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Original Article

Investigation of *Lupinus angustifolius* Extracts against High-Fructose Diet-Induced Metabolic Syndrome in Male Wistar Rats

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ABSTRACT

Background: Metabolic syndrome is a global health concern, affecting a significant portion of the population worldwide. According to the World Health Organization (WHO), the prevalence of metabolic syndrome is increasing globally due to the rising of obesity and physical inactivity. **Objectives:** In the present study, we evaluated *Lupinus angustifolius* seeds for their potential in alleviating metabolic abnormalities associated with metabolic syndrome induced by High Fructose Diet. **Materials and Methods:** Hexane and ethyl acetate seeds extracts of *L. angustifolius* (HELA and EAELA) were screened for phytochemical constituents. Normoglycemic male albino Wistar rats were distributed into 6 groups with 6 in each. Fructose solution (10% w/v) administered p.o. for 8 weeks caused induction of Metabolic Disorder. The metabolic parameters were assessed and recorded before and after the administration of 200 mg/kg of extracts over 8 weeks. **Results:** Preliminary screening demonstrated the presence of flavonoids, alkaloids, glycosides among others from both extracts. HELA and EAELA showed a significant (p<0.001) decrease in obesity. They also led to significantly (p<0.001) decreased expression of inflammatory mediators like IL-6, TGFβ, and TNF-α. Hematological parameters exhibited improvement whereas HbA1C expressed a significant (p<0.001) increase in plasma insulin and HOMA IR revealed a significant (p<0.001) decrease in insulin resistance when compared to disease control. Metabolic hormone levels also showed a positive change after administration where leptin and adiponectin levels are decreased (p<0.001 and p<0.1 respectively) and gherlin levels were increased non-significantly (p<0.05). Antihyperlipidemic, antioxidant potential of the extracts was more proficient. **Conclusion:** The EAELA at 200 mg/kg displayed significant capacity in inhibiting the Metabolic Syndrome induced by High fructose diet when compared to that of HELA at 200 mg/kg which was effective in comparison with Standard.

Keywords: *Lupinus angustifolius*, Simvastatin, Oxidative stress, Hepatic lipid regulation, Hematological parameters.

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INTRODUCTION

The Metabolic Syndrome (MS) is a cluster of metabolic abnormalities that occur together and increase the risk of developing cardiovascular disease and type-2 diabetes. These abnormalities include elevated blood pressure, high blood glucose, and excess body fat around the waist, abnormal cholesterol levels, insulin resistance, and glucose intolerance.[1] In terms of worldwide statistics, it is estimated that 20-25% of adults have MS.[2] The prevalence of MS is higher in developed countries, where it affects up to 35% of adults. It is also more common in older adults and in individuals who are overweight or obese. As per the literature reports, the prevalence of MS in India is estimated to be around 20-25% among developing countries.[3] The study also found that the prevalence of MS is higher in urban areas compared to rural areas and that it is more common in older adults and in individuals who are overweight or obese. The molecular mechanisms behind the MS are complex and not fully understood. However, several key pathways have been identified that contribute to the development of the condition. One of the main molecular mechanisms is insulin resistance, which is a state in which the body's cells do not respond properly to the hormone insulin. Insulin resistance can lead to high blood sugar levels and an increased risk of type 2 diabetes. Another important mechanism is inflammation, which is a response of the immune system to injury or infection.[4] Chronic low-grade inflammation is associated with MS and has been linked to the development of insulin resistance, obesity, and atherosclerosis. Adipocyte dysfunction leads to the abnormal accumulation of fat in adipose

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tissue, which can result in the release of pro-inflammatory signaling molecules and contribute to the development of insulin resistance and inflammation. Finally, oxidative stress, which is a disparity between the production of Reactive Oxygen Species (ROS) and the body's ability to neutralize them, is also associated with MS. ROS can damage cells and contribute to the development of insulin resistance, inflammation, and atherosclerosis.[5] All these mechanisms are interconnected and contribute to the development of the MS. However, more research is desired to fully understand the underlying causes and identify new targets for the effective treatment. There are several factors that can increase the risk of developing MS; some of these predisposing factors include: Obesity, especially having excess abdominal fat, physical inactivity, aging, genetical, ethnicity, unhealthy diet, smoking, alcohol consumption, certain medical circumstances such as polycystic ovary syndrome, sleep apnea, and certain medications (glucocorticoids) can increase the risk of MS.[6] It is important to note that the development of MS is a multifactorial process, and the presence of multiple risk factors increases the risk. One of the treatment strategies for MS typically involves lifestyle changes. Medications are being prescribed to control blood pressure, blood sugar, and cholesterol levels. The goal of treatment is to reduce the risk of developing cardiovascular disease and diabetes. Natural products, including plant-based compounds and traditional remedies, have been used to treat a wide range of diseases for centuries. In recent years, there has been a renewed interest in the use of natural products to treat diseases in the modern world, and there are several reasons why these products are considered important: Safety, effectiveness, cost-effectiveness, sustainability, complementarity with modern medicine and cultural relevance.[7]

Lupinus angustifolius (*L. angustifolius*), also known as narrow-leaved lupin, is a plant species that belongs to the *Fabaceae* family. It is native to the Mediterranean region, also usually cultivated in many parts of the world, including Europe, North and South America, and Australia. *L. angustifolius* is a hardy and drought-resistant plant that can be grown in a variety of soils and climates. The plant has a variety of uses, including as a food crop and as a source of industrial and medicinal compounds. *L. angustifolius* is a good source of protein, and its seeds are used as a food ingredient in many countries, particularly in Europe. The present study is described the evaluation of effect of seed extracts towards High-Fructose Diet-Induced Metabolic Syndrome in animal models.

MATERIALS AND METHODS

Materials

The reagents used in this study were commercially available kits. The other chemicals used like Ethanol, Hexane, Ethyl acetate, Paraffin, Xylene, Fructose, Metformin, Simvastatin, Ketamine hydrochloride and Formalin were purchased from SD fine Chemicals, India.

Plant material collection

L. angustifolius seeds were procured from Mohan Alva, Moodabidri Pilikula Nisarga Dhama, Dakshina Kanmada District, Karnataka and were authenticated botanically at Sