

Effect of Beetroot juice supplementation on the physical and biochemical profiles of induced metabolic syndrome in rats

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Abstract: Beetroot is a vegetable containing phytochemical ingredients with potent antioxidant, hypoglycaemic and anti-inflammatory properties. It is a key driver in the pathophysiology of some metabolic syndromes. Its effect on the progress of induced metabolic syndrome has, however, hardly been investigated. Thus, this study aims to determine the effect of beetroot extract on various biophysical components of metabolic syndrome in rat. Following ethical approval, 50 Wister albino rats were used in this study and divided into three groups: Group A: rats were put on a high-fat-high-fructose (HFHF) diet; Group B: rats were put on an HFHF + fresh beetroot extract while group C: rats were the control group and were given a normal diet. The animals' body weights and fasting blood sugar were taken fortnightly. Five rats from each group were then sacrificed at weeks 4, 8, 12 and 16 and the following parameters were measured: liver volume, fasting lipid profile, hepatic transaminases and blood platelet level. Compared to the HFHF group, beetroot supplementation resulted in a significant attenuation of overall weight gain (56.91% vs. 34.51%; $p < 0.001$) and absolute liver volume ($p < 0.01$) but minimal effect on the body-mass normalized liver volume. Over time, the HFHF+B group similarly had a significantly lower time-dependent elevation in fasting blood sugar ($p < 0.001$) and considerably lower triglycerides, low-density lipoproteins, high-density lipoproteins, cholesterol, liver enzyme levels (AST and ALT) ($p > 0.05$). Beetroot supplementation further ameliorated the thrombocytopenia caused by an HFHF. Beetroot juice supplementation attenuated the attendant effects of induced metabolic syndrome which might contribute towards averting the consequential cardiovascular sequel. Thus, lifestyle modification comprising beetroot intake as a dietary supplementation may alleviate metabolic syndrome and may offer a potential non-medical adjunct in the routine management of metabolic syndrome.

Introduction

Metabolic syndrome comprises a collection of interrelated factors that considerably increase the risk of cardiovascular diseases. It is characterized by the presence of either three of central obesity, diabetes,



hypertension, elevated body mass index or dyslipidaemia [1]. Its prevalence has been increasing and currently stands at above 30.0% with the greatest burden among persons aged between 20 and 30 years [2]. The specific prevalence of metabolic syndrome among different populations, however, varies and it is known to be significantly higher in certain ethnic groups, persons of a higher socioeconomic background and people living with certain chronic conditions [3]. Of the complications of metabolic syndrome, non-alcoholic fatty liver disease remains one of the most prevalent and presents with attendant derangement of liver enzymes and blood platelet disorders [4]. Similarly, the association of metabolic syndrome with increased predisposition to cardiovascular and cerebrovascular diseases makes it an important public health concern and thus obviating necessary and critical interventions. Established components of metabolic syndrome are routinely managed by various pharmacological agents. However, cost implications, side effect profiles and patient adherence have hindered the effectiveness of medical interventions [5]. Various non-medical interventions have been instituted with synergistic effects on medical therapies and have remarkably been shown to alleviate metabolic syndrome. These lifestyle modifications include a reduction in alcohol consumption, increased physical activity and enhanced consumption of a diet rich in fruits and vegetables [6]. Of these vegetables, beetroot is known to have remarkable antioxidant, anti-inflammatory and anti-neoplastic properties, qualities that have attracted a lot of interest to it as a medicinal plant. Previous studies have documented the hypoglycaemic [7], lipid-lowering [8], hepato-protective [4] and gut flora modifying [9] effects of beetroot. These are among the key drivers of the pathophysiological processes in the development of metabolic syndrome. Earlier studies have further successfully induced non-alcoholic steatohepatitis in various rat and other rodent species and reproduced biochemical and physical changes similar to those reported in human patients with metabolic syndrome [10]. However, several studies did not individually investigate the whole range of the metabolic syndrome profile and have the shortcoming of reporting on the relatively shorter duration of the study [4, 7-9]. Similarly, past *in vitro* studies on beetroot have mainly investigated the possible mechanism of action of the various phytochemicals found in beetroot [11]. This study aims to document the effect of beetroot juice supplementation on various parameters of induced metabolic syndrome in a rodent model.

Materials and methods

The ethical approval to conduct this study was obtained from the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (FVM BAUEC/2020/266), Kenya. This study included 50 Albino Wister rats obtained from the Department of Zoology, University of Nairobi. Rats were housed in standard well-labelled cages measuring 109 cm by 69 cm by 77.5 cm and placed under 12/12 hours of light/dark diurnal cycle. Best animal practices were upheld during the entire time of the study. Selected study rats were then divided into three groups: Group A: rats were put on a high-fat-high-fructose (HFHF) diet; Group B: rats were put on an HFHF diet plus fresh beetroot extract (HFHF+B) while Group C: rats were the control group and were fed on a normal diet composed of standard rat pellets and free access drinking water. The beetroot juice (*Beta vulgaris vulgaris*-cultivar 'Alto', gotten from the same farm in Nakuru County, Kenya) was prepared by mixing whole beetroot and clean water in a 1: 1 ratio and blending it using a kitchen blender. The extract was then freeze-dried for three days. The powder was then mixed in distilled water and given by gavage at a dose of 200 mg/kg [12]. As a control measure, Groups A and C were similarly fed by gavage an equitable amount of normal saline. The body weights of the rats were measured weekly. The HFHF diet was prepared as described in [12] and as briefly described below. Saturated fat (Cowboy™ manufactured by Bidco™ industries) -20.0% w/w was added to standard rat chow obtained from Unga Feeds Limited and containing protein (29.8%), fat (13.4%), carbohydrates (56.7%), fibre (5.3%) and vitamins and minerals in appropriate quantities. Fructose (30.0%) was added to the drinking water under the HFHF diet. The food given to the rats was prepared freshly and by the same person to maintain uniformity and the quality of the feeds. Rats were fed ad libitum and the pattern of feeding was monitored daily. Any

food remaining from the previous day was weighed and discarded as per the animal house guidelines. Fasting blood glucose levels were obtained fortnightly following eight hours of fasting.

Five rats from the control group were used for baseline results. Similarly, five from each group were euthanized at weeks 4, 8, 12 and 16. Before euthanasia, the rats were starved for eight hours and euthanasia was done according to established guidelines. Briefly, rats were put in an airtight gas chamber containing cotton wool soaked in halothane gas (1.0%-3.0%). Rats were then removed when they showed an absent pupillary reflex and elicited a minimal response to pain. A midline incision was made, and the heart was accessed while it was still pumping. At each harvesting period and during euthanasia, blood was collected directly from the right atrium for the biochemical analysis of fasting blood glucose, triglyceride, high-density lipoproteins, blood platelet and aspartate aminotransferase and alanine aminotransferase. Following euthanasia, rats were examined for subcutaneous, visceral and abdominal adiposity and liver volumes were determined using Scherle's method [13]. Body mass normalized liver volumes were calculated by dividing the average liver volumes by the average of the rat weights sacrificed in the respective week and expressed as a percentage. Data thus collected were recorded, coded and entered into the SPSS version. The descriptive data were calculated and analysed by one-way ANOVA test to compare the differences in means among the groups. Post-hoc test was used for further analysis to find differences between the groups.

Results

All the rats recruited into the current study survived and no significant differences were noted in the feeding habits. There was, however, an obvious body-weight gain in the interventional groups with the greatest increase noted in rats fed on the HFHF diet alone. The total body weight of the control rats gradually increased throughout the study. By the 16th week, the rats weighed on average 383.0 ± 28.94 gm with a 38.3% increase from the baseline. On the contrary, rats fed on an HFHF had a marked increase in the body weight with a maximum body weight of 477.4 ± 34.86 gm attained by the 16th week with a 56.9% increase from the baseline. The addition of fresh beetroot extract to the HFHF diet resulted in attenuation of this body weight gain (overall increase from baseline of 34.5%) and which was comparable to the body weight in the control group ($p > 0.05$) (Figure 1). Furthermore, the HFHF diet group was significantly heavier than the HFHF+B for weeks 10th, 12th and 16th (heavier by 23.6%, 21.1% and 24.4%, respectively; $p < 0.001$ on all the occasions). At post-mortem, rats fed on an HFHF diet alone were found to have significant subcutaneous abdominal and visceral fat deposition compared to the HFHF+ B and the control groups.

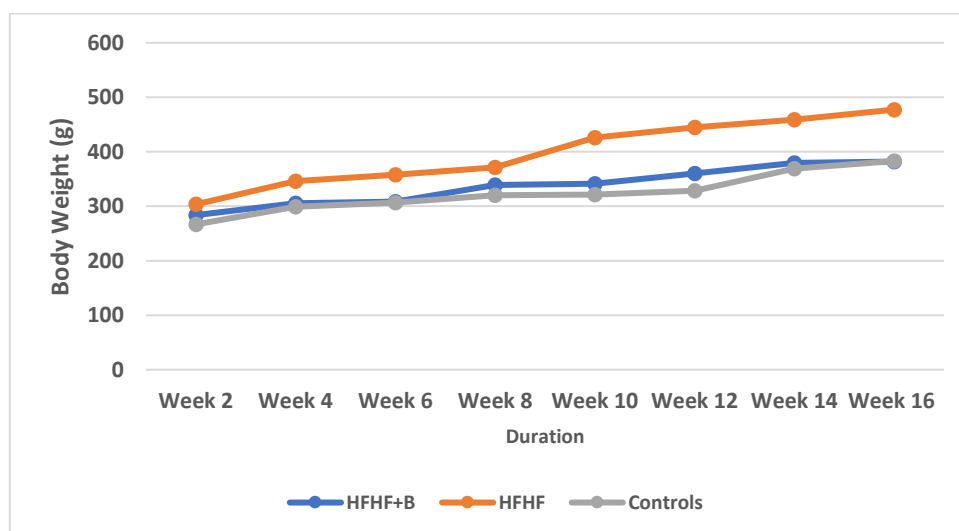


Figure 1: Body weight for the high-fat-high-fructose (HFHF) and high-fat-high-fructose plus beetroot (HFHF+B)

Absolute liver and body-mass-normalized liver volume: The control group had the least absolute and body-mass-normalized liver volume with the absolute liver volume showing a moderate increase over time. On the other hand, there was a significant increase in liver volume for the HFHF group ($p < 0.01$) with significant differences registered between weeks four and eight ($p = 0.01$) and weeks eight and 12 ($p < 0.05$). The HFHF+B group on the contrary, had a more blunted increase in liver volume over time (**Figure 2**). The body-mass-normalized liver volume for the HFHF and the HFHF+B were, conversely, comparable to each other but marginally greater than that of the control group. They similarly remained relatively constant throughout the study (**Figure 3**).

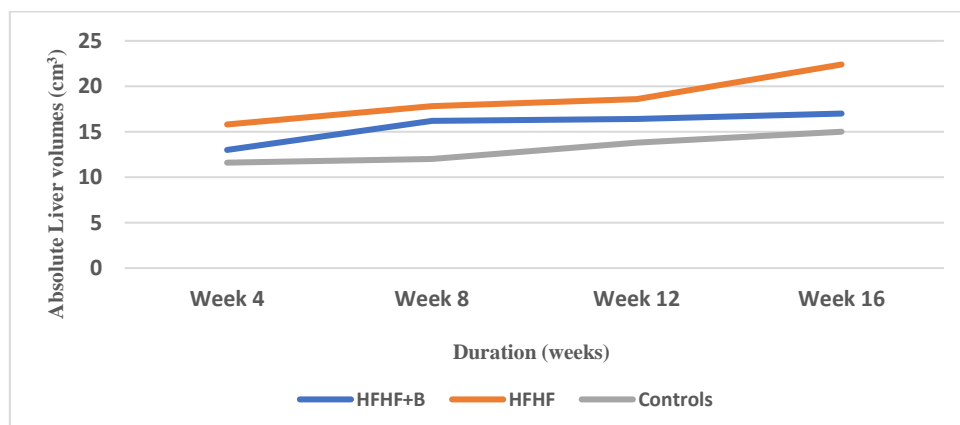


Figure 2: Liver volume for the HFHF and HFHF+B– groups recorded over 16 weeks period

