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Rumex usambarensis (*Dammer*) Leaf and Stem Extract Effect on Body Weight and Lipid Profile: A Study in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OPE designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author NH collected the plant, carried out extraction and conducted laboratory experiments for the effect of the extract on weight and lipid profile and author ND carried out the phytochemical analysis and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to determine the effect of *Rumex usambarensis* plant extract on body weight and lipid profile of fattened albino rats.
Study Design: This was an *in-vivo* experimental study in laboratory animal model.
Place and Duration: The study was conducted at the Faculty of Medicine, Mbarara University of Science and Technology, Uganda, from January to May 2015.

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Methodology: *Rumex usambarensis* stems and leaves were collected from Kabale district in western Uganda during rainy season for the study while one whole plant was brought to the university for authentication by a university botanist. The fresh stems and leaves weighing 8.1 kg were blended in 1.045L of distilled water and filtered through a muslin cloth before evaporating in an oven set at 55℃ to a constant weight of dry mass. A portion of the dry mass was screened for presence of phytochemicals groups while the remaining portion was dissolved in distilled water and administered to fattened wistar albino rats at the doses of 125 mg/kg, 250 mg/kg and 500 mg/kg corresponding to group 1, 2 and 3 respectively daily by gavage for 21 days. The Control group received equivalent volume of distilled water by gavage daily. Mean body weights at baseline and at day 21 were statistically compared using ANOVA followed by sidak statistical test at p=0.05. Lipid profiles obtained on day 21 were also compared using the same statistical tests.

Results: *Rumex usambarensis* extract significantly reduced body weight of fattened rats from baseline at all the doses tested i.e 338.7±141.4 Vs 323.4±135, p=0.03, for 125 mg/kg dose, 335.9±140.1 Vs 322.7±134.6, p=0.03 for 250mg/kg dose, 339.1±141.5 Vs 324.7±135.6, p=0.03 for 500 mg/kg dose while the control group had no significant change in body weight i.e 338.7±141.3 Vs 338.3±141.1, p=0.35.The extract groups at lower dose levels had significantly higher High Density Lipoprotein level i.e. 29.1±4.8 for 125 mg/kg dose (p= 0.002) and 29.1±6.8 for 250 mg/kg dose 4 (p=0.03) compared to 21.4±5 for the control group and 25.1±6.5 for the 500 mg/kg group.

Conclusion: *Rumex umsambarensis* extract decreased weight of fattened animals and may be useful in reduction of body weight in overweight and obese persons.

Keywords: Rumex usambarensis; satiety; overweight; obesity.

1. INTRODUCTION

According to World Health Organisation (WHO), Overweight and obesity are defined as abnormal or excessive fat accumulation in the body that presents a risk to health [1]. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height in meters. A person with a BMI of 30 or more is generally considered obese. A person with a BMI between 25 and 30 is considered overweight [2]. A study done in the United States of America that examined the trends in obesity among adults showed that between 2007 and 2008, the prevalence of obesity was 32.2% among men and 35.5% among women [3]. Overweight and obesity are no longer problems of the developed countries only. Increased rates of overweight and obesity including childhood obesity, as well as associated chronic diseases such as hypertension and diabetes, have been recently observed in many developing countries [4,5]. While modern drugs have been developed for body weight and lipid reduction in overweight and obese persons, a number of medicinal plants or herbal medicines are also used and some have been shown to have weight reduction effects or causing satiety effect [6]. Traditionally, in Uganda some plants are chewed or eaten as foods to effect satiety and hence avoid eating of food. One such plant is *R. usambarensis* which in western part of Uganda, it is chewed by cattle keepers with claim that it alleviates desire for food. This effect may explain why most cattle keepers in western Uganda are of lean body weight. A review of ethnobotanical studies and pharmacological studies of the plant only report *R. usambarensis* uses as an antihelmintic, [7], for treatment of mastitis, induction of abortion, treatment of liver and stomach conditions [8]. There was no study reporting its use or effect in weight control or management of obesity or induction of satiety. In this paper we report for the first time the effect of the plant extract on the weight and lipid profile of fattened albino rats.

