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Lower feed and water consumptions and body weight in rats consuming aqueous extracts or ground *leptadenia pyrotechnica*

Bushra S. Alsahafi, Sawsan H. Mahassni*

Department of Biochemistry, Faculty of Science, King Abdualziz University, Jeddah, Saudi Arabia

**Corresponding author: sawsanmahassni@hotmail.com*

Abstract

Leptadenia pyrotechnica (Forssk.) Decne. (LP) is used in folk medicine for the treatment of different ailments. No published studies exist on the effects of ground LP while only one study exists on the effects of aqueous LP extract. Thus, this study is the first to investigate the difference between using ground or aqueous extract of LP on body weight parameters and consumptions of feed and water in young adult Wistar albino rats. Four groups (one rat of either sex per each group) were administered with 25%, 50% and 75% ground LP mixed with the regular feed for 7 days, while the control rats were given the regular diet. Five groups (two rats of either sex per each group) were orally gavage with 3, 9, 15 and 20 g LP extract/kg body weight, while the control group was gavage with water, daily for 14 days. Findings were compared with the control groups. The mean body weight loss and feed inefficiency ratio s for the ground LP groups were higher, leading to lower feed and water intakes. Rats that consumed 15 and 20 g LP extract/kg had higher mean body weight loss and feed inefficiency ratio and lower water and feed intakes for the first but not for the second week. Therefore, both forms of LP affect weights and consumptions of water and feed during the first week. Thus, while using LP for any medicinal or therapeutic uses in humans, any effects on weight or feed and water consumptions may last only for a week.

Keywords: Body weight; feed intake; *Leptadenia pyrotechnica*; rats; water intake.

1. Introduction

Worldwide, between 1980 and 2013, the prevalence of overweight and obesity increased with 2.1 billion individuals being overweight or obese (Ng *et al.,* 2014). According to the World Obesity Federation (2018), by the year 2025 around 3 billion people around the world will be overweight or obese. Obesity is associated with heightened inflammation, which leads to an increased risk for inflammatory and other types of diseases, such as cardiovascular diseases, atherosclerosis, type 2 diabetes, and some types of cancer.

Many plants and seeds have been used in folk and traditional medicinal systems as natural methods for the reduction of weight and lipid levels. There are several published studies on the effectiveness and safety of certain medicinal plants, seeds, and herbs for weight reduction, such as ginger (Nguyen, 2012), nigella sativa (Hasani-Ranjbar *et al.,* 2013), rhubarb, coptis, cassiae semen, and *citrus aurantium* (Zhou *et al.,* 2014), and for

reducing levels of lipids like *eurycoma longifolia* (Lim *et al.,* 2019).

Leptadenia pyrotechnica (Forssk.) Decne. (LP) belongs to the family of Asclepiadaceae, which is a desert plant that grows in the sandy regions in countries of the Arabian Gulf and equatorial regions of Asia and Africa. LP is a shrub that has many green branches with a height of 0.5 to 2.6 meters (Khasawneh *et al.,* 2015). All parts of the plant contain five major active components, which are alkaloids, flavonoids, tannins, saponins (Munazir *et al.,* 2015) and cardiac glycosides (Youssef *et al.,* 2009). The stem contains polyphenolic compounds (Preet & Chand 2018) along with some other chemical components, such as fatty acids, steroids and terpenes (Youssef *et al.,* 2007).

The decoction extract of the young stems of LP is used in Saudi Arabian local traditional medicine for the treatment of flu and as a tussive (Randa & Youssef, 2013), in India it is used to treat tuberculosis (Patel *et al.,* 2014), rheumatism and gout and in Pakistan it is used as an antihistaminic and expectorant (Bhabootra, 2016). In Sudan, the decoction extract of LP roots is used to treat constipation (Bhabootra, 2016), while in Yamen the stem paste is used for the treatment of wounds (Upadhyay *et al.,* 2007) and the sap of young steams is used for diabetes, eczema and other skin diseases (Katewa & Galav, 2006). None of these effects has been proven scientifically.

Only one previous study, done by us (Alsahafi & Mahassni, 2021), used the aqueous LP extract and it determined its effects on the immune system, daily body weights, and feed and water consumptions. After an extensive search in the internet, no other research studies using aqueously extracted or ground LP in animals or humans were found. In addition, there is only one study on the effect of the alcoholic extract of LP on weights. This study used LP alcoholic extracts on rats for two months finding no effects on weights (Soliman *et al.,* 2012). Since there are no published studies on the effectiveness of aqueous extracts or ground LP for weight reduction in humans and animals, this study aimed to determine the suitability of using LP (aqueously extracted or ground) to suppress appetite and/or to reduce body weight (BW) in rats.

