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THERAPEUTIC POTENTIAL OF *CATHARANTHUS ROSEUS* LEAVES AND LOTUS ROOT IN A HIGH-FAT DIET-INDUCED NAFLD MODEL

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ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) is a worldwide health concern identified by excessive liver fat retention. The nutritional strategies are more effective than other available treatments to treat liver problems. The current study examines the therapeutic efficacy of *Catharanthus roseus* leaves and Lotus (*Nelumbo nucifera*) root in mice models of NAFLD. The NAFLD was induced by administering a high-fat diet (HFD) for six weeks, followed by a two-week intervention with plant extracts. Albino mice were separated into four groups: Control, HFD, HFD + Lotus root aqueous extract (LRAE) 400 mg, and HFD + *Catharanthus roseus* leaves aqueous extract (CRLAE) 500 mg. In the high-fat diet (HFD) group, there was significant elevation in the levels of liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and bilirubin values. The values increased to 56.76±1.27 U/L, 157.94±6.12 U/L, 142.24±1.74 U/L, 0.84±0.04 mg/dl respectively. Nevertheless, the values of ALT, AST, ALP and bilirubin significantly reduced in CRLAE group. The values are 31.6±3.0 IU/L, 104.25±6.84 IU/L, 125.45±3.55 IU/L, 0.51±0.03 mg/dl respectively. The improvement in Lipid profiles was seen in both intervention groups. The values were significantly improved in group treated with CRLAE. The values of total cholesterol (TC) 86.61±3.18 mg/dl, high-density lipoprotein (HDL) 38.22±2.61 mg/dl, low-density lipoprotein (LDL) 27.14±1.05 mg/dl, very low-density lipoprotein (VLDL) 21.25±1.07 mg/dl, triglycerides (TG) 106.24±4.57 mg/dl and glucose content 95.88±5.59 mg/dl. The CBC parameters declined in the HFD group, while improved after treatment. Moreover, the WBC count and renal profile showed variations in the HFD group. Histopathological examination showed declined inflammation and steatosis after intervention. These results contribute the potential of both CRLAE and LRAE as efficacious natural treatment for the management of NAFLD, and also enlighten their role in improving body functions.

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INTRODUCTION

The occurrence of metabolic disorders is prevailing worldwide nowadays. The diseases, including fatty liver disease, diabetes, obesity and renal problems, are prevalent globally, causing public health concerns if left untreated (1). Among all of them, NAFLD affects approximately 38% people worldwide. In Pakistan, it affected 47 % of the population, having obesity and other metabolic disorders (2). The reason behind the elevation of this disease is consuming high fat foods, lack of physical activity, and sugary food consumption. NAFLD is characterized by high fat buildup in liver cells, possibly causing various complications including inflammation of liver, liver fibrosis, and liver carcinoma (3). NAFLD is asymptomatic in initial stages, as it is progressing towards advance stages it showed symptoms of having fatigue and swelling. It also indicated abnormal values of lipid profile, renal profile and CBC parameters. Escalated levels of liver enzymes including ALP, AST and ALT all linked to NAFLD (4). Furthermore, insulin contributed to progression of liver malfunction resistance and inflammation damages liver

tissues by imposing oxidative stress that potentiates NAFLD. NAFLD damages gut microbiota that can impair metabolism of body, by which various other bodily functions are disturbed (5).

Treatment by natural strategies including lifestyle and dietary modifications is now becoming more popular due to less adverse effects profile. Herbal therapies have the ability to treat various metabolic disorders including NAFLD and other problems related to health (6). Although, the natural compounds obtained from various plants have been used for centuries for their therapeutic potential against various illnesses. *Catharanthus roseus* commonly known as sada bahar famous for having anti-cancer potential. In Ayurveda, leaves, roots and stems are significantly used for many purposes including healing of wounds, diabetes, asthma, cardiovascular problems, hypertension and reproductive health (7). *C. roseus* leaves contain bioactive compounds like vincristine, vinblastine, vindoline that are important in improving health and used in various medicines to treat the metabolic disorders (8).

Nelumbo nucifera root is commonly found in Asian countries and widely used as food and traditional medicine in various localities. It is famous for having dyslipidemic properties. *N. nucifera* root is also used to improve immunity and boost memory. In Ayurveda, it is used to treat problems including skin diseases, diarrhea, ulcer and bleeding. Polyphenols and flavonoids found in *N. nucifera* root contribute anti-inflammatory, anti-oxidant and hypolipidemic properties (9). This study examines the therapeutic potency of *C. roseus* leaves and *N. nucifera* root in mitigating NAFLD in mice model.

METHODOLOGY

Collection of raw materials

C. roseus leaves and Lotus root were collected from Multan and identified by Department of Botany, Bahauddin Zakariya University, Multan, Pakistan. The leaves were cleaned than shade dried for eight days. *N. nucifera* root was washed thoroughly, sliced, and sun-dried for four days. Then both samples were grind into a fine powder by grinder (Model BJ-9176). Both powders were stored in a sealed plastic container for further analysis in Department of Human Nutrition, BZU, Multan (10).

Water extract preparation

The aqueous extract of the plants was prepared using a method adapted from (11), with some minor adjustments. The Lotus root and *C. roseus* leaves powder was mixed in 200 mL distilled water and place them in an orbital shaker (Model, BSOT-604) for six hours. After being left to settle overnight, the extracts were filtered through Whatman No. 4 filter paper and then concentrated using a rotary evaporator (Model, RE202) and stored at 4°C for further use.

Housing and grouping of Animals:

The 20 male mice, aged four weeks (weighed 18 to 25 g) were procured from Department of Pharmacy, BZU, Multan, kept in a controlled setting with temperature of 25 ± 2℃, and a relative humidity of 55 ± 3 and 12-hour light and dark cycle. The mice were grouped into four groups and initially fed a standard control diet and provided with water for acclimatization. Throughout this study, ethical considerations were rigorously adhered to, and all procedures were conducted in full compliance with the guidelines established by the Bioethical Committee at BZU, Multan (12).

Induction of NAFLD:

Mice were fed a high-fat diet (HFD) for six weeks to induce NAFLD. The diet composition included high saturated fats (60%); simulating conditions conducive to liver fat accumulation (13).

Physical and Behavior Examination

The body weight was measured every week using a weighing balance (GX-600, Japan), behavior and feed and water intake was monitored daily to observe the changes.

Dissection of Animals

The rats were anesthetized using ketamine hydrochloride (50 mg/kg BW) and then euthanized using a dissection blade number 24. According to (14), blood samples were drawn in EDTA and blood serum tubes, centrifuged (Model, Z326K) for 5 minutes at 3000 rpm and stored at -20°C. The serum was examined for biochemical parameters, organs were removed and weighed (GX-600, Japan).

Serum Biochemistry:

Biochemical analysis included assessments of kidney function, serum glucose levels, and lipid profile. An automated serum analyzer (FA-LF100B, Zhejiang, China) by following the method of (15) was used to evaluate the liver function test (LFT) and renal function test (RFT). For analyzing glucose and serum lipid profile, a biochemical analyzer (BC400, Hebei, China) was used.

Effect on Hematology Parameters

Hematological parameters, including RBCs and WBCs were evaluated using an automated hematology analyzer (YSTE120C, Guangdong, China) (15).

Histopathological Examination:

The morphological changes in liver tissues were evaluated by histopathological examination. After the dissection, the live samples were immersed in 10 % formalin buffer solution at room temperature as described by (16). By using microtome, the tissues were embedded in paraffin. The thin pieces were then placed on glass slides and stained with various dyes enlighten the structure of tissues. After staining, these slides were placed under the light microscope and the digital images were captured for thorough analysis.

STATISTICAL ANALYSIS

The data was analyzed using SPSS 25 version. Results were presented as mean ± standard deviation (SD). For comparisons among multiple groups, one-way ANOVA was employed, followed by the Tukey test for post-hoc analysis. Statistical significance was determined at a p-value of less than 0.05.

RESULTS

Feed and Water Intake

Daily measurements of water and feed consumption were recorded throughout the study and summarized weekly in Figure 1 (a, b). Results demonstrated that the high-fat diet (HFD) group maintained higher consumption levels of both feed and water consistently during the experimental period. In contrast, treated groups exhibited reduced feed intake. Mice administered *Nelumbo nucifera* root aqueous extract (NNRAE) and *Catharanthus roseus* leaves aqueous extract displayed the most significant reductions in final body weight, cumulative weight gain, and food intake, suggesting potential appetite-suppressing and weight-regulating properties. A pronounced decline in body weight was observed in intervention groups between weeks 5 and 8 (Figure 1a). Notably, treated groups showed elevated water intake, likely linked to metabolic adjustments and alterations in taste perception (Figure 1b).

Body weight and organ weight

Body and organ weight underwent significant changes due to the high-fat diet (HFD). The results for organ and body weights are presented in Table 1. After 56 days, a notable reduction ($p<0.05$) in body weight was observed in LRAE group at 28.49±1.24 g and CRLAE group at 26.92±1.09 g, compared to the HFD group at 31.97±1.61 g. This difference is attributed to the HFD's tendency to promote weight gain through increased fat storage and reduced energy utilization. The liver weight ratio increased substantially in response to the high-fat diet.

Following treatment with 400 mg of NNRAE and 500 mg of CRLAE, a significant decrease was observed in liver weight ratios, reaching 1.28±4.09 g and 1.25±3.18 g, respectively. However, no significant differences were found in the weights of the heart, lungs, spleen, or kidneys across the groups.

Lipid Profile

The lipid profile analysis showed significant changes in serum lipid and glucose levels among the treatment groups compared to the control group: The results are represented in Table 2. In the HFD group, levels of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), triglycerides (TG), and glucose were elevated. In contrast, these parameters were decreased in the intervention groups. The decreased values in Group 3 supplemented with LRAE 400 mg are (91.45±4.09 mg/dl), (37.30±1.49 mg/dl), (30.50±1.08 mg/dl), (23.65±0.58 mg/dl), (118.24±5.61 mg/dl), and (102.34±6.90 mg/dl), respectively. The highest values improved in Group 4 treated with CRLAE 500 mg and the values are (86.61±3.18 mg/dl), (38.22±2.61 mg/dl), (27.14±1.05 mg/dl), (21.25±1.07 mg/dl), and (106.24±4.57 mg/dl), and (95.88±5.59 mg/dl) respectively. The results are shown in Table 2

Liver Function Test

Liver function tests (LFTs) assess key enzymes (e.g., ALT, AST, ALP) and bilirubin levels to detect hepatic injury and evaluate functional capacity, serving as essential tools for diagnosing and monitoring liver health. In this study, therapeutic administration of *lotus root aqueous extract (LRAE)* and *Catharanthus roseus leaf aqueous extract (CRLAE)* demonstrated beneficial effects on LFT parameters. Mice on a high-fat diet (HFD) exhibited elevated serum levels of ALT, AST, ALP, and bilirubin—biomarkers indicative of liver stress and cellular damage. However, these markers were markedly reduced in treated groups receiving LRAE and CRLAE interventions, as illustrated in Figure 2.

Renal Parameters: Renal function parameters revealed notable variations in serum creatinine and blood urea nitrogen (BUN) levels across all experimental groups (Table 3). The high-fat diet (HFD) group exhibited markedly elevated renal markers, with peak serum creatinine levels at 2.71±0.15 mg/dL and urea concentrations reaching 35.34±0.16 mg/dL. In contrast, intervention groups treated with *Nelumbo nucifera* root aqueous extract (NNRAE) and Catharanthus roseus leaf aqueous extract (CRLAE) demonstrated significant reductions in creatinine levels, registering 1.05±0.02 mg/dL and 1.26±0.02 mg/dL, respectively.

BUN levels also improved in treated mice, with NNRAE and CRLAE groups showing values of 19.75±0.39 mg/dL and 18.36±0.17 mg/dL, respectively. These results indicate that neither extract negatively impacted kidney function, as evidenced by the normalization of key renal biomarkers compared to the HFD group. The comprehensive renal profile data are presented in Table 3.

Hematological Indices

Hematological parameters across all treatment groups showed no significant differences, as detailed in Table 4. After the consumption of HFD, the mean values for hemoglobin (Hb), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated. HFD group showed increased hemoglobin levels at 12.85±0.37 g/dL. In contrast, the NNRAE and CRLAE groups exhibited improved levels of hemoglobin 11.83±0.43 g/dL and 12.11±0.26 g/dL, respectively. Additionally, elevated levels were shown in RBCs (11.09±0.52 10^6 cells/µl), HCT (41.55±2.86 %), MCV (37.46±2.68 fl), MCH (11.58±0.52 ρg), MCHC (30.93±1.26 g/dl) group that followed HFD when compared to LRAE (10.18±0.52 10^6 cells/µl), CRLAE (10.60±0.29 10^6 cells/µl) in RBC, (38.64±1.52 %), (35.88±1.22 %) in HCT, (37.95±1.42 fl), (33.84±0.63 fl) in MCV, (11.62±0.50 ρg), (11.42±0.21 ρg) in MCH, (30.62±2.11 g/dl), (33.75±1.19 g/dl) in MCHC groups respectively. These results demonstrated the efficacy of intervention groups that played protective role in managing health concerns.

WBC count

The mean values of WBC parameters including lymphocytes, neutrophils, monocytes, and eosinophils, are shown in Table 5. All the values were calculated after the consumption of intervention groups. The HFD group revealed significant increment in WBC (9.23±0.43 10^3/µl), lymphocytes (62.84±2.63 %), neutrophils (28.33±1.13 %), monocytes (5.10±0.49 %), eosinophil (2.63±0.13 %), and basophil (1.10±0.01 %). However, NNRAE and CRLAE groups showed improved levels of WBC (7.83±0.24 10^3/µl), (7.22±0.47 10^3/µl), lymphocytes (58.65±3.27 %), (54.77±5.25 %), neutrophils (36.18±1.02 %), (39.23±1.86 %), monocytes (3.00±0.23 %), (3.70±0.19 %), eosinophil (1.60±0.07 %), (1.40±0.09 %), and basophil (0.57±0.02 %), (0.90±0.04 %) correspondingly.

Histopathology

In Fig (a) normal diet group, histopathological examination showed normal histology of liver. In Fig (b) the group with HFD, histological examination of liver revealed foamy alterations including hepatocytes degeneration and hepatic sinusoids. In the Fig (c) NNRAE showed a lesser degree of ballooning degeneration. In the Fig (d) CRLAE revealed reduced steatosis and inflammation, indicating hepatoprotective effects.