2. METHODOLOGY

2.1 Study Material and Phytochemical Analysis of the Extract

Heathy mature fresh leaves and stems of *Rumex usambarensis,* were obtained from Kabale district in south Western Uganda about 150 km from Mbarara University of Science and technology, and about 400 km from Kampala the capital city of Uganda. The whole plant sample collected was authenticated by the university botanist Dr. Eunice Olet of the faculty of applied sciences and given reference Voucher specimen number Nattabi 001, the specimen was deposited at the university herbarium. Fresh

leaves and stems of the plant (8.1 kg) were washed with distilled water, blended with 1.045 L of distilled water and filtered using a muslin cloth. The filtrate was concentrated in an oven at temperature of 55℃ to a constant weight of dry mass.

Dry extract (5 g) obtained was dissolved in distilled water (40 mls) to get a solution which was used for various phytochemical tests according to procedures described in Trease and Evans' pharmacognosy text book [9] and briefly described below:

Test for alkaloids: 1 ml of extract was evaporated in a water bath. The residue was then dissolved in 1 ml HCl and 2-3 drops of Wagner's reagent added while shaking. Orange precipitate confirmed presence of alkaloids.

Test for flavonoids: To extract solution (1ml) was added ethanol (5 mls, 95%) and concentrated hydrochloric acid (3 drops) and then magnesium (0.5 g) added. Pink colour confirmed presence of the flavonoids.

Test for saponins: Distilled water (5 mls) was added to extract solution (1 ml) and the mixture was shaken strongly for 5 minutes. Formation of a foam on surface of the solution which persisted for 5 minutes indicated presence of saponins.

Test for tannins: Extract solution (1 ml) was boiled with distilled water (5 mls). The mixture was filtered and a portion of the filtrate was diluted with sterile distilled water in a ratio of 1:4 and Iron III chloride (10%, 3 drops), added. Formation of a blue green precipitate indicated presence of tannins.

Test for phenolic compounds: Ferric chloride (5%, 3 drops) was added to the extract solution (1 ml). Formation of the red wine color indicated presence of Phenolic compounds.

Test for glycosides: To the extract solution (2mls), sodium picrate (2 mls) was added. A yellow or orange colour indicated the presence of glycosides.

Test for steroids and glycosides: To the extract solution (2 ml) was added chloroform followed by acetic anhydride (2 ml). Concentrated sulphuric acid (2 drops) were added from the side of the test tube. A reddish brown colouration on the interface of the test tube indicated presence of steroids and terpenoids.

2.2 Determination of Extract Effect on Body Weight and Lipid Profile of Fattened Rats

2.2.1 Experimental animals

Thirty (30) healthy female Wistar rats weighing between 98 g to 180 g were obtained from the animal facility of Mbarara University of Science and Technology. The animals were left to acclimatize in the pharmacology laboratory for one week feeding on rat pellets and allowed water at liberty, twelve hours of day light and twelve of hours of darkness. Six (6) rats were randomly picked and fed on normal rat diet for use as control to determine weight changes during fattening phase of the study. The other 24 rats were fed on high fat diet that consisted of Kent extra virgin olive oil and cheese and rat pencil for 30 days according to method previously described for induction of obesity in rat models [10,11,12]. Kent Extra Virgin Olive Oil (200 mls) was added to 400 g of the pellets daily and given to the animals. Composition of the extra virgin olive oil per 100 g was: polyunsaturated fats (10.8 g), monounsaturated fats (71.4 g), saturated fats (17.8 g), fats (100 g), energy (820-920 kcal) and 18% oleic acids. It did not contain any proteins, carbohydrates or cholesterol. Cheese 100 g was also given to each animal every morning. The 24 animals gained between 20% to 37% of their body weight after one month while those that fed on normal diet did not gain weight. Sampling of the 24 animals into groups was done by simple randomization method to distribute the fattened animals to groups 1, 2, 3 and 4. One researcher was blinded and he randomly picked each of the fattened animals without replacing, placing them into the 4 groups (1,2,3, and 4) of 6 rats each. The groups pre-labelled 1 to 4 in which 1, 2, 3 and 4 represented treatment groups 125 mg/kg, 250 mg/kg, 500 mg/kg and water respectively. Healthy rats that had gained at least 20% of baseline body weight during fattening phase were included while rats that became pregnant or sick were excluded.

2.2.2 Administration of the extract, measurement of weight and lipid levels

The dry extract 1.5 g was weighed and dissolved in distilled water (15 mls) to produce 100 mg//ml concentration. To group 1 was given 125 mg/kg, to group 2 was given 250 mg/kg and to group 3 was given 500 mg/kg orally by gavage once a day for 21 days. The fourth group received

distilled water only (1 ml) orally by gavage serving as a control. On the day of treatment, food was withdrawn for at least 1 hour before and after extract administration to allow absorption of the extracts. The animals were weighed every three days and weights used in dose calculation till day 21. On day 21, blood was drawn from the venaecava of each rat under general anaesthesia with ether and the blood samples sent to clinical chemistry laboratory for determination of lipid profile. After the blood draws, all the animals were humanely sacrificed under chloroform vapour and incinerated.

2.3 Data Analysis

All data for weights and lipid profiles obtained were entered in excel 2007. The data was imported to STATA program version 10.0 and analyzed. Data was summarized as Mean±Standard Error of Mean. ANOVA was used to detect difference in means followed sidak test. Lipid profile mean values for the groups at day 21 were analyzed by ANOVA for difference followed by sidak test. In all statistical comparisons, a difference in means was considered statistically significant if p value was less than 0.05.

2.4 Ethical Approval and Animal Care

The research proposal was approved by the Pharmacy and Pharmaceutical Sciences Departments Research committee and Faculty of medicine Institutional Review and Ethics Committee (IREC) of Mbarara University of Science and Technology. All animals were cared for as per National Institute for Health (NIH) guidelines for care and use of laboratory animals in teaching and research. Proper animal handling techniques were followed as regards to feeding, housing and general handling throughout the entire study period of 2 months. After the study, the animals were humanely sacrificed by exposing them to chloroform vapour and then taken to the university hospital incinerator for proper disposal.

3. RESULTS

The water extract was found to be rich in alkaloids, tannins, saponin, steroidal glycosides and flavanones but lacked starch, anthocyanosides and anthracenosides (see Table 1). The extracts at all those levels caused significant reduction in body weights of the fattened rats (Table 2). Analysis of the lipid profile showed that only High Density Liprotein and triglycerides were significantly elevated and this occurred at doses of 125 mg/kg and 250 mg/kg (see Table 3).

Table 1. Results of phytochemical analysis of the water extract

Phytochemical group	Results
Alkaloids	+
Tannins	+
Saponnins	+
Steroids	+
Flavanones	+
Starch	–
Anthocyanosides	–
Anthracenosides	–

(+) Phytochemical group present, (-) Phytochemical group absen

4. DISCUSSION

The cattle keepers in western Uganda chew this plant to maintain satiety. The phytochemical analysis showed absence of starch in the plant extract indicating that the satiety effects reported by the cattle keepers is probably not due to sugars but other bioactive compounds. Starches are known to induce satiety [13]. The plant also has no sweet taste, it tastes salty and it is the salty that makes people like to chew it. Studies reporting phytochemical profiles of *R. usambarensis* were not available as per our literature search, however comparison with Rumex *dentatus* L. water extract done in India [14] showed similarity in phytochemicals with exception of saponins that were present in the *R. usambarensis* extract in our study.

Table 2. Effect of the treatment on body weight of fattened wistar albino rats

Treatment group (N=7)	Average weight at day 0 (g) ±S.E	Average weight at day 21 (g) ±S.E	Pvalue
125 mg/kg of extract	338.7±141.4	323.4±135	0.034*
250 mg/kg of extract	335.9±140.1	322.7±134.6	0.029*
500 mg/kg of extract	339.1±141.5	324.7±135.6	0.0324*
Distilled water (control)	338.7±141.3	338.3±141.1	0.3522

**difference statistically significant at p=0.05*

Table 3. Blood lipid profile for the treatments of fattened wistar albino

Treatment group (N=6)	Lipid profile (Mean± SD)			
	Total cholesterol	High density lipoproteins	Low density lipoproteins	Triglycerides
125 mg/kg	75.9±18.1	29.1±4.8***	5.2±0.5	220.5±68.3*
250 mg/kg	63.5±9.0	29.1±6.8**	6.2±1.0	155.5±44.5
500 mg/kg	68.5±15.9	25.1±6.5	5.1±0.6	191.5±46.6
Distilled water	60.2±16.4	21.4±5.4	5.3±0.7	144.5±57.8

****p=0.002, **p=0.03, *p=0.04*

The extract caused a significant reduction in body weight of the fattened rats at all the three dose levels tested with dose of 250 mg/kg being better than 125 mg/kg and 500 mg/kg (Table 2).

The phytochemical groups found in the plant extract are similar to those that have been established in other plants with anti-obesity activity. Alkaloids in particular such as capsaicin found in red pepper has been shown to attenuate obesity-induced inflammation, obesity related metabolic disorders, and liver diseases, reduce food intake and increase energy expenditure and lipid oxidation [15,16]. Examples of such plants with similar alkaloids include: *Khat edhulis and Caralluma fimbriata. Khat edhulis* which has notable amounts of alkaloids (cathionine, cathine), is widely chewed by Somalis and long distance track drivers in East Africa with claim of having satiety effect. The people who chew these plants are indeed of lean body weight but the Khut edulis is claimed to be addictive and so its use is highly discouraged. *Caralluma fimbriata* whose key ingredients are pregnane glycosides has been demonstrated in clinical trials to cause appetite reduction, reduction in waist circumference and also reduced lipogenesis in overweight and centrally obese human subjects [17,18], Although the bioactive compounds against obesity in *R. usambarensis* remain to be discovered, the presence of alkaloids and saponins in the water extract provides leads for the observed weight reduction activity of the plant extract. Discovery of the specific phytochemical groups and compounds in *R. usambarensis* causing the weight reduction may help in its development into medicines for weight reduction.

Rumex usambarensis extract at dose level of 125 mg/kg and 250 mg/kg body weight in this study raised High Density Lipoprotein levels (HDL) significantly as well as total cholesterol and Low Density Lipoproteins (LDL) levels. These findings are similar to studies on *Rumex patientia* seed in diabetic rats in which also serum total cholesterol were not significantly raised while the HDL were significantly raised [19]. Raised HDL is clinically beneficial especially in overweight and obese persons as this helps to reduce the risk of heart diseases and stroke. However, elevation of total cholesterol and LDL is undesirable as these may predispose the users to atherosclerosis and other cardiovascular diseases [20]. Discovery and removal of the phytochemical group and compounds causing elevation of bad cholesterol from the extract may help reduce the observed undesired rise in total cholesterol and Low Densisty Lipoproteins (bad cholesterol).

In this study, the elevation of total cholesterol and LDL were however not significant implying that with right diet, the plant could be used to reduce weight in overweight and obese persons but clinical studies are needed to confirm such benefit. The major limitation of our study is that we were not able to determine the effect of the extract on the body fat of the animals and individual animal food intake. While we can drew conclusion on the effect of the plant extract on over-weight subjects, we are not able to predict the possible mechanism of action of the extract and its effect in obese persons.

5. CONCLUSION

Despite the limitations stated, the study established for the first time that *R. usambarensis* used by some communities in Uganda for satiety effect contains phytochemicals that cause weight reduction in fattened albino rats and may be useful in reducing weight in overweight individuals.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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