2. Materials and methods

2.1 Rats and diet

Female and male Wistar albino rats used for this study were all provided with the regular rats feed ad libitum. The diet (Grain Silos and Flour Mills Organization, Jeddah, KSA) contained 20.0 % protein, 4.0 % fat, 5.0 % fiber, 6% ash, 1.0% vitamin mix (vitamin A, D and E), 3.50% mineral mix (calcium, phosphorus, cobalt, copper, iodine, iron, manganese, selenium and zinc), 0.25% choline chloride, and 60.25% cornstarch to complete the 100%. One kilogram of this diet provides 2850 kcal.

2.2 Preparation of the ground LP diets

Freshly collected young LP stems were washed with water and allowed to air dry. Then the stems were coarsely ground and mixed with the ground rat feed at three different

concentrations, as described below. Subsequently, these diets were repelleted, with the aid of a small amount of water, and allowed to air dry.

2.3 Collection of LP stems and preparation of the aqueous extract

Young LP stems were harvested from the Khulais governorate, Makkah, KSA, and then washed with distilled water. Subsequently 500 g of stems were boiled in 1 L of water for 5 minutes (Patel *et al.,* 2014). The hot water extract was filtered using cotton balls and it was allowed to air dry for two days. The extract resulted in a greenish brown colored semisolid precipitate. The evaporation of 30 ml of extract resulted in 18 g of semisolid precipitate.

The LP dose used for the rats was calculated (Reagan-Shaw *et al.,* 2008; Pandy, 2020) based on the previously used human dose (30 ml/day) (Patel *et al.,* 2014).

2.4 Study design

Fourteen female and fourteen males healthy Wistar albino rats, weighing between 170-250 g, were allowed to adapt to the laboratory for one week, after which, they were divided into two groups, with one administered ground LP while the other administered the aqueous LP extracts. For each group, the overall mean BW loss, percent relative overall BW loss, feed and water consumptions and feed inefficiency ratio (FIR) were calculated daily and weekly.

Three ground LP groups (LPG) and a control group (one female and one male per group) were administered, daily for one week, with ground LP at different concentrations (LPG 1: 25%, LPG 2: 50% and LPG 3: 75% ground LP mixed with the regular ground rat feed) while the control rats were administered with the regular feed.

Four LP aqueous extract groups (LPE) and a control group (two females and two males per group) were used. Based on the results of a previous unpublished acute toxicity study (Mahassni & Alsahafi, 2022), four different doses of dried LP extract, dissolved in 3 ml water, were orally gavaged (LPE 1: 3, LPE 2: 9, LPE 3: 15, and LPE 4: 20 g LP/kg rat BW) while the control was gavaged with 3 ml of water, daily for two weeks.

2.5 Statistical analysis

The Mega Stat statistical program (Version 9.4, Butler University, and Indianapolis, Indiana, USA) was used for the statistical analysis. The pairwise *t*-test was used for the significance testing between groups for all parameters. The statistical difference was considered significant for $P < 0.05$, highly significant for $P < 0.01$ and non-significant for $P \geq 0.05$.

3. Results and discussion

There are no previous research studies in humans or laboratory animals on the effects of ground LP and only one published study (Alsahafi & Mahassni, 2021), done in rats, on the effects of the aqueous LP extracts on BW gain/loss, feed and water consumptions, and FIR

in humans or laboratory animals. Therefore, this is the first study to determine these effects for different concentrations of ground LP, mixed with the regular diet of rats, and aqueous LP extract gavage to rats. In addition, this study is the first to investigate the differences in the effects of ground and aqueous LP extracts on weights and weight related parameters, and feed and water consumptions. Thus, we were unable to compare any of our evaluations and findings with previous studies.

The amounts of ground LP used were not based on any previous studies since it was never used before. This is also the case for the amount used for the extract, although a toxicity study using the extract was done previously by us (Mahassni & Alsahafi, 2022). In this unpublished study, the extract was found to be safe up to a dose of 40 g/kg body weight. Therefore, the highest dose used in the present study (20 g/kg) is well below the highest safe dose.

3.1 Daily physiological evaluation

The mean overall BW loss (Table 1) for the rats in the LPG 2 and 3 groups were significantly higher compared with the control. In addition, the mean percent relative overall BW loss was significantly higher for the LPG 1, 2 and 3 rats. The mean daily feed and water consumptions for the LPG 1, 2 and 3 groups were significantly lower compared with the respective control. Mean daily FIRs were significantly higher for the LPG 1, 2 and 3 groups compared with the control.

It is noteworthy that as the percent of LP mixed with the diet increases so does the BW loss, while the feed and water consumptions decrease and the feed inefficiency increases. Thus, the increased BW loss for the ground LP groups may be due to the reduced feed consumption resulting from the possibility that the diets with the higher amounts of LP were non-palatable. In fact, the rats showed signs of dislike for the diet since they scattered it in the cage instead of eating it. In addition, this was more apparent for the higher percent of LP. This reduced feed consumption may have subsequently resulted in a reduced need for water consumption as evidenced by the results.

The mean overall BW loss (Table 2) for the rats of the LPE 3 and 4 groups were significantly higher compared with the control. In addition, compared to the control, the mean percent relative overall BW loss was significantly higher for all LPE rats. Compared with the control, the mean daily feed consumptions for the LPE 2, 3, and 4 groups were significantly lower. The LPE 3 and 4 groups showed significantly lower mean water consumptions and significantly higher mean FIR compared with the respective controls.

Table 1. Physiological evaluation for the ground LP groups.

Parameter	Group	Mean±SD	P-value
Daily BW (g)	Control	198.00±19.80	
	LPG 1	174.50±20.51	0.285
	LPG 2	168.50±23.33	0.196
	LPG 3	171.00±9.90	0.229
Overall BW loss (g)	Control	2.00±2.83	
	LPG 1	20.00±22.63	0.330
	LPG 2	51.00±18.38	0.039*
	LPG 3	72.00 ±14.14	0.012*
Percent relative overall	Control	1.00±1.41	
BW loss (%)	LPG 1	19.50±0.71	0.010*
	LPG 2	25.00±5.66	0.004**
	LPG 3	34.00±5.66	0.001**
Daily feed consumption (g)	Control	22.00±0.71	
	LPG 1	19.50±1.41	0.001**
	LPG 2	10.00±2.83	0.000**
	LPG 3	11.10±1.41	0.000**
Daily water consumption	Control	20.50±3.54	
(ml)	LPG 1	13.50±0.71	0.026*
	LPG 2	12.00±2.83	0.021*
	LPG 3	8.50±0.71	0.005**
	Control	0.10±0.00	
Daily FIR	LPG 1	0.30±0.00	0.017*
	LPG 2	0.50±0.28	0.034*
	LPG 3	1.50±0.42	0.009**

LPG: ground LP group; *Significant; **highly significant

Table 2. Daily and weekly physiological evaluation for the extract groups.

Parameter	Group	Daily		Weekly			
				First week		Second week	
		Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value
BW (g)	Control	208.50±21.21		207.00±23.23		209.75±19.72	
	LPE 1	200.00±18.79	0.626	200.00±20.51	0.620	200.50±18.72	0.592
	LPE 2	197.50± 26.89	0.518	190.75±14.57	0.276	194.75±25.12	0.388
	LPE 3	196.25±13.00	0.472	191.30±11.62	0.290	179.00±13.14	0.088
	LPE 4	195.00±32.69	0.429	207.50±29.86	1.000	199.30±36.14	0.543
BW loss (g)	Control	2.25±2.63		2.75±2.63		0.50±0.58	
	LPE 1	7.50±5.69	0.238	6.75±7.89	0.311	0.00±0.00	0.308
	LPE 2	8.25±4.79	0.103	10.50±1.73	0.060	0.00±0.00	0.308
	LPE 3	17.25±3.86	0.000**	15.25±7.41	0.005**	0.70±0.96	0.605
	LPE 4	15.57±6.55	0.000**	17.00±4.32	0.002**	0.50±1.00	1.000
Percent relative BW loss (%)	Control	1.00±1.15		1.25±1.19		0.24±0.28	
	LPE 1	4.50±3.70	0.029*	3.12±3.61	0.331	0.00±0.00	0.371
	LPE 2	4.80±1.71	0.021*	5.20±1.24	0.051	0.00±0.00	0.371
	LPE 3	8.25±1.71	0.000**	7.43±3.17	0.005**	0.41±0.53	0.542
	LPE 4	9.50±0.58	0.000**	7.97±2.96	0.002**	0.29±0.59	0.854
Feed consumption (g)	Control	23.50±2.31		26.00±5.77		28.50±8.66	
	LPE 1	22.00±1.15	0.323	26.00±1.15	1.000	25.00±2.31	0. 384
	LPE 2	20.00± 0.00	0.030*	26.50±1.73	0.849	26.00±3.46	0. 531
	LPE 3	19.50± 2.31	0.015*	22.50±2.89	0.197	23.00±5.77	0.220
	LPE 4	19.25±2.87	0.011*	24.00±4.62	0.452	23.50±5.20	0.179
Water consumption (ml)	Control	30.00± 2.31		29.00±1.15		25.50±2.89	
	LPE 1	28.50±0.58	0.582	26.00±2.31	0.179	26.00±1.73	0.876
	LPE 2	28.50±5.20	0.582	25.00±3.46	0.079	26.50±0.00	0.755
	LPE 3	20.00±2.31	0.002**	19.00±2.31	0.000**	22.00±6.93	0.285
	LPE 4	24.00±5.77	0.040*	24.00±4.62	0.033*	23.50±6.35	0.535
FIR	Control	0.06±0.04		0.10±0.10		0.02±0.02	
	LPE 1	0.08±0.03	0.514	0.25±0.29	0.464	0.00±0.00	0.262
	LPE 2	0.08±0.02	0.464	0.39±0.07	0.173	0.00±0.00	0.262
	LPE 3	0.15±0.05	0.012*	0.58±0.47	0.032*	0.03±0.03	0.818
	LPE 4	0.17±0.05	0.002**	0.74±0.28	0.006**	0.03±0.05	1.000

LPE: LP extract group; *Significant; **highly significant

3.2 Weekly physiological evaluation for the extract groups

As shown in (Figure 1), the weight loss for the first week for the LP groups increased with the increase of the extract dose, while for the second week the differences between the groups, compared to the control, were much smaller. The mean for all groups (including the control) for the first week was nearly the same as the mean for the LPE 2 group, while for the second week the mean for all groups was the same as for the control group. Therefore, the weight loss for the LP groups for the first week were higher than for the control, meaning the extract led to a reduction in the BWs of the rats. On the other hand,

the weight losses for the LP groups for the second week were negligible and nearly the same as for the control rats.

In the first week, the mean weekly BW loss and its mean percent and FIR (Table 2) were significantly higher for the LPE 3 and 4 groups compared with the respective controls. The mean water consumptions for the LPE 3 and 4 groups were significantly lower for the first week compared with the respective control.

When comparing the mean weekly BW loss and it's percent between the two weeks for all LP aqueous extract groups, shown in (Tables 3 and 4), it was revealed that there were significant decreases for the second week compared to the first week for LPE 2, 3 and **4.** As for the feed and water consumptions (Tables 5 and 6), there were no significant differences between the weeks for all groups. Comparing the mean weekly FIR between the two weeks for all extract groups (Table 7), there were a significant decrease for the LPE 2, 3 and 4 groups for the second week.

