0.96	10.7±1.8	10.6±1.2	10.6±0.4
	Cl (mmol/L)	123±0.5	122±0.7	132±9.9	120±0.7
Serum lipids	Cholesterol (mg/dl)	59.4±4.9	59.9±2.1	42.8±4.8	50.9±2.4
	Triglycerides (mg/dl)	56.5±6.7	66.7±5.6	42.8±6	42.8±4.5
Serum proteins	Total proteins (g/dl)	67.4±2.2	70.7±1.3	73.8±2.6	68.8±1.8
	Albumin (g/dl)	35.2±0.9	37.1±0.8	36.9±0.6	36.3±0.9
Females					
Liver function tests	GOT (IU/L)	241±37	234±23	186±11	180±7
	GPT (IU/L)	83.5±12.3	102±14.7	81.9±12.1	68.4±7.1
	GGT (IU/L)	1.6±0.5	3.7±1.6	0.9±0.3	2.1±0.5
	ALP (IU/L)	234±69	258±69	256±135	224±81
Kidney function tests	Urea (mg/dL)	48.5±2.4	52.9±3.5	48.3±2.2	47.6±1.2
	Glucose (mg/dl)	58.9±15.7	108±16.5	167±34	92.7±39
	Creatinine (mg/dl)	0.61±0.05	0.67±0.04	0.6±0.03	0.55±0.05
	Na (mmol/L)	165±1.9	180±7.1	163±1.2	161±1
	K (mmol/L)	8.8±0.6	10.6±1.2	8.1±1	9.3±0.9
	Cl (mmol/L)	122±15.4	126±4	123±0.5	120±1
Serum lipids	Cholesterol (mg/dl)	58.2±5.2	70.2±13.8	58.4±3.9	62.5±4.3
	Triglycerides (mg/dl)	124±15.4	89.3±10.3	95.1±5.4	105±10.3
Serum proteins	Total proteins (g/dl)	66.7±13	67.5±10.8	47.3±18.1	47.4±17
	Albumin (g/dl)	47.4±2.4	44.6±3	46.9±1.3	48.4±0.5

Data points are expressed as mean±SEM (n = 5). GOT: glutamic-oxaloacetic transaminase; GPT: glutamate pyruvate transaminase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase.

treat hemorrhoids, irritations, minor pains and itching. Such preparations are currently marketed in many pharmaceutical dosage forms, but most commonly as witch hazel water or distilled witch hazel extract. However, witch hazel water is a steam distillate of the extract and therefore, it does not contain tannins (Barnes et al., 2007). Since tannins have been reported to be responsible for astringent and hemostatic properties and antioxidant activity, the use of the whole plant extract may be preferred for treating hemorrhoids.

Tannins have been also proven to enhance tissue regeneration and prevent gastric ulcers (Vasconcelos et

Table 6. Hematological parameters of Males and Females rats post 28 days of repeated rectal administration of different doses *Hamamelis virginiana* dry leaf extract.

Parameter	Treatment			
	Placebo	20 mg/kg	100 mg/kg	300 mg/kg
Males				
RBC ($x10^{12}$/L)	7.9±0.3	8.5±0.2	8.8±0.1	6.6±1.5
Hb (g/dl)	14.8±0.9	15.7±0.4	15.9±0.1	15.4±0.3
PCV (%)	43.1±2.8	45.6±1.1	46.4±0.8	44.8±0.6
MCV (fL)	54.7±1.2	53.9±0.6	52.5±0.4	54.9±0.7
MCHC (g/dl)	34.3±0.2	34.6±0.4	34.4±0.3	34.4±0.5
WBC ($x10^{6}$/L)	6.1±1.3	6.1±0.9	7.1±1.3	9.5±1.7
Neutrophils (%)	28.3±2.2	25.8±1	30.6±2.7	28.6±1.7
Lymphocytes (%)	54.8±1.8	60±0.9	54.4±2.5	57±2.1
Monocytes (%)	17±0.7	14.2±0.7	15±1.3	14.4±1
Females				
RBC ($x10^{12}$/L)	8.2±0.3	7.8±0.4	7.3±0.3	7.6±0.1
Hb (g/dl)	15.6±0.4	15.3±0.9	14.3±0.5	15.1±0.1
PCV (%)	46.4±1.3	44.7±3	41.8±1.7	43.7±0.3
MCV (fL)	56.6±0.6	56.1±1	55.8±1.3	57.6±0.8
MCHC (g/dl)	33.8±0.3	34.3±0.2	34.2±0.3	34.7±0.3
WBC ($x10^{6}$/L)	6.3±0.6	6.9±1.4	6.9±2.1	5.6±0.9
Neutrophils (%)	24.2±3.7	31.8±7.3	19.7±1.2	25±2.5
Lymphocytes (%)	61±3.9	52.8±6.9	67.7±1.7	59.3±4.7
Monocytes (%)	14.8±1	15.4±0.7	13.7±1.3	16±2.5