DISCUSSION

A high-fat diet (HFD) plays a critical role in the progression of NAFLD by inducing a series of metabolic disturbances that lead to liver damage. The excessive intake of fats results in lipid accumulation within liver cells, triggering cytotoxic processes that cause inflammation and hepatocellular injury. This lipid overload elevates liver enzyme levels, indicating hepatocellular damage. The current research aimed to investigate the therapeutic potential of the *N. nucifera* root and *C. roseus* leaves on the NAFLD rodent model. The eight-week study revealed a significant escalation in liver parameters of the positive control group and an improvement in the treatment groups. The treatment groups exhibited significantly reduction in body weight gain and feed consumption relative to the control group, which aligns with previous studies indicating that herbal supplements can modulate weight gain associated with high-fat diets. This reduction in body weight may be attributed to the appetite-suppressing effects of bioactive compounds present in both plants. The bitter taste of NNRAE and CRLAE may have contributed to increased water consumption, as bitter flavors can enhance the sensation of thirst. Moreover, plant leaves include high protein content which activates sympathetic system and results in increased water intake. These plants are rich in bioactive components that support weight management and overall health (17). However, liver weight and size increased due to HFD consumption but reduced after being treated with NNRAE and CRLAE. Other body organs also showed minor modifications but improved after treatment advocated by the findings of (18, 19). For instance, *C. roseus* has been shown to enhance metabolic rates and fat oxidation, contributing to weight management (20). Similarly, *N. nucifera* root is rich in dietary fiber, which promotes satiety and may help regulate caloric intake (9). Serum Glucose and lipid profile showed significant improvements as serum glucose and lipid parameters increased after HFD consumption. Hepatic insulin resistance is linked with NAFLD. Blood glucose promotes hepatic lipid buildup, and this resistance promotes lipolysis. HFD increases the risk of oxidative stress by disrupting the lipoprotein balance, elevating LDL and lowering HDL levels (21).

The NNRAE appears to impede the intestinal absorption of dietary fats. Additionally, its antioxidant properties may help protect LDL cholesterol from oxidative damage. Similarly, CRLAE seems to enhance bile acid production, which can facilitate cholesterol elimination. The administration of CRLAE might also increase the activity of lecithin-cholesterol acyltransferase (LCAT), potentially aiding in blood lipid regulation

(22). Animals given 400 mg of NNRAE and 600 mg of CRLAE for two weeks showed significant reductions in glucose, LDL, TG, TC, and VLDL levels, alongside improvements in HDL levels (23, 24).

Because of liver damage, the HFD group's levels of liver enzymes were significantly elevated. Liver inflammation is caused by cytotoxic processes triggered by lipid buildup in the liver cells. Because of the hepatocellular damage brought on by this lipid overload, the hepatic enzyme levels of ALT and AST are elevated. Persistent inflammation markers (NF-κB, IL-1β, IL-6, TNF-α, and CRP) and oxidative stress, which are often linked with HFD, further degrade liver cells and cause NAFLD, cirrhosis, hepatocellular carcinoma (HCC), and cardiovascular risk because decreased bile flow and hepatocyte injury lead to elevated levels of ALP and bilirubin (25). A high-fat diet has the potential to change the composition of the gut microbiota, which can enhance intestinal permeability and cause bacterial endotoxins to enter the bloodstream. Systemic inflammation and toll- like receptors can worsen the liver damage and increase liver inflammation. Sterol regulatory element-binding proteins (SREBPs) and peroxisome proliferator-activated receptors (PPARs) play important role in altering lipid metabolism. The administration of NNRAE 200 mg for seven days reduced the liver enzyme levels as reported by (9). Moreover, supplementation of CRLAE 600 mg for 15 days exhibited Hepatoprotective effects as described by (23).

The treatment groups showed significant improvements in renal parameters. High-fat diet consumption impaired kidney function which lead towards improper removal of waste products and causing stress on kidneys. The basic function of kidney is to remove toxins such as urea and creatinine from the body. HFD can cause inflammation, oxidative stress, lipid accumulation and metabolic disruptions which increased the levels of serum urea and creatinine. Research has shown that incorporating 5% *N. nucifera* powder into the standard diet of rats for four weeks can improve serum renal parameters, as reported by (26). Moreover, oral administration of *C. roseus* leaf ethanolic extract 400 mg for two weeks had been shown to improve serum creatinine and blood urea nitrogen levels (25).

Significant changes have been noted in the hematological profile of NAFLD induced mice, especially in the RBCs, Hb, and HCT levels. HFD abnormally increased the production of red blood cells and causes inflammation as reported by (Kim et al., 2020). The results of the study exhibited improved hematological profile in mice due to administration of NNRAE and CRLAE, as they contain anti-inflammatory and antioxidant compounds (27, 28). This suggests that both plants have the potential to treat blood related disorders. HFD raised WBC levels which indicated increased inflammation and abnormal immune response. The potential of *C. roseus* improved WBC count as reported by (29). HFD caused the destruction of RBCs which produce stress and inflammation. The results investigated that after treated with both plant extracts improved the immunity and inflammatory response.

Histopathological examination showed that both plant extracts significantly reduced inflammation and steatosis in liver tissues. Treatment with CRLAE significantly improved liver histology. This finding is similar with existing literature that enlightens the hepatoprotective potential of both of these plants. The antioxidant properties present in plants causing the reduction in liver fat and ameliorate oxidative stress which potentiates NAFLD (24, 30).

CONCLUSION

This research evaluates the therapeutic efficacy of NNRAE and CRLAE in managing NAFLD in a mice model. Both extracts improved body weight, lipid profiles, liver enzymes, and histology, with *Catharanthus roseus* leaves aqueous extract showing the most significant liver function test results. However, *Nelumbo nucifera* root also

improved all parameters that ameliorate NAFLD. These findings support the inclusion of herbal plants in diet for management of NAFLD, suggesting their potential as safe and effective natural therapies. Further research should focus on highlighting their mechanisms on other body organs to verify therapeutic potential in metabolic disorders.