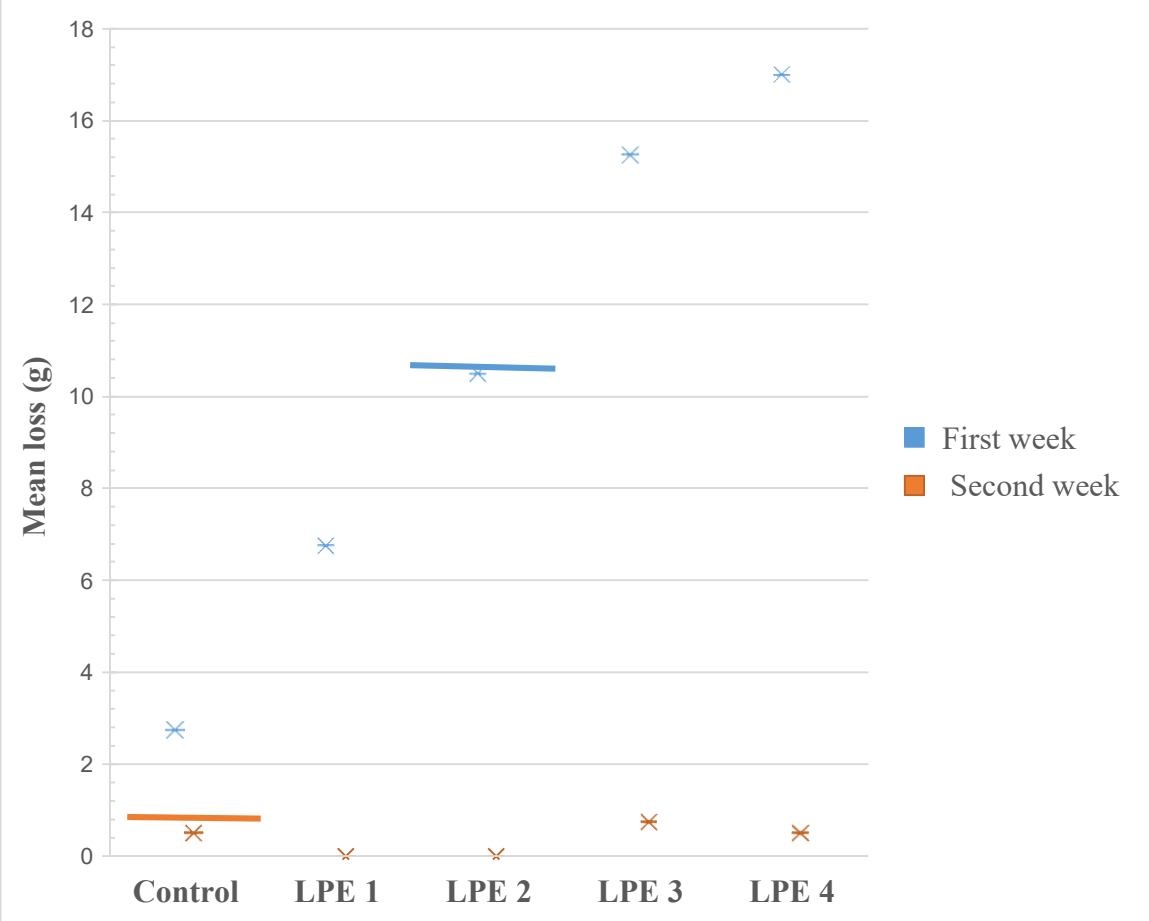

Fig. 1. Mean weekly BW loss (g) for each group for the first and second weeks (stars) and the overall mean for all groups for each week (lines).

Table 3. Comparing the weekly BW loss for the extract groups between the two weeks.

Group	Week	Weekly BW loss (g)		
		Mean±SD	Mean difference±SD	P-value
Control	First	2.75±2.63	2.25±2.75	0.200
	Second	0.50±0.58		
LPE 1	First	6.75±7.89	6.75±7.89	0.185
	Second	0.00±0.00		
LPE 2	First	10.50±1.73	10.50±1.73	0.001**
	Second	0.00±0.00		
LPE 3	First	15.25±7.41	14.50±8.34	0.040*
	Second	0.75±0.96		
LPE 4	First	17.00±4.32	16.50±4.12	0.004**
	Second	0.50±1.00		

LPE: LP extract group; *Significant; ** highly significant

Table 4. Comparing the percent relative weekly BW loss for the extract groups between the two weeks.

Group	Week	Percent relative weekly BW loss (%)		
		Mean±SD	Mean difference±SD	P-value
Control	First	1.25±1.19	1.00±1.47	0.266
	Second	0.24±0.28		
LPE 1	First	3.12±3.61	3.12±3.61	0.181
	Second	0.00±0.00		
LPE 2	First	5.20±1.24	5.20±1.24	0.003**
	Second	0.00±0.00		
LPE 3	First	7.43±3.17	6.95±3.69	0.032*
	Second	0.41±0.53		
LPE 4	First	7.97±2.96	7.67±2.518	0.008**
	Second	0.29±0.59		

LPE: LP extract group; *Significant; **highlysignificant

Table 5. Comparing the weekly feed consumption for the extract groups between the two weeks.

Group	Week	Weekly feed consumption (g)		
		Mean±SD	Mean difference±SD	P-value
Control	First	26.00±5.77	-2.50±2.88	0.181
	Second	28.50±8.66		
LPE 1	First	26.00±1.15	1.00±3.46	0.604
	Second	25.00±2.31		
LPE 2	First	26.50±1.73	0.50±1.73	0.604
	Second	26.00±3.46		
LPE 3	First	22.50±2.89	-0.50±8.66	0.915
	Second	23.00±5.77		
LPE 4	First	24.00±4.62	0.50±9.81	0.925
	Second	23.50±5.20		

LPE: LP extract group

Table 6. Comparing the weekly water consumption for the extract groups between the two weeks.

