



Effect of Juice Extract of Beetroot, Lemon, Pawpaw Leaf and Their Combinations on Weight and Lipid Profile of Albino Wistar Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IG, LW and SBM designed the study. Authors IG, SWK and SBM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ERE and AAH managed the analyses of the study. Authors IG and SWK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Fruits and vegetables have been found to contain important phytochemicals that have been implicated in preventing overweight and obesity. This study is aimed at investigating the effects of lemon, pawpaw leaves, beetroots juices and their combinations on rates of urination, defecation, and weight changes of albino wistar rats were examined at interval. Also, the effects of the juice extracts on cholesterol, triglyceride, high density lipoprotein and low density lipoprotein were examined. Juice was extracted using a laboratory extractor. Results obtained indicate an increase in weight in groups A, B, C, D, E, F and G. But a decrease in weight was noticed in group H, which is the group administered all juice extracts at equal proportion. A reduction in the volume of urine was noticed in group A, B, C, D, F, G and H. Urine level was seen to increase in group E. The

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quantity of feces was observed to reduce in groups A, E, F, G and H. with the exception of groups B,C and D where the feces level was seen to increase. Cholesterol level was observed to reduce drastically in group D compared with other groups. Triglyceride level was observed to be low in group B compared with other groups. HDL level was very low in group H compared to other groups. LDL level was very low in group D compared with other groups. Group D also had a very high effect on HDL and LDL by increasing and reducing HDL and LDL respectively. This is only second to control group. The above evidences suggests that some fruit juices directly inhibits weight gain by providing the needed calories required by the body for its activity. This implies that consumption has a potential role in the prevention of overweight and obesity.

Keywords: Fruit; juice; weight; regulation.

1. INTRODUCTION

Obesity is a global epidemic with multifactorial causes. It occur as a result of an imbalance between energy intake and energy consumption [1]. With the exception of having a gene which predisposes an individual to overweight and obesity, they are usually associated with environmental factors, among which are sedentary lifestyle and over-consumption of high-energy yielding foods which are the main contributors [1]. Many local and globally recognized food-based dietary guidelines recommend an increase in the consumption of fruits and vegetables to prevent the emergence of several diseases such as cardiovascular diseases and certain types of cancer [2,3,4]. The scientific evidence for an inverse association between the risk of being overweight and obese, and the consumption of fruits and vegetables, was considered convincing [2].

There is generally a positive view about absolute fruit juices, some public health bodies have raised concerns about their effects on body weight, specifically in relation to overconsumption and sugar content. Consumption of 100% fruit juice In Europe, the average daily consumption of beverages during childhood and adolescence is 1455 ml, which is mostly represented by water. On average, drinks provide 1,609 kJ (385 kcal), about 30% was found come from sugar-sweetened beverages, 21% was found to come from sugar-sweetened milk, and 18% was found to come from fruit juices [5]. Industry published data indicates that the average intake of 100% fruit juice in Europe is just 31 ml daily [6], which is said to be equivalent to 13 kcal daily, it is also one third of a portion of fruit. These statistics suggest that the assertion that overconsumption of fruit juice are misplaced. If the daily consumption is rose to 150 ml, the amount that is equal to one portion of fruit in some European countries, daily energy intake

from absolute fruit juice would only be 62 kcal or 3% of the Reference Intake (2000 kcal). Research results on this subject are inconsistent, owing to the fact that differing approaches have been used. Furthermore, most researchers do not differentiate between absolute fruit juice and juices that contain added sugars. In most studies the control is represented by the consumption of sugar-sweetened carbonated drinks. Few researchers have considered the specific impact of 100% fruit juice on adult body weight and the literature is predominantlyly observational data which cannot determine cause and effect. The outcomes of these researches differ from positive correlations between adult weight gain, albeit clinically insignificant amounts and 100% fruit juice intake [7,8] to inverse associations between body mass index, waist circumference and 100% fruit juice intake [9]. A study of a large European population shows no association between the combined consumption of 100% fruit juice and fruit nectars and body mass index [10]. A similar observation was established in the Food4Me survey of European adults from seven different countries [11].

It is imperative to note that fruit juices consumption tends to be statistically associated with beneficial health behaviours which includes reduced alcohol consumption, not smoking and increased physical activity.