Data points are expressed as mean±SEM (n=5). RBC: red blood cells; Hb, hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MHCH: mean corpuscular hemoglobin concentration; WBC: white blood cells.

al., 2010; de Jesus et al., 2011; Li et al., 2011). The external astringency and soothing effect of tannins is believed to be associated with its capability of forming protein complexes which in terns enclose the external layers of the exposed or damaged tissues. This action tightens up superficial cell layers and shrinks colloidal structures, thus protecting underlying layers of cells (Lamont and Strong, 2006). Although witch hazel has a long therapeutic use in folk medicine, few reports regarding its efficacy and mechanism of action have been published. Furthermore, literature lacks chronic systemic toxicity studies to support the safe consumption of witch hazel.

Although the oral LD_{50} in rats could not be found by Bernard et al. (1972) thus indicating its safety, the rectal LD_{50} had never been reported for *H. virginiana* before this current study was conducted. Therefore, it was decided to administer the test animals rectally with three dose levels of the formulated suppositories, where the highest dose was 60 times higher than the dose intended for human use.

In the present study, none of the selected dose levels of rectally administered *H. virginiana* dry leaf extract induced death in the tested animals. Both rabbits and rats appeared to tolerate the administered dose well without any significant changes in their body weights or organ weights. There were no treatment or gender related toxicological changes observed in the animals and no notable behavioural changes. Generally, changes in the hematological system have a higher predictive value for human toxicity whereas the liver and kidney function tests reflect possible organ toxicities (Gautam et al., 2012).

Therefore, complete blood count along with the liver and kidney function tests was conducted in both acute and repeated dose studies. However, some other parameters such as glucose, proteins and lipids were only investigated in the 28 day repeated dose study (Table 5). Such parameters also reflect the normal metabolism and excretion functions in the tested animals post repeated hamamelis extract administration. As a result,

none of the tested clinical and hematological parameters were significantly changed in both studies ($p > 0.05$).

All checked internal organs showed normal morphology in all treated groups compared to control groups. Rectal, kidney and liver biopsies from treated rats did not reveal any histopathological concerns. Based on these results, the “no observed adverse effect level” (NOAEL) for the rectal administration of *H. virginiana* dry leaf extract preparation is reported to be 300 mg/kg.

It has been reported that polyphenols have an oral bioavailability in the range of 2 to 20% (Hu, 2007). Most polyphenols are considered potentially too hydrophilic to penetrate the gut wall by passive diffusion, but the membrane carriers that could be involved in polyphenol absorption have not been identified (Manach et al., 2004). In addition, the biological effects of tannins, as large polyphenolic compounds, usually depend on its grade of polymerisation and solubility. Highly polymerised tannins exhibit low bio-accessibility in the small intestine and low fermentability by colonic microflora (Serrano et al., 2009). For example, the high molecular weight proanthocyanidins, with oligomers larger than trimers, are unlikely to be absorbed in the small intestine in their native form (Manach et al., 2005; Pandey and Rizvi, 2009).

Therefore, it is expected that the rectal adminis-tration of *H. virginiana* dry leaf extract would not induce harmful systemic toxicity due to the limited absorption of its active principles from the anorectal area. Neverthe-less, the current study was justified due to the presence of other bioavailable small phenolic compounds in the extract such as tannic, gallic, and cafeic acids. For example, a subchronic toxicity study of gallic acid in rats for 13 weeks resulted in NOAEL doses of 119 and 128 mg/kg/day, respectively for male and female rats (Niho et al., 2001). Therefore, exceeding the daily recommended dose of the suppositories is not recommended without consulting a health practitioner until full toxicokinetic studies of such compounds become available to support the safe use of witch hazel.

Conclusion

The results obtained from the evaluation of the presented toxicity indicate that the rectal administration of *H. virginiana* dry leaf extract formulated in suppositories was discharged from any rectal systemic toxicity in the tested animals *in vivo* up to a dose of 300 mg/kg. Nevertheless, due to the presence of multiple phenolic compounds, chronic systemic oral and rectal toxicity studies are still warranted to further assure the safety of consumption of *H. virginiana* extracts or any of its isolated active principals.

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