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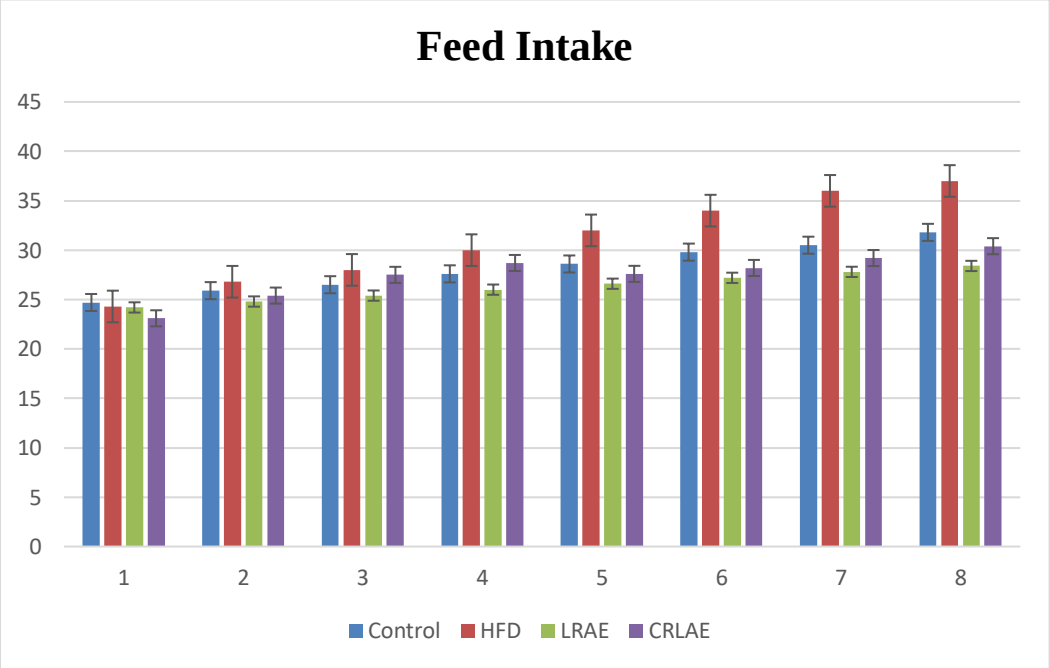
Experimental Design:

Group of Mice	Diet	Dose
Group I (Control)	Normal diet	**-**
Group II (Control positive)	HFD	**-**
Group III (NNRAE)	HFD + LRAE	**400mg/kg /b. wt. dilute in distilled water**
Group IV (CRLAE)	**HFD + CRLAE**	**500mg/kg /b. wt. dilute in distilled water**

HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract, CRLAE = *Catharanthus roseus* leaves aqueous extract



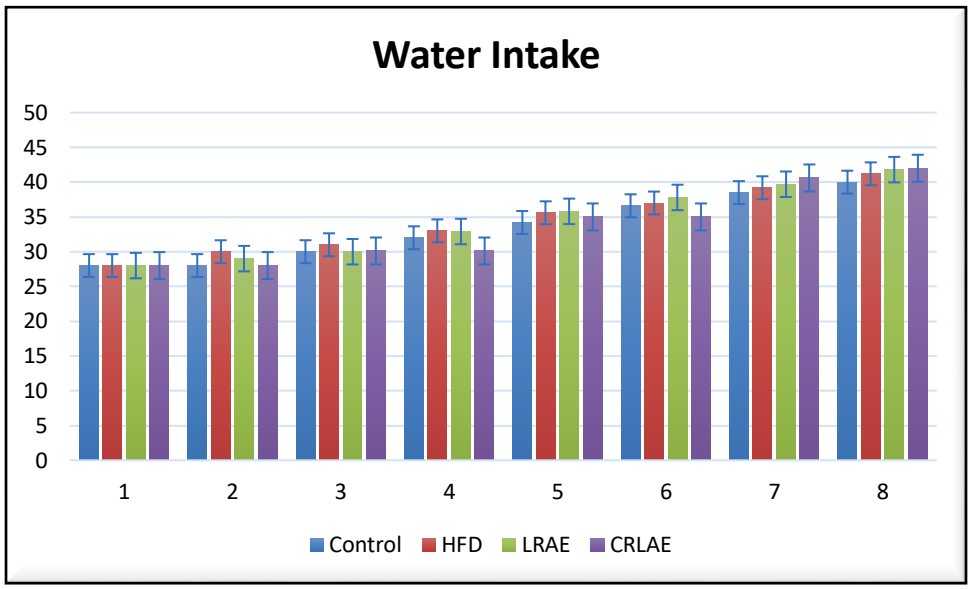


Figure 1. (a) Feed Intake (b) water intake

Table 1. Effect of Catharanthus leaves and Lotus roots on body weight and organ weight