Consequently, a few randomised controlled researches that are available, show no impact on weight management, even at consumption up to 500 ml daily. A recent review on citrus fruit juices indicates that "Based on clinical intervention studies, the addition of orange juice or grapefruit juice to a habitual or study diet did not result in weight change, these suggest that individuals likely compensated with other dietary choices" [12]. A similar findings in a 2017 randomised controlled trial in which 500 ml of orange juice was consumed daily for 12 weeks as part of

calorie-controlled diet [13]. The sugars content of 100% fruit juice has come under close observation with regards to weight addition and risk of obesity. These sugars are contained in the fruit used to make the juice. Following a substantial systematic review, a group of WHO commissioned researchers established that excess calories were responsible for weight gain, not sugars specifically [14]. Therefore, when energy consumption is balanced with energy expenditure through activities like exercises and other physical activities, dietary sugars from 100% fruit juice do not increase the risk of excess weight gain. Fruit juice consumption and weight in children Data from 12 of the 19 studies identified in the literature failed to establish a significant correlation between habitual consumption of fruit juices and BMI.

Furthermore, there are three studies that shows that, when the intake of absolute fruit juices increased, the risk of adding more weight and obesity statistically and significantly decreased; meanwhile, three other researches establish that a significant relationship between consumption of fruit juices and weight gain exist. However, the intake levels in these researches were particularly high and included drinks other than 100% fruit juice. Another study established that higher juice intake levels were only associated with a further risk of overweight in children who were already gaining weight or predisposed to it. Conversely, more consumption of fresh fruit juice was associated with a lower increase in body weight [15]. A 2016 systematic review supported this result by establishing that 22 studies in children and adolescents found no significant associations between intake of absolute fruit juice and weight increase after controlling for energy intake [16].

In view of the different reports of the various researches mentioned above, this study is focusing on investigating the effects of different fruit juices and their combination on weight changes and lipid profile of albino wistar rat. This is to verify the various claims for and against the fact that fruit juice can increase or decrease body weight.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty (40) wistar strain albino rats were purchased from the animal house of the University of Jos.

2.2 Treatment of Experimental Animals

The animals were randomly selected (to avoid bias) and divided in Eight (8) groups, A, B, C, D, E, F, G and H of 4 animals per group.

- A. Normal control (normal animals given standard feed and water).
- B. Normal animals given standard feed, water and Lemon juice.
- C. Normal animals given standard feed, water and Beetroot.
- D. Normal animals given standard feed, water and Paw-paw leaf juice.
- E. Normal animals given standard feed, water and Lemon + beetroot juice.
- F. Normal animals given standard feed, water and Lemon + paw-paw leaf juice.
- G. Normal animals given standard feed, water and Beetroot + paw-paw leaf juice.
- H. Normal animals given standard feed, water and Lemon + beetroot + paw-paw leaf juice.

2.3 Juice Extraction

Juices of Beat Root, Lemon and Pawpaw Leaf were extracted using laboratory homogenizer at room temperature.

2.4 Biochemical Parameters

Biochemical parameters assayed; Total cholesterol concentration, serum HDL cholesterol and triacylglyceride were done using Randox diagnostic kits. LDL-cholesterol was estimated using Friedewald formula [17].

2.5 Statistical Analysis

Data were subjected to analysis using graph pad prism 7 statistical tool. One way analysis of variance (ANOVA) was used for comparison of different groups and values were considered significant at $P < 0.05$. Results were presented as the means \pm SEM.

3. RESULTS AND DISCUSSION

Results from Table 1 showed the effects of different fruit juices and their combination on increase or decrease in weight of the rats at different interval. Group A which is a control group was not administered any fruit juice, which accounts for the steady increase in the body weight of the rats as a result of the caloric intake from the continuous feeding of the rat.

Group B, C, D, E and H were administered lemon juice, beetroot, pawpaw leaf, lemon plus beetroot, beetroots plus pawpaw leaf and Lemon plus beetroot plus pawpaw leaf juice respectively at different interval. After administration for a period of time, a fluctuation in weight was observed, that is a decrease and subsequent increase in weight or increase, then decrease and subsequently increase in weight was later observed too. This observation contradicts the assertion by [6] that even if the quantity consumed per day is increased, the caloric addition remains the same. Our observation about a decrease and a subsequent increase in the body weight could be as a result of the constant volume maintained in our work. Telling us that as the animal grow in size and increases its feed consumption, it predisposes it to weight addition even though its initial weight was reduced based on the interaction of caloric intake and the various juices consumed. Also, the differences in weight fluctuation observed in this groups could be as a result of the different composition of the various fruit juices used in this research.