Group	Week	Weekly water consumption (g)		
		Mean±SD	Mean difference±SD	P-value
Control	First	29.00±1.15	3.50±3.10	0.109
	Second	25.50±2.89		
LPE 1	First	26.00±2.31	0.00±2.30	1.000
	Second	26.00±1.73		
LPE 2	First	25.00±3.46	-1.50±3.87	0.495
	Second	26.50±0.00		
LPE 3	First	19.00±2.31	-3.00±9.23	0.562
	Second	22.00±6.93		
LPE 4	First	24.00±4.62	0.50±7.85	0.906
	Second	23.50±6.35		

LPE: LP extract group

Table 7. Comparing the weekly FIR for the extract groups between the two weeks.

Group	Week	Weekly FIR		
		Mean±SD	Mean difference±SD	P-value
Control	First	0.10±0.10	0.08±0.10	0.239
	Second	0.02±0.02		
LPE 1	First	0.25±0.29	0.25±0.29	0.183
	Second	0.00±0.00		
LPE 2	First	0.39±0.07	0.39 ±0.07	0.001**
	Second	0.00±0.00		
LPE 3	First	0.58±0.47	0.53±0.32	0.044*
	Second	0.03±0.03		
LPE 4	First	0.74±0.28	0.71±0.29	0.016*
	Second	0.03±0.05		

LPE: LP extract group; *Significant; **highly significant

Results of the present study showed that both the ground and extract LP diets resulted in higher overall and percent relative body weight losses for the groups, which was linked to lower feed and water consumptions and higher feed inefficiency ratios. These effects increased as the amount of ground or LP extract administered increased. In addition, these effects for the LP extract were apparent for the first week only, but not for the second week. Therefore, any effects of LP on weight might only be during the first week.

The significant decrease in BW, feed intake and significant increase in weight loss in the rats may be due to the phenolic compounds, such as tannin, that are naturally present in vegetables and plants (Manzoor *et al.,* 2021) including LP (Munazir *et al.,* 2015) and has been shown to reduce weight and feed intakes in rats (Chung *et al.,* 1998; Nakamura *et al.,* 2001; Manzoor *et al.,* 2021). This is in addition to the previously mentioned undesirability of the feed mixed with the LP.

As stated above, there are no previous studies that can be compared with the current results with the exception for the previously mentioned study on BW using the alcoholic extract and on BW gain/loss, feed and water consumptions and FIR using aqueous LP extracts. Thus, studies on medicinal plants, other than LP, and seeds are compared here. The current results disagree with a previous study in rats consuming ground *lepidium sativum* seeds mixed with the regular diet for 39 days that showed higher overall BW gain percent and lower consumed feed and water compared to the control (Mahassni & Nabulsi, 2020). Moreover, a previous study (Gauthaman *et al.,* 2003) using different concentrations of the puncturevine plant extract on male rats for two months showed an increase in BW. A previous study (Mahassni & Khudauardi, 2017) also using an aqueous extract of *lepidium sativum* seeds on mice for 19-21 days showed an increase in BW. An increase in

the body weights of Wistar rats following oral administration of methanol extract of *ganoderma lucidum* for 21 days was found in a previously study (Shamaki *et al.,* 2017).

On the other hand, the present findings agree with our previous study (Alsahafi & Mahassni, 2021) on the aqueous LP extract gavaged to rats for three weeks that resulted in a significantly lower BW and feed and water intakes and significantly higher BW loss and FIR. Another study in agreement with the current findings is a study (Eddouks *et al.,* 2005) on aqueouse *lepidium sativum* extract administered for two weeks to healthy rats, which showed lower BW and feed intake. Additionally, findings of a previous study (Alsoodeeri, *et al.,* 2020) in rats administered with *cinnamomum cassia* for 30 days showed a reduction in BW gain, feed intake and feed efficiency ratio. A previous study (Manal *et al.,* 2016) using ethanol and ethyl acetate extracts of *maerua psuedopetalosa* administered to Wistar rats for a week also showed a reduction in BW.

4. Conclusions

The ground and LP extracts resulted in higher overall and percent relative BW losses for the groups, which was linked to lower feed and water consumptions and higher FIRs. These effects increased as the amount of ground or LP extract administered increased. In addition, the effects for the LP extract were apparent for the first week only, but not for the second week. Therefore, any effects of LP on weight might only be during the first week. The same may be expected to happen in humans. Thus, while using LP for any medicinal or therapeutic uses, any effects on weight or feed and water consumptions may last only for the first week of usage.

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