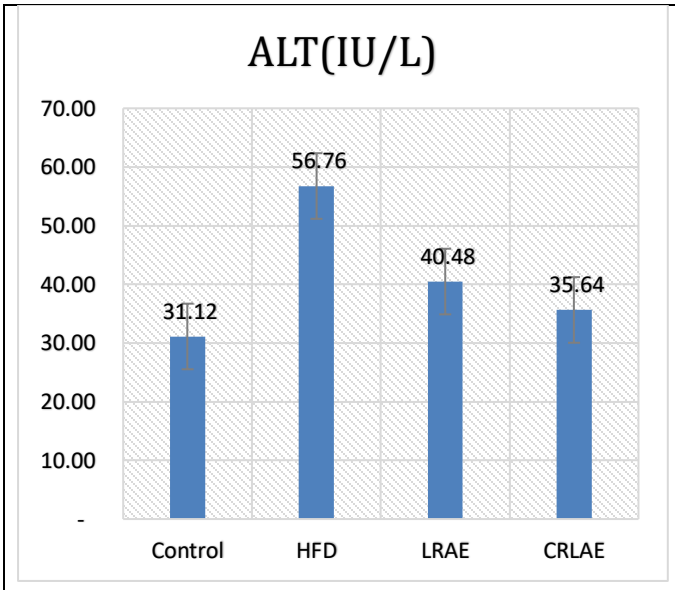
	Body weight and organ weight			
	Control	**HFD**	**NNRAE**	**CRLAE**
Initial body weight (g)	21.23±0.82^{ab}	21.78±0.91^{a}	21.01±0.83^{ab}	**20.44±0.93^{b}**
Final body weight (g)	25.39±0.95^{c}	31.97±1.61^{a}	28.49±1.24^{b}	**26.92±1.09^{bc}**
Weight gain (g)	4.16±0.17^{d}	10.19±0.35^{a}	7.48±0.21^{b}	**6.48±0.24^{c}**
Liver(g)	1.21±5.23^{b}	1.56±5.69^{a}	1.28±4.09^{b}	**1.25±3.18^{b}**
Heart(g)	0.34±2.31^{b}	0.37±1.13^{a}	0.36±1.03^{ab}	**0.35±1.86^{ab}**
Lungs (g)	0.50±0.04^{bc}	0.62±0.01^{a}	0.48±0.03^{c}	**0.53±0.04^{b}**
Spleen(g)	0.16±0.16^{b}	0.18±0.35^{a}	0.17±0.21^{ab}	**0.17±0.24^{ab}**
L-Kidney(g)	0.20±0.03^{c}	0.23±0.03^{a}	0.22±0.02^{ab}	**0.21±0.03^{bc}**
R-Kidney	**0.19±0.01^{b}**	**0.23±0.02^{a}**	**0.23±0.02^{a}**	**0.22±0.01^{a}**

HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract. Data are presented as the mean ± SD at $p < 0.05$. The same letters represent the insignificant difference between the value

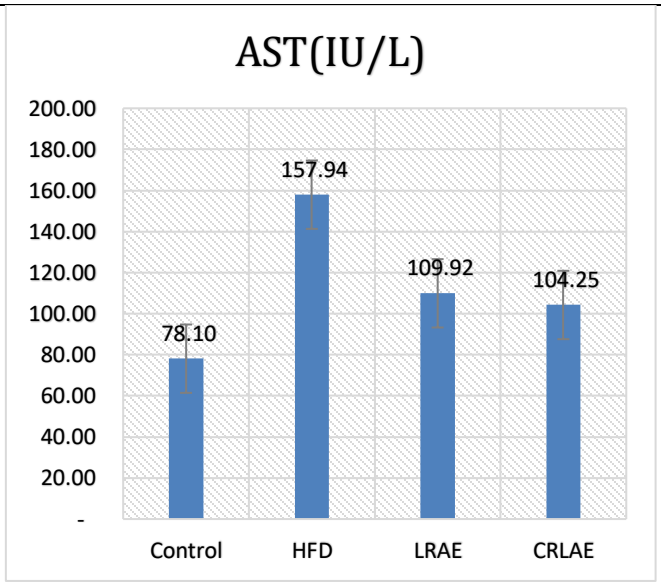
Table 2. Effect of *Catharanthus* leaves and Lotus roots on serum lipid profile (mg/dl)

Treatments	Lipid Profile					
	TC	**HDL**	**LDL**	**VLDL**	**TG**	**Glucose**
Control	79.35±5.23^{c}	35.25±1.83^{b}	24.25±1.03^{d}	19.85±0.87^{d}	99.25±5.39^{c}	**90.42±5.54^{c}**
HFD	110.69±5.70^{a}	39.26±1.73^{a}	41.36±1.17^{a}	30.07±1.16^{a}	150.35±5.42^{a}	**134.21±4.35^{a}**
NNRAE	91.45±4.09^{b}	37.30±1.49^{ab}	30.50±1.08^{b}	23.65±0.58^{b}	118.24±5.61^{b}	**102.34±6.90^{b}**
CRLAE	**86.61±3.18^{b}**	**38.22±2.61^{a}**	**27.14±1.05^{c}**	**21.25±1.07^{c}**	**106.24±4.57^{c}**	**95.88±5.59^{bc}**

HFD = High-fat diet, LRAE = Lotus root aqueous extract, and CRLAE = *Catharanthus roseus* leaves aqueous extract. Results are expressed as mean ± SD at $p < 0.05$. Values denoted by the same letter indicate no significant difference between them.