Group F was administered lemon plus pawpaw leaf juice. A steady increase in body weight was observed in this group. This observation agreed with the assertion by 12, 13 and 14 that 100% juice intake does not reduce body weight. This implies that the constant increase in weight observed is as a result of the calories obtained from the feeds given to the rats. Furthermore, it also indicates that the caloric intake is almost equal to the energy expel and as the animal grow in size, the caloric intake increases. This enables the rat to maintain a constant increase in weight as the age increases.

The fluctuations and differences observed in the different groups could be attributed to the differences in type of carbohydrate contained in the different fruits and their combination. This assertion agrees with the finding by [18] which states that "The type and amount of fruits and vegetables may affect satiety and intake".

Table 2 shows the volume of urination with interval across the various groups.

Table 1. Weight changes recorded in albino wistar rats with interval

Group	Treatment	Day 23/7	Day 24/7	Day 29/7	Day 30	Day 1/8	Day 2/8	Day 5/8	Day 8/8
A	Control	99.63± 12.863	92.50± 11.715	94.23± 12.656	95.66± 12.560	114.27± 15.519	105.67 ±11.570	106.60± 14.442	111.90± 15.840
B	Lemon juice	121.13± 4.511 ^b	115.10± 6.334 ^b	118.50± 6.495 ^b	128.53± 4.418 ^b	133.33± 5.052 ^b	134.77 ±4.206 ^b	127.13± 5.861 ^b	134.00± 7.057 ^b
C	Beetroot	111.58± 8.618 ^b	104.35± 7.952 ^b	109.45± 8.459 ^b	120.28 ±10.184 ^b	124.23± 9.438 ^b	125.68 ±9.648 ^b	126.70± 8.625 ^b	136.38± 9.147 ^b
D	Paw-paw leaf juice	108.50± 5.828 ^b	119.78± 17.233 ^b	101.18± 8.227 ^b	105.70± 8.755 ^b	114.15± 8.463 ^a	115.18 ±8.271 ^b	108.33± 8.436 ^b	120.25± 9.411 ^b
E	Lemon + beetroot juice	119.93± 6.018 ^b	109.18± 4.570 ^b	103.98± 4.745 ^b	108.9± 3.737 ^b	111.90 ±4.688 ^a	113.50± 4.049 ^b	117.58± 4.111 ^b	125.85± 4.884 ^b
F	Lemon + paw-paw leaf juice	92.32± 27.826 ^a	110.80± 6.500 ^b	111.40± 6.949 ^b	117.28± 7.592 ^b	125.75 ±8.265 ^b	125.68± 9.430 ^b	119.13± 12.963 ^b	135.83± 9.231 ^b
G	Beetroot + paw leaf juice	90.92± 14.300 ^a	112.07± 2.046 ^b	103.67± 2.368 ^b	116.83± 5.019 ^b	114.97 ±4.484 ^b	123.27± 4.201 ^b	120.37± 4.088 ^a	134.13± 3.805 ^b
H	Lemon + Beet root + paw-paw leaf juice	96.32± 8.999 ^a	88.45± 7.336 ^a	85.45± 6.887 ^a	97.50± 9.607 ^b	99.82 ±9.076 ^a	102.38± 8.935 ^a	103.80± 9.617 ^a	116.23± 16.610 ^b
p-values	-	0.6336	0.2834	0.1337	0.1552	0.2575	0.1618	0.5116	0.5254

If p-value is greater than 0.05, mean values are not statistically significant ($p < 0.05$)

Values are expressed as mean ± SEM, n=5.

^aValues are significantly low when compared to control ($p < 0.05$)

^bValues are significantly high when compared to control ($p < 0.05$)