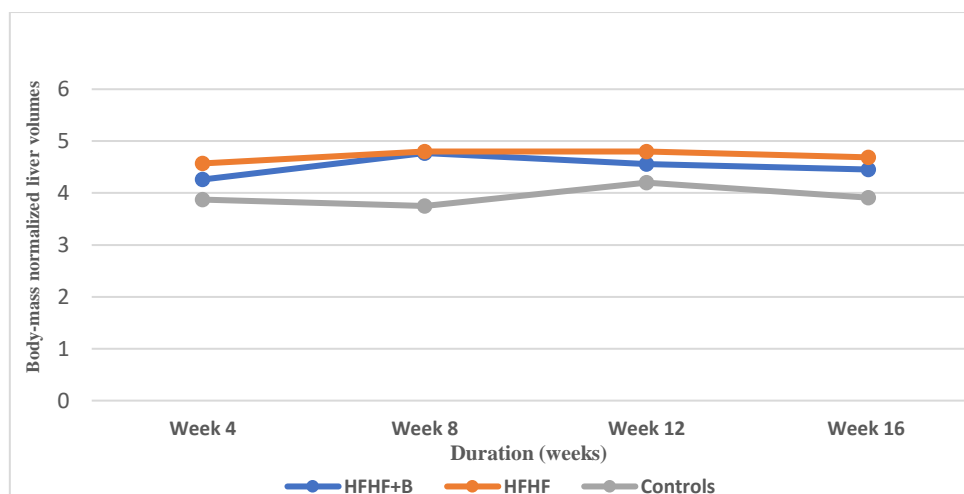


Figure 3: Body-mass normalized liver volume for the HFHF and high- HFHF+B – group

Fasting blood sugar: The level of fasting blood sugar of the control group remained relatively constant (between 6.0 mmol/dL and 6.5 mmol/dL) over the entire duration of the study. The mean fasting blood sugar was, however, higher for the HFHF+B group (ranged between 6.83 mmol/dL and 7.56 mmol/dL) and highest for the HFHF group (ranged between 7.64 mmol/dL and 8.33 mmol/dL). There were no changes in the fasting blood sugar measurements within the groups over time except between weeks six and eight for the HFHF+B group and between weeks eight and ten for the HFHF group (**Figure 4**). However, there were significant differences noted between the various groups over time for every corresponding week ($p < 0.05$) (**Table 1**).

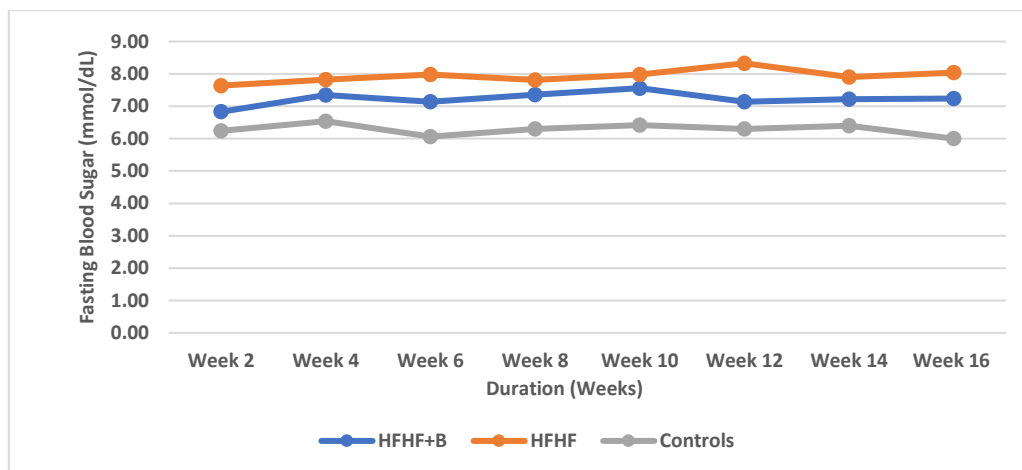


Figure 4: Fasting blood sugar level over time

Table 1: The fasting sugar levels amongst the three groups and over time

Weeks	Fasting blood sugar (mmol/dL)					
	Control	HFHF	P vs Control	HFHF+B	P vs Control	P vs HFHF
2	6.24±0.81	7.64±0.79	0.0009	6.83±0.81	0.240	0.07
4	6.54±0.71	7.82±0.83	0.0016	7.35±0.71	0.058	0.36
6	6.06±0.93	7.98±0.67	0.0001	7.14±0.97	0.032	0.11
8	6.30±0.913	7.81±0.55	0.0005	7.36±0.85	0.015	0.43
10	6.42±0.75	7.98±0.72	0.0050	7.56±0.94	0.043	0.6
12	6.30±0.61	8.33±0.72	0.0002	7.14±0.88	0.120	0.02
14	6.40±0.75	7.90±0.37	0.0300	7.22±1.08	0.270	0.39
16	6.00±0.69	8.04±0.73	0.0005	7.24±0.27	0.017	0.13

Lipid profile: Throughout the study, the control rat group registered a favourable lipid profile reflected by lower levels of triglyceride, cholesterol, low density lipoproteins and high-density lipoproteins. On the contrary, rats fed on HFHF diet only had significantly elevated levels of serum triglyceride, cholesterol and low-density lipoproteins compared to the control group. The addition of beetroot juice to the HFHF diet resulted in a lower increase in the mentioned lipid profile markers. It, nonetheless, resulted in a greater increase in the high-density lipoproteins levels compared to the control group and the HFHF group (**Table 2**).

Hepatic aminotransferase: Both interventional groups had significantly elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities compared to the control group (**Figures 5 and 6**). Rats fed on the HFHF diet alone, however, had the greatest increase that remained elevated over the entire course of the study.

Blood platelet counts: The count for the control group was higher compared to those of both interventional groups and showed a natural decline throughout the study. The decline has however been not statistically significant. A similar decline was seen in the HFHF group albeit in a more pronounced manner. Rats in the HFHF+B group showed a progressive increase in the platelet counts until a decline in the 16th week. This decline has however been less compared to that of the HFHF group. All the changes in the platelet counts have however been not significant (**Figure 7**).