(a)



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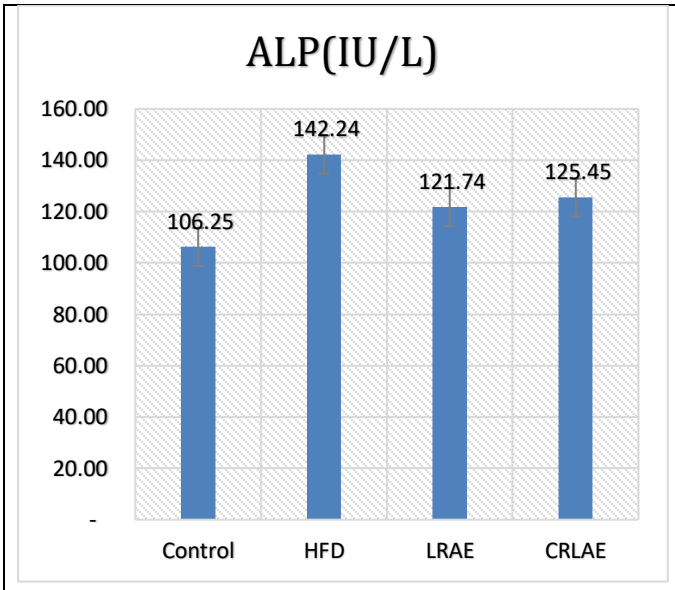

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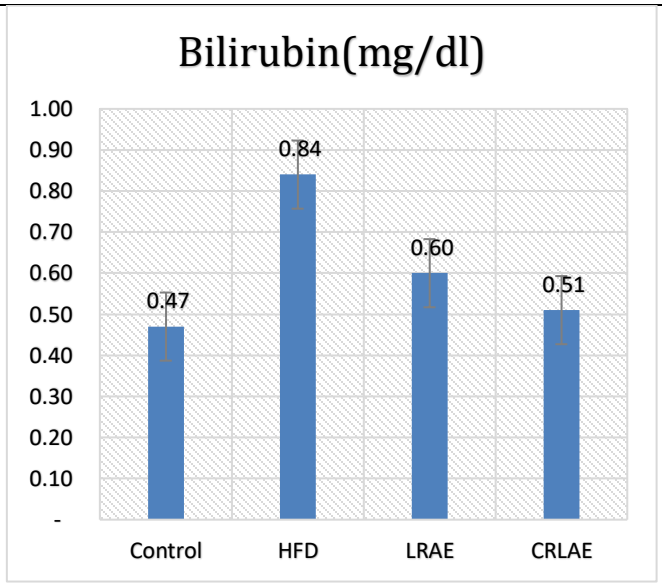

(d)

Figure 2. HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract, and CRLAE = *Catharanthus roseus* leaves aqueous extract. Data are presented as the mean ± SD at $p < 0.05$.

Table 3. Effect of *Catharanthus roseus* leaves and *Nelumbo nucifera* roots on renal parameters

Treatments	Renal parameters	
	Creatinine (mg/dl)	**BUN (mg/dl)**
Control	0.73±0.03^{d}	**17.25±0.63^{d}**
HFD	2.71±0.05^{a}	**35.34±1.39^{a}**
NNRAE	1.26±0.12^{b}	**23.75±1.67^{b}**
CRLAE	**1.05±0.04^{c}**	**19.36±0.67^{c}**

Results are expressed as mean ± SD at $p < 0.05$. Values denoted by the same letter indicate no significant difference between them. HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract, and CRLAE = *Catharanthus roseus* leaves aqueous extract.

Table 4. Effect of CRLAE and NNRAE on hematology profile

Treatments	Hematological Indices					
	Hb (g/dl)	RBC (10^6 cells/µl)	HCT %	MCV (fL)	MCH (ρg)	MCHC (g/dl)
Control	12.32±0.45^{b}	10.40±0.28^{b}	36.89±1.79^{bc}	35.47±1.03^{bc}	11.84±0.52^{a}	**33.40±1.23^{a}**
HFD	12.85±0.37^{a}	11.09±0.52^{a}	41.55±2.86^{a}	37.46±2.68^{ab}	11.58±0.52^{a}	**30.93±1.26^{b}**
NNRAE	11.83±0.43^{b}	10.18±0.52^{b}	38.64±1.52^{b}	37.95±1.42^{a}	11.62±0.50^{a}	**30.62±2.11^{b}**
CRLAE	**12.11±0.26^{b}**	**10.60±0.29^{ab}**	**35.88±1.22^{c}**	**33.84±0.63^{c}**	**11.42±0.21^{a}**	**33.75±1.19^{a}**

HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract, and CRLAE = *Catharanthus roseus* leaves aqueous extract. Results expressed as mean ± SD at $p < 0.05$. Values denoted by the same letter indicate non-significant difference.