Table 2. Changes in urine excretion

Group	Treatment	22/07	24/07	26/07	29/07	31/07	2/08	5/08	7/08
A	Control	12.62± 0.0115	9.06± 0.037	10.10± 0.063	6.61± 0.012	3.04± 0.026	3.82± 0.014	8.94± 0.032	2.13± 0.100
B	Lemon juice	10.08± 0.049 ^a	10.51± 0.015 ^b	12.54± 0.020 ^b	2.62± 0.017 ^a	9.04± 0.030 ^b	7.15± 0.078 ^b	12.02± 0.024 ^b	1.07± 0.039 ^a
C	Beetroot	5.33± 0.025 ^a	4.06± 0.032 ^a	8.24± 0.023 ^a	10.04± 0.020 ^b	6.05± 0.032 ^b	9.06± 0.034 ^b	2.04± 0.029 ^a	3.53± 0.027 ^b
D	Paw-paw leaf juice	6.05± 0.031 ^a	2.52± 0.014 ^a	16.50± 0.003 ^b	10.63± 0.025 ^b	2.03± 0.020 ^a	18.10± 0.060 ^b	12.03± 0.024 ^b	3.11± 0.050 ^b
E	Lemon + beetroot juice	3.31± 0.008 ^a	8.06± 0.048 ^a	11.09± 0.057 ^b	16.61± 0.013 ^b	5.52± 0.015 ^b	2.81± 0.008 ^a	3.10± 0.053 ^a	6.56± 0.048 ^b
F	Lemon + paw-paw leaf juice	9.05± 0.035 ^a	2.50± 0.043 ^a	3.54± 0.026 ^a	8.32± 0.018 ^b	4.13± 0.081 ^b	22.03± 0.017 ^b	11.08± 0.067 ^b	0.53± 0.023 ^a
G	Beetroot + paw-paw leaf juice	7.31± 0.010 ^a	5.55± 0.029 ^a	11.09± 0.057 ^b	10.63± 0.025 ^b	6.05± 0.032 ^b	2.03± 0.020 ^a	3.10± 0.053 ^a	4.05± 0.028 ^b
H	Lemon + beetroot + paw-paw leaf juice	7.07± 0.060 ^a	3.47± 0.039 ^a	17.08± 0.040 ^b	10.04± 0.020 ^b	6.53± 0.020 ^b	6.13± 0.091 ^b	2.04± 0.029 ^a	6.56± 0.048 ^b
p-value	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

If p-value is greater than 0.05, mean values are not statistically significant ($p < 0.05$)

Values are expressed as mean \pm SEM, n=5.

^aValues are significantly low when compared to control ($p < 0.05$)

^bValues are significantly high when compared to control ($p < 0.05$)

There were consistent fluctuation in volume of urine in all the treatment groups. Groups A, B, C, D, F, G and H showed a high volume of urine initially before consistent fluctuation was observed. While group E showed a low volume of urine before consistent fluctuation was observed. The reason for consistent fluctuation of urination is unclear but it may not be unconnected to the fact that the rate of feeding fluctuates as a result of the calories [18] absorbed from the juice which in turn affects the level of thirst per time and urine excretion.

Table 3 shows the quantity of fecal material excreted with interval across groups. There were consistent fluctuation in quantity of fecal material in all the treatment groups. This fluctuations may not be unconnected to the rate of feed consumption with interval as the fruit juice administration progresses.

Over weight and or obesity is associated with high level of lipid in the body. Table 4 shows the effect of different fruit juice consumption and lipid profile.

Group H had little or no effect on cholesterol compare to control which increases the risk of cardiovascular disease in this group while group D had significant effect on cholesterol which reduces the risk of cardiovascular disease in this group. This agreed with assertion by [19] that an increase in blood cholesterol of overweight person could lead to atherosclerosis and cardiovascular diseases.

Group E and F which were administered Lemon + beetroot juice and Lemon + paw-paw leaf juice had little or no effect on triglyceride considering how close they are with control (group A). This results are in agreement with the assertion that triglycerides are the most common fat molecule in our bodies. The body converts any excess calories you eat into triglycerides for storage. Blood triglyceride levels are significantly impacted by diet and lifestyle as well as other genetic factors. Diets high in refined carbohydrates (such as sugar and white bread), and alcohol signal the liver to produce more triglycerides. Saturated fat from the diet also raises triglyceride levels [20].