Table 2: Differences in the various lipid profile parameters over time

Weeks	Lipid profile					
	Control	HFHF	P vs control	HFHF+B	P vs control	P vs HFHF
Triglyceride level (mg/dL)						
4	73.69±13.04	194.42±62.28	0.0018	188.66±35.25	0.002	0.97
8	92.81±12.09	252.80±81.47	0.0011	202.31±36.88	0.016	0.30
12	101.5±13.03	230.29±55.18	0.0004	198.23±33.94	0.004	0.40
16	86.27±10.68	262.86±74.63	0.0048	195.22±95.34	0.072	0.31
Cholesterol level (mg/dL)						
4	56.84±6.48	63.73±9.91	0.630	53.29±16.52	0.880	0.37
8	60.25±12.12	65.58±3.40	0.560	62.41±5.72	0.900	0.81
12	52.05±11.52	68.99±6.53	0.043	68.06±10.46	0.056	0.99
16	57.62±11.46	75.02±10.77	0.045	81.29±7.56	0.008	0.6
LDL levels (mg/dL)						
4	9.51±4.8	21.13±8.64	0.046	12.45±6.28	0.77	0.15
8	9.51±5.4	21.36±6.16	0.19	19.33±15.39	0.31	0.97
12	11.21±6.79	22.74±6.04	0.17	18.18±13.54	0.49	0.73
16	13.07±7.23	19.95±6.35	0.43	18.18±10.97	0.62	0.94
HDL levels (mg/dL)						
4	23.67±6.47	28.07 ± 3.26	0.64	22.43±10.94	0.96	0.49
8	24.52±6.55	35.04 ± 4.99	0.02	35.81±3.65	0.013	0.97
12	23.67±5.0	30.47±2.94	0.052	30.78±4.02	0.042	0.99
16	21.12±5.9	37.74±9.08	0.003	41.14±0.8	0.0008	0.68

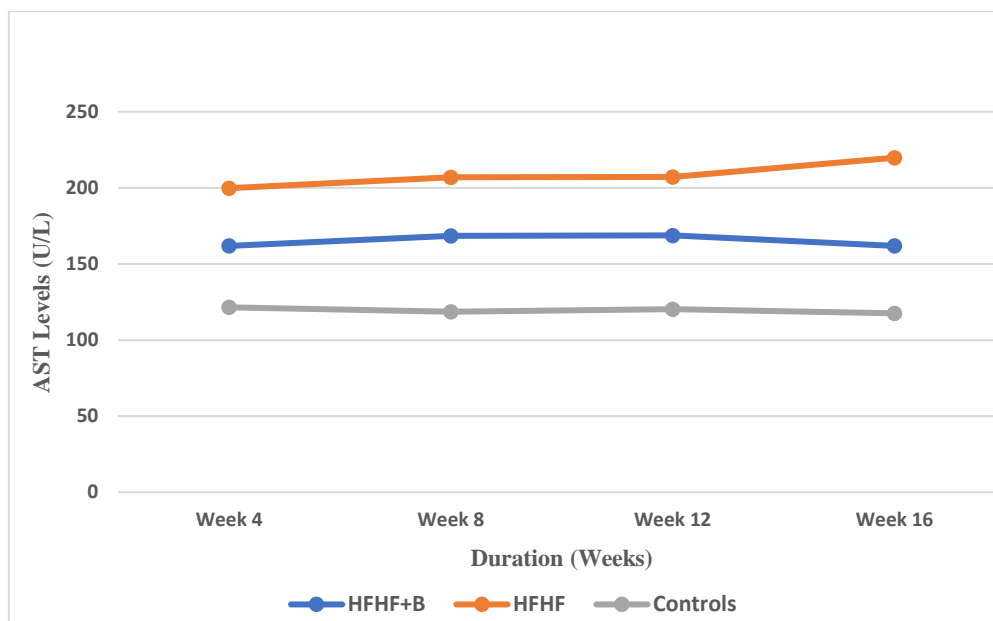


Figure 5: Serum aspartate aminotransferase level recorded over time

Table 3: Differences in liver enzyme activities between the groups

Weeks	Aminotransferase activities					
	Control	HFHF	P vs control	HFHF+B	P vs control	P vs HFHF
aspartate aminotransferase levels (U/L)						
4	121.5±18.11	199.8±43.73	0.002	161.86±9.36	0.100	0.120
8	118.56±18.3	207.4±2.95	0.003	171.6±30.63	0.056	0.230
12	120.32±18.65	207.12±74.4	0.030	168.78±27.71	0.270	0.430
16	117.56±13.3	219.92±6.18	0.001	161.86±41.07	0.040	0.008
alanine aminotransferase levels (U/L)						
4	43.14±16.38	77.96±6.45	0.001	62.64±9.94	0.052	0.140
8	41.82±17.17	85.7±21.15	0.002	63.4±5.06	0.120	0.110
12	43.44±15.1	84.66±11.64	0.001	69.12±12.67	0.020	0.190
16	43.14±15.64	81.84±5.57	0.003	79.78±18.36	0.004	0.970