Table 5. Impact of *Catharanthus roseus* leaves and *Nelumbo nucifera* roots on white blood cells (WBC) count

Treatments	WBC count					
	WBC (10^3/µl)	Lymphocytes %	Neutrophil %	Monocytes %	Eosinophil %	Basophil %
Control	8.46±0.43^{b}	53.00±1.91^{c}	40.00±2.32^{a}	4.80±0.47^{a}	1.50±0.06^{bc}	**0.70±0.04^{c}**
HFD	9.23±0.43^{a}	62.84±2.63^{a}	28.33±1.13^{c}	5.10±0.49^{a}	2.63±0.13^{a}	**1.10±0.01^{a}**
NNRAE	7.83±0.24^{c}	58.65±3.27^{ab}	36.18±1.02^{b}	3.00±0.23^{c}	1.60±0.07^{b}	**0.57±0.02^{d}**
CRLAE	**7.22±0.47^{d}**	**54.77±5.25^{bc}**	**39.23±1.86^{a}**	**3.70±0.19^{b}**	**1.40±0.09^{c}**	**0.90±0.04^{b}**

Results are expressed as mean ± SD at $p < 0.05$. Values denoted by the same letter indicate no significant difference between them. HFD = High-fat diet, NNRAE = *Nelumbo nucifera* root aqueous extract, and CRLAE = *Catharanthus roseus* leaves aqueous extract.

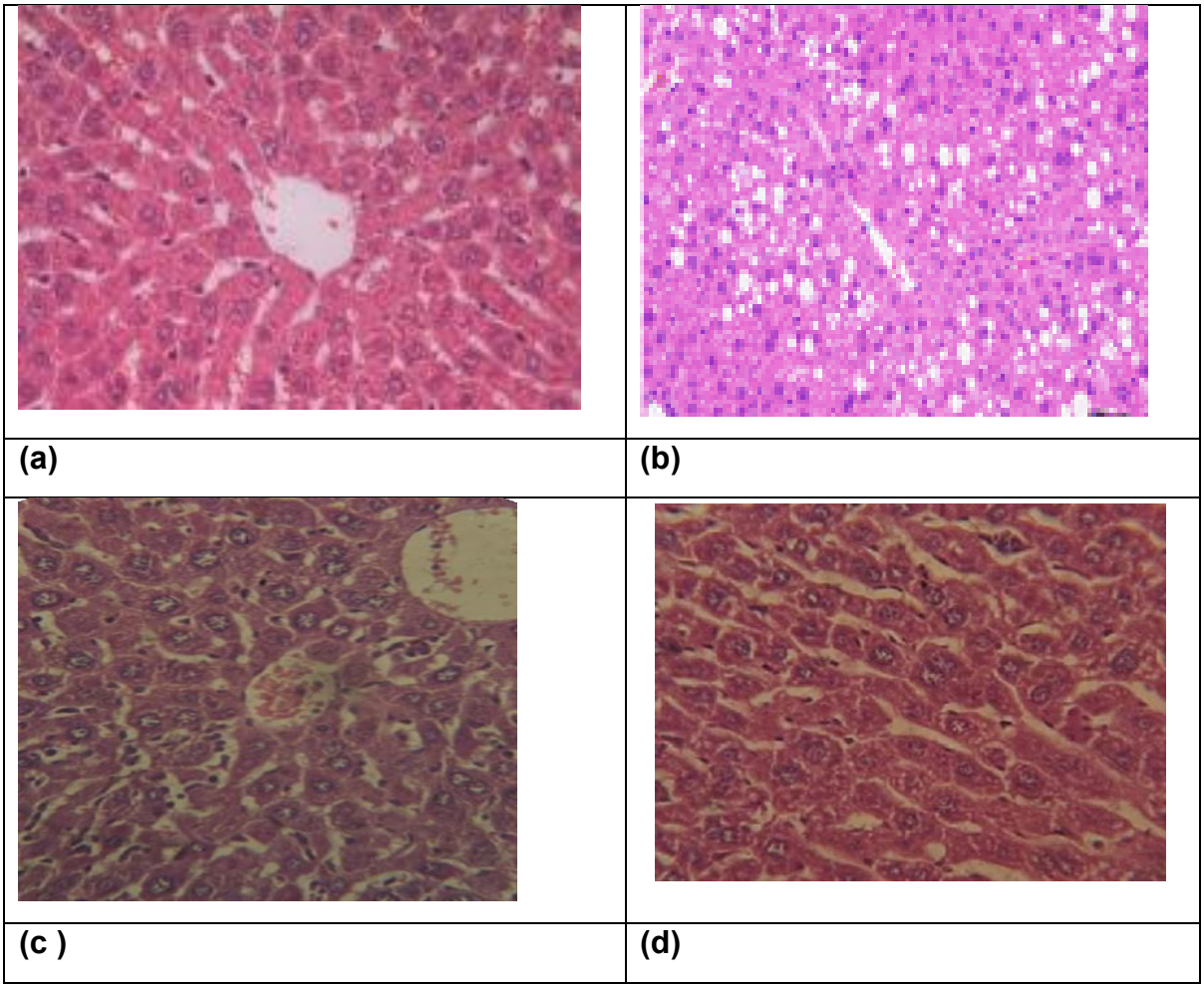

Figure 3. (a) Normal control (b) HFD (c) NNRAE (d) CRLAE