Table 3. Changes in feces excreted

Group	Treatment	Day 23/7	Day 24/7	Day 29/7	Day 30	Day 1/8	Day 2/8	Day 5/8	Day 8/8
A	Control	25.23± 0.024	35.12± 0.060	41.81± 0.095	37.09± 0.057	26.96± 0.045	31.18± 0.135	24.87± 0.171	22.60± 0.421
B	Lemon juice	32.60± 0.003 ^b	32.63± 0.031 ^a	27.77± 0.134 ^a	92.45± 0.028 ^b	45.05± 0.035 ^b	51.77± 0.136 ^b	81.13± 0.142 ^b	81.71± 0.178 ^b
C	Beetroot	41.60± 0.003 ^b	36.15± 0.039 ^b	46.13± 0.023 ^b	49.69± 0.162 ^b	25.68± 0.046 ^a	39.59± 0.260 ^b	35.03± 0.062 ^b	50.01± 0.012 ^b
D	Paw-paw leaf juice	47.60± 0.146 ^b	57.26± 0.032 ^b	32.07± 0.050 ^a	64.54± 0.104 ^b	33.97± 0.051 ^b	31.18± 0.135 ^c	39.18± 0.046 ^b	76.83± 0.103 ^b
E	Lemon + beetroot juice	23.43± 0.008 ^a	42.33± 0.049 ^b	39.13± 0.038 ^a	68.54± 0.041 ^b	34.18± 0.046 ^b	57.36± 0.037 ^b	36.34± 0.029 ^b	38.09± 0.283 ^b
F	Lemon + paw-paw leaf juice	48.94± 0.032 ^b	38.25± 0.038 ^b	11.85± 0.035 ^a	52.57± 0.253 ^b	28.31± 0.162 ^b	24.16± 0.088 ^a	32.87± 3.312 ^b	37.68± 0.159 ^b
G	Beetroot + paw-paw leaf juice	45.62± 0.021 ^b	38.96± 0.031 ^b	57.47± 0.243 ^b	52.16± 0.037 ^b	45.23± 0.020 ^b	37.68± 0.160 ^b	50.76± 0.134 ^b	38.52± 0.021 ^b
H	Lemon + beetroot + paw-paw leaf juice	36.35± 0.026 ^b	41.74± 0.037 ^b	22.36± 0.350 ^a	25.91± 0.076 ^a	23.64± 0.041 ^b	34.16± 0.040 ^b	37.90± 0.064 ^b	29.13± 0.025 ^b
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

If p-value is greater than 0.05, mean values are not statistically significant ($p < 0.05$)

Values are expressed as mean \pm SEM, n=5.

^aValues are significantly low when compared to control ($p < 0.05$)

^bValues are significantly high when compared to control ($p < 0.05$)

^cValue is equal to control ($p < 0.05$)

Table 4. Lipid profile assay of rat serum sample

Group	Treatment	Cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
A	Control	75.31±4.710	121.90±1.700	69.03±2.290	37.12±4.595
B	Lemon juice	81.18±2.920 ^b	86.26±19.035 ^a	69.82±2.160 ^b	49.96±1.320 ^b
C	Beetroot	71.35±3.335 ^a	87.88±10.800 ^a	62.02±2.460 ^a	41.37±1.665 ^b
D	Paw-paw leaf juice	69.01±0.270 ^a	90.64±13.560 ^a	57.84±8.500 ^a	38.81±0.790 ^b
E	Lemon + beetroot juice	81.08±4.730 ^b	132.15±10.150 ^b	60.68±0.500 ^a	42.51±6.660 ^b
F	Lemon + paw-paw leaf juice	86.20±3.280 ^b	129.79±30.115 ^b	59.41±0.910 ^a	48.36±9.120 ^b
G	Beetroot + paw-paw leaf juice	72.02±5.450 ^a	88.98±17.420 ^a	52.65±6.310 ^a	43.69±7.675 ^b
H	Lemon + beetroot + paw-paw leaf juice	94.56±1.230 ^b	94.09±15.710 ^a	47.21±1.370 ^a	66.30±4.100 ^b
p-values	-	0.0133	0.3007	0.0488	0.0732

If p-value is greater than 0.05, mean values are not statistically significant ($p < 0.05$)

Values are expressed as mean \pm SEM, n=5.

^aValues are significantly low when compared to control ($p < 0.05$)

^bValues are significantly high when compared to control ($p < 0.05$)

Group H which is the combination of all the juices had little or no effect on HDL and LDL because it had the lowest and highest figures respectively compare to control. This implies that the risk of

cardiac arrest in this group is very high implying that this combination is not suitable for overweight individuals. In contrast, group D which was administered pawpaw leaf juice had a

very high effect on HDL and LDL which is only second to control group. This implies that this juice is suitable for overweight individuals due to its effect on HDL and LDL. This result agrees with the findings by [21]. There was significant reduction in triglyceride in groups B, C and G.

4. CONCLUSION

From the observations and tests recorded in this research, it is obvious that consuming 100% fruit juice is effective on weight loss even though the mechanism of action is unclear. The continuous fluctuation of weight, urine and fecal material over a short interval is prove that these juices affects the weight of the rats in the different groups. This is indicative of the fact that if it is consumed consistently over a long period of time, the result could impact more on the animal.

ETHICAL APPROVAL

The animals were fed with standard feed throughout the period of the research. All experiments on animals were in accordance with the guidelines of both the University's ethical committee and the international guidelines for handling of laboratory animals [6].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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