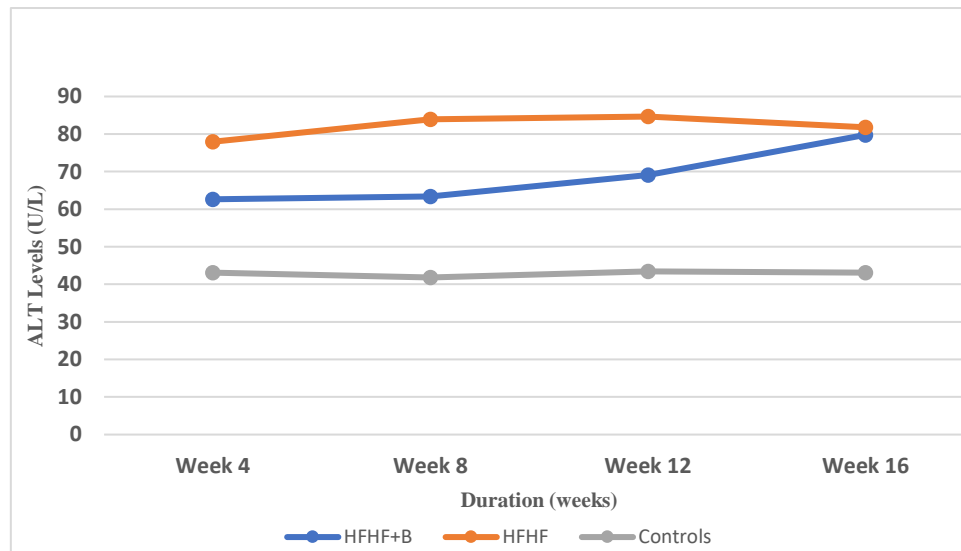


Figure 6: Liver alanine aminotransferase activities over time

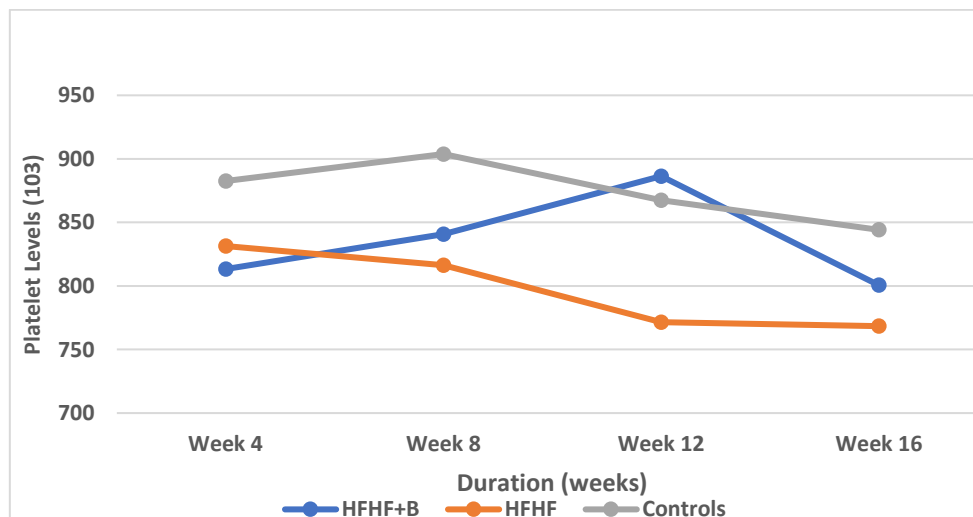


Figure 7: Platelet number trends over time for the three groups

Discussion

Lifestyle changes were touted as key initial pillars in the management of metabolic syndrome [5]. Beetroot contains phytochemicals with antioxidant, hypoglycaemic and anti-inflammatory properties [14]. This makes it an attractive target for investigating its potential to check the development of metabolic syndrome. The potential of other medicinal herbs and oils in treating certain conditions was investigated before including the use of alpha-lipoic acid in iron overload states [15] and green tea, red clover and hibiscus in hypertension [16]. Changes associated with HFHF diet were shown to occur within eight weeks of intervention [17]. Currently, these changes were evidenced by an increase in body-weight that significantly peaked after ten weeks. In mice, a comparable increase in body weight with attendant insulin resistance was brought about by increased visceral and subcutaneous fat deposition [10] which is in line with the current study. The weight increases nonetheless seemed to plateau by 12 weeks with an apparent improvement thereafter. A similar finding in weight gain was reproduced in a porcine model with associated increases in body weight, liver volume and habitus adiposity [18]. Other studies have reported minimal weight gain in animals fed on diabetogenic diets despite histological evidence of hepatic steatosis [19]. The duration of the study and feed preparation may be attributed to these findings. The increase in visceral and subcutaneous fat deposition is attributed to increased hepatic *de novo* lipogenesis secondary to the high fructose load and is accompanied by considerable hepatomegaly which was reflected by the greater liver volume at post-mortem [20]. Co-administration of beetroot juice attenuated this increase in body weight and liver volume. Many mechanisms were fronted with involving the antioxidant properties of flavonoids contained in beetroot that are hepato-protective against lipid peroxidation and thereby helping to effectively maintain the liver's ability to carry out its energy metabolism [14]. Beetroot increases glucose uptake by skeletal muscle and adipose tissues thereby mopping out the substrates required for *de novo* lipogenesis and causing a reduction in visceral and subcutaneous fat deposition [21]. These are accompanied by better glycaemic control and favourable lipid profiles with interventions involving beetroot supplementation [21]. Such weight gain induced by an HFHF diet has likewise been attenuated by the concomitant administration of conophylline, a vinca alkaloid extracted from the pinwheel flower [10].

An HFHF diet has previously been credited with an increase in plasma glucose concentration [22] which is in line with the present data on persistent hyperglycaemia in interventional rats. The rise in blood sugar in rats fed on a diabetogenic diet may result in a concomitant rise in plasma insulin levels [7, 23]. This elevation of insulin in response to prolonged fructose intake reverses with the development of Non-alcoholic fatty liver disease which might reflect the exhaustion of pancreatic beta cells, the onset of insulin resistance and ultimately frank diabetes mellitus [24]. Co-administration of beetroot juice with a HFHD resulted in lower blood sugar. Such a decline in glucose following beetroot juice intake occurred within 15 minutes and was sustained by up to 180 minutes post-prandial [25]. A lower blood sugar with beetroot intake was also accompanied by a decline in HbA_{1c} showing the favourable euglycaemic effects of its supplementation [8, 26]. Its supplementation in diabetic rats moderated hyperglycaemia by increasing skeletal muscular glucose uptake by 40% facilitated by arteriolar dilatation and thus enhanced blood flow. Beetroot causes increased expression of glucose transporter protein type-4 transporter proteins in skeletal muscles and adipose tissues [21] whose up-regulated expression increases glucose uptake into the target tissues further helping achieve better glycaemic control [27]. Inhibition of α -glucosidase by flavonoid glycosides, has similarly been shown to reduce hyperglycaemia in mice which might have further attenuated the rise in plasma glucose by reducing intestinal absorption of glucose [28]. Other plasma glucose lowering mechanisms of beetroot, and that are mediated by betanin, include lowering the activity of gluconeogenic enzymes and increasing the expression of glycolytic enzymes [29].



Dyslipidaemia following an HFHF diet was reflected by elevated triglyceride, cholesterol and low-density lipoproteins. Dyslipidaemia has also been reported in mice fed on a high-fat-high-calorie diet and was associated with up to 7-fold rise in triglyceride and free fatty acid content [30]. These observations are supported by others who found that while dietary fat was the key driver of Non-alcoholic fatty liver disease development, fructose exerted its steatogenic effect by increasing the circulating pool of lipids [31]. A diet combining a HFHF content has a synergistic effect on the elevated lipid profile and a more deleterious effect on the development of hepatic steatosis [23, 32]. Thus, consumption of raw red beetroot among diabetic patients resulted in a decrease in apo-lipoprotein B100 pointing to a reduced rate of hepatic *de novo* lipogenesis [8]. Also, by up-regulating the expression of glucose transporter protein type-4 transporter proteins in target tissues and inhibiting α -glucosidase, beetroot causes a reduction in plasma glucose and reduces the substrates necessary for the *de novo* lipogenesis. Instead, concomitant administration of obeticholic acid, a semi-synthetic bile acid analogue, was found to attenuate hypercholesterolemia in rats fed on a Western diet [18]. Obeticholic acid has anti-fibrotic and anti-inflammatory effects in the liver, causes increased bile acid production and enhances the entero-hepatic circulation of bile salts by directly influencing the enterocytes and hepatocytes [33]. An influence on the entero-hepatic circulation of bile acids on a hyperlipidaemic diet and co-fed on beetroot stalks resulted in lower plasma levels of short-chain fatty acids [34]. Elevated levels of liver aminotransferase enzymes following a steatogenic diet have been reported [4, 23]. Currently, similar results with the significant and sustained rise of the aspartate aminotransferase and alanine aminotransferase following a HFHF diet were found. This increase is brought about by hepatic inflammation secondary to steatosis and is partly mediated by mitochondrial dysfunction that culminates in oxidative stress secondary to increased lipid peroxidation and cellular injury [11]. As a result, there is an enhanced release of chemokines that induce peri-sinusoidal macrophage infiltration and hepatic stellate cell activation [4]. The presence of such an inflammatory hepatic milieu leads to an increased hepatocyte susceptibility to apoptosis and serves as a positive feedback loop to enhanced insulin resistance and hepatic inflammation with reduced hepatic ability to maintain adequate energy balance [32]. Co-administration of beetroot juice resulted in a diminution of hepatic transaminases. This ability of raw beetroot supplementation to lower elevated transaminase enzyme among diabetic patients was reported and attributed to the antioxidant capacity of the various flavonoids contained in beetroot and its abundant amounts of nitrates [8]. Thrombocytosis is associated with metabolic syndrome and was suggested as a preclinical surrogate marker [35]. This was shown in this study by a rise in platelet counts in rats fed on HFHF diet and beetroot juice. Such an increase in platelets occurs in tandem with hepatic steatosis, is associated with an increased mean platelet and is partly driven by the attendant insulin resistance and hypertriglyceridemia [36]. A greater mean platelet is a marker of thrombocyte activation and is associated with increased agglutination and collagen binding [37]. Patients with metabolic syndrome and have high platelet and thrombocytosis had a greater predisposition to cardiovascular diseases [38]. Other drivers of the reactive thrombocytosis seen in metabolic syndrome include a local hepatic inflammatory milieu as evidenced in the current data by the elevated hepatic transaminase. Conversely, established NAFLD has been associated with thrombocytopenia which occurs in between 3.0%-25.0% of affected patients [39]. This occurs as hepatic steatosis progresses to steatohepatitis and mirrors increased hepatic fibrosis [40]. Such an observation might explain the occurrence where there was marked thrombocytopenia in rats only fed on an HFHF diet and which recorded a greater rise in liver enzyme activities, and had worse hyperglycaemia and dyslipidaemia. Oxidative stress is secondary to the dysglycaemia and dyslipidaemia seen in metabolic syndrome was fronted to result in increased platelet activation and aggregation and their increased pooling in the liver where they serve as key drivers of the inflammation and fibrotic processes [41]. Thrombocytopenia has equally been thought to be antibody-mediated and routinely accompanied by elevated liver transaminases [40]. Thrombocytopenia occurs in other chronic liver diseases with reduced hepatic production of thrombopoietin and increased splenic destruction being attributed to the causality [42].



Conclusion: Beetroot juice supplementation in the setting of a high-fat-high-fructose diet, a substrate of the metabolic syndrome complex, diversely attenuates the attendant effects and contributes towards averting the consequential cardiovascular complications. Healthy lifestyle changes inclusive of beetroot intake as a dietary supplementation may offer a potential non-medical adjunct in the routine management of metabolic syndrome.

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Author contribution: JM, AM & MO conceptualized the work and drafted the manuscript. JM, VK & DO performed the experiment and collected and analyzed the data. All authors revised the manuscript and approved the final version of the manuscript and agreed to be accountable for its contents.

Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission have completely been observed by authors.

Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.

Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

References

1. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith Jr SC (2009) Harmonizing the metabolic syndrome. *Circulation*. 120 (16): 1640-1645. doi: 10.1161/CIRCULATIONAHA.109.192644
2. Hirode G, Wong RJ (2020) Trends in the prevalence of metabolic syndrome in the United States, 2011-2016. *JAMA*. 323 (24): 2526-2528. doi: 10.1001/jama.2020.4501
3. Liang XP, Or CY, Tsoi MF, Cheung CL, Cheung BMY (2021) Prevalence of metabolic syndrome in the United States National Health and Nutrition Examination Survey (NHANES) 2011-2018. *European Heart Journal*. 42 (S1): ehab724.2420. doi: 10.1093/eurheartj/ehab724.2420
4. Gabbia D, Roverso M, Guido M, Sacchi D, Scaffidi M, Carrara M, Orso G, Russo FP, Floreani A, Bogiallo S, De Martin S (2019) Western diet-induced metabolic alterations affect circulating markers of liver function before the development of steatosis. *Nutrients*. 11 (7): 1602. doi: 10.3390/nu11071602
5. Marchisello S, Di Pino A, Scicali R, Urbano F, Piro S, Purrello F, Rabuazzo AM (2019) Pathophysiological, molecular and therapeutic issues of nonalcoholic fatty liver disease: an overview. *International Journal of Molecular Sciences*. 20 (8): 1948. doi: 10.3390/ijms20081948
6. Van Wormer JJ, Boucher JL, Sidebottom AC, Sillah A, Knickelbine T (2017) Lifestyle changes and prevention of metabolic syndrome in the heart of new Ulm project. *Preventive Medicine Reports*. 6: 242-245. doi: 10.1016/j.pmedr.2017.03.018
7. Ochoa M, Val-Laillet D, Lallès JP, Meurice P, Malbert CH (2016) Obesogenic diets have deleterious effects on fat deposits irrespective of the nature of dietary carbohydrates in a Yucatan minipig model. *Nutrition Research*. 36 (9): 947-954. doi: 10.1016/j.nutres.2016.07.003
8. Aliahmadi M, Amiri F, Bahrami LS, Hosseini AF, Abiri B, Vafa M (2021) Effects of raw red beetroot consumption on metabolic markers and cognitive function in type 2 diabetes patients. *Journal of Diabetes and Metabolic Disorders*. 20 (1): 673-682. doi: 10.1007/s40200-021-00798-z
9. Capper TE, Houghton D, Stewart CJ, Blain AP, McMahon N, Siervo M, West D (2020) Whole beetroot consumption reduces systolic blood pressure and modulates diversity and composition of the gut microbiota in older participants. *NFS Journal*. 21: 28-37. doi: 10.1016/j.nfs.2020.08.001
10. Ohashi T, Nakade Y, Ibusuki M, Kitano R, Yamauchi T, Kimoto S, Inoue T, Kobayashi Y, Sumida Y, Ito K, Nakao H, Umezawa K, Yoneda M (2019) Conophylline inhibits high fat diet-induced non-alcoholic fatty liver disease in mice. *PLoS ONE*. 14 (1): e0210068. doi: 10.1371/journal.pone.0210068
11. García-Berumen CI, Ortiz-Avila O, Vargas-Vargas MA, Del Rosario-Tamayo BA, Guajardo-López C, Saavedra-Molina A, Rodríguez-Orozec AR, Cortes-Rojo C (2019) The severity of rat liver injury by fructose and high fat depends on the degree of respiratory dysfunction and oxidative stress induced in mitochondria. *Lipids in Health and Disease*. 18 (1): 78. doi: 10.1186/s12944-019-1024-5



12. Macharia FK, Mwangi PW, Yenesew A, Bukachi F, Nyaga NM, Wafula DK (2019) Hepatoprotective effects of *Erythrina abyssinica* Lam Ex Dc against non-alcoholic fatty liver disease in Sprague Dawley rats. *bioRxiv*. 577007. doi: 10.1101/577007
13. Munguti JK, Obimbo MM, Odula PO, Sibuur OO, Cheruiyot IK (2018) Hypervitaminosis A causes reversible liver volume changes independent of body mass in Albino rats (*Rattus Norvegicus*). *Austin Journal of Anatomy*. 5 (3): Id: 1086. doi: Nil.
14. Clifford T, Howatson G, West DJ, Stevenson EJ (2015) The potential benefits of red beetroot supplementation in health and disease. *Nutrients*. 7 (4): 2801-2822. doi: 10.3390/nu7042801
15. Sibuur W, Gwala F, Munguti J, Obimbo M (2018) Alpha-lipoic acid attenuates iron-overload-induced structural changes in the liver of the laboratory mouse (*Mus musculus*). *Anatomy*. 12 (3): 118-123. doi: 10.2399/ana.18.074
16. Ushiroda C, Naito Y, Takagi T, Uchiyama K, Mizushima K, Higashimura Y, Yasukawa Z, Okubo T, Inoue R, Honda A, Matsuzaki Y, Itoh Y (2019) Green tea polyphenol (epigallocatechin-3-gallate) improves gut dysbiosis and serum bile acids dysregulation in high-fat diet-fed mice. *Journal of Clinical Biochemistry and Nutrition*. 65 (1): 34-46. doi: 10.3164/jcbn.18-116
17. Lorizola IM, Furlan CPB, Portovedo M, Milanski M, Botelho PB, Bezerra RMN, Sumere RMN, Rostagno MA, Capitani CD (2018) Beet stalks and leaves (*Beta vulgaris* L.) protect against high-fat diet-induced oxidative damage in the liver in mice. *Nutrients*. 10 (7): 872. doi: 10.3390/nu10070872
18. Sun G, Jackson CV, Zimmerman K, Zhang LK, Finneary CM, Sandusky GE, Zhang G, Peterson RG, Wang Y-X (2019) The FATZO mouse, a next generation model of type 2 diabetes, develops NAFLD and NASH when fed a Western diet supplemented with fructose. *BMC Gastroenterology*. 19: 41. doi: 10.1186/s12876-019-0958-4.
19. Henkel J, Alfine E, Saín J, Jöhrens K, Weber D, Castro JP, König J, Stuhlmann C, Vahrenbrink M, Jonas W, Keinridders A, Pucshel GP (2018) Soybean oil-derived poly-unsaturated fatty acids enhance liver damage in nafld induced by dietary cholesterol. *Nutrients*. 10 (9): 1326. doi: 10.3390/nu10091326
20. Fouret G, Gaillet S, Lecomte J, Bonafos B, Djohan F, Barea B, Badia E, Coudray C, Feillet-Coudray C (2018) 20-Week follow-up of hepatic steatosis installation and liver mitochondrial structure and activity and their interrelation in rats fed a high-fat-high-fructose diet. *The British Journal of Nutrition*. 119 (4): 368-380. doi: 10.1017/S0007114517003713
21. Gheibi S, Jeddi S, Carlström M, Gholami H, Ghasemi A (2018) Effects of long-term nitrate supplementation on carbohydrate metabolism, lipid profiles, oxidative stress, and inflammation in male obese type 2 diabetic rats. *Nitric Oxide: Biological Chemistry*. 75: 27-41. doi: 10.1016/j.niox.2018.02.002
22. Wang KW, Xiao BQ, Li BH, Liu YY, Wei ZY, Rao JH, Chen JH (2019) Effects of fat-to-sugar ratio in excess dietary energy on lipid abnormalities: a 7-month prospective feeding study in adult cynomolgus monkeys. *Lipids in Health and Disease*. 18 (1): 1. doi: 10.1186/s12944-018-0950-y
23. Jensen VS, Hvid H, Damgaard J, Nygaard H, Ingvorsen C, Wulff EM, Lykkestedt J, Fledelius C (2018) Dietary fat stimulates development of NAFLD more potently than dietary fructose in Sprague-Dawley rats. *Diabetology and Metabolic Syndrome*. 10: 4. doi: 10.1186/s13098-018-0307-8
24. Mu W, Cheng X-f, Liu Y, Lv Q-z, Liu G-l, Zhang J-g, Li X-y (2022) Potential nexus of non-alcoholic fatty liver disease and type 2 diabetes mellitus: insulin resistance between hepatic and peripheral tissues. *Frontiers in Pharmacology*. 9: 1566. doi: 3389/fphar.2018.01566
25. Chang PY, Hafiz MS, Boesch C (2018) Beetroot juice attenuates glycaemic response in healthy volunteers. *Proceedings of the Nutrition Society*. 77 (OCE4): E165. doi: 10.1017/S0029665118001714
26. Clifford T, Constantinou CM, Keane KM, West DJ, Howatson G, Stevenson EJ (2017) The plasma bioavailability of nitrate and betanin from *Beta vulgaris rubra* in humans. *European Journal of Nutrition*. 56 (3): 1245-1254. doi: 10.1007/s00394-016-1173-5
27. Klip A, McGraw TE, James DE (2019) Thirty sweet years of GLUT4. *The Journal of Biological Chemistry*. 294 (30): 11369-11381. doi: 10.1074/jbc.REV119.008351
28. Assefa ST, Yang EY, Chae SY, Song M, Lee J, Cho MC, Jang S (2020) Alpha glucosidase inhibitory activities of plants with focus on common vegetables. *Plants*. 9 (1): 2. doi: 10.3390/plants9010002
29. Dhananjayan I, Kathioli S, Subramani S, Veerasamy V (2017) Ameliorating effect of betanin, a natural chromoalkaloid by modulating hepatic carbohydrate metabolic enzyme activities and glycogen content in streptozotocin - nicotinamide induced experimental rats. *Biomedicine and Pharmacotherapy*. 88: 1069-1079. doi: 10.1016/j.biopha.2017.01.146
30. Liu XJ, Duan NN, Liu C, Niu C, Liu XP, Wu J (2018) Characterization of a murine nonalcoholic steatohepatitis model induced by high fat high calorie diet plus fructose and glucose in drinking water. *Laboratory Investigation; a Journal Technical Methods and Pathology*. 98 (9): 1184-1199. doi: 10.1038/s41374-018-0074-z



31. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, Nakagawa T, Kuwabaara M, Sato Y, Kang DH, Tolan DR, Sanchez-Lozada LG, Rosen HR, Lanaspa MA, Deihl AM, Johnson RJ (2018) fructose and sugar: a major mediator of nonalcoholic fatty liver disease. *Journal of Hepatology*. 68 (5): 1063-1075. doi: 10.1016/j.jhep.2018.01.019
32. Henkel J, Coleman CD, Schraplau A, Jöhrens K, Weber D, Castro JP, Hugo M, Schulz TJ, Kramer S, Schuramnn A, Puschel GP (2017) Induction of steatohepatitis (nash) with insulin resistance in wild-type b6 mice by a western-type diet containing soybean oil and cholesterol. *Molecular Medicine*. 23: 70-82. doi: 10.2119/molmed.2016.00203
33. Sclair SN, Little E, Levy C (2015) Current concepts in primary biliary cirrhosis and primary sclerosing cholangitis. *Clinical and Translational Gastroenterology*. 6 (8): e109. doi: 10.1038/ctg.2015.33
34. Chen L, Zhu Y, Hu Z, Wu S, Jin C (2021) Beetroot as a functional food with huge health benefits: Antioxidant, antitumor, physical function, and chronic metabolomics activity. *Food Science and Nutrition*. 9 (11): 6406-6420. doi: 10.1002/fsn3.2577
35. Fang KC, Cheng YL, Su CW, Wang YJ, Lan KH, Huo TI, et al. (2017) Higher platelet counts are associated with metabolic syndrome independent of fatty liver diagnosis. *Journal of the Chinese Medical Association*. 80 (3): 125-132. doi: 10.1016/j.jcma.2016.07.003
36. Ding Q, Wang F, Guo X, Liang M (2021) The relationship between mean platelet volume and metabolic syndrome in patients with type 2 diabetes mellitus: A retrospective study. *Medicine (Baltimore)*. 100 (13): e25303. doi: 10.1097/MD.00000000000025303
37. Rodriguez BAT, Johnson AD (2020) Platelet measurements and type 2 diabetes: investigations in two population-based cohorts. *Frontiers in Cardiovascular Medicine*. 7: 118. doi: 10.3389/fcvm.2020.00118
38. Ozhan H, Aydin M, Yazici M, Yazgan O, Basar C, Gungor A, Onder E (2010) Mean platelet volume in patients with non-alcoholic fatty liver disease. *Platelets*. 21 (1): 29-32. doi: 10.3109/09537100903391023
39. Liu F, Zhou H, Cao L, Guo Z, Dong C, Yu L, Wang W, Liu C, Xue Y, Liu X, Xu Y (2018) Risk of reduced platelet counts in patients with nonalcoholic fatty liver disease (NAFLD): a prospective cohort study. *Lipids in Health and Disease*. 17 (1): 221. doi: 10.1186/s12944-018-0865-7
40. Lin YY, Hu CT, Sun DS, Lien TS, Chang HH (2019) Thioacetamide-induced liver damage and thrombocytopenia is associated with induction of antiplatelet autoantibody in mice. *Scientific Reports*. 9 (1): 17497. doi: 10.1038/s41598-019-53977-7
41. Dalbeni A, Castelli M, Zoncapè M, Minuz P, Sacerdoti D (2022) Platelets in non-alcoholic fatty liver disease. *Frontiers in Pharmacology*. 13: 842636. doi: 10.3389/fphar.2022.842636
42. Rivera-Álvarez M, Córdova-Ramírez AC, Elías-De-La-Cruz GD, Murrieta-Álvarez I, León-Peña AA, Cantero-Fortiz Y, Olivares-Gazca J, Ruiz-Delgado GJ, Ruiz-Arguelles GJ (2022) Non-alcoholic fatty liver disease and thrombocytopenia IV: its association with granulocytopenia. *Hematology, Transfusion and Cell Therapy*. 44 (4): 491-496. doi: 10.1016/j.htct.2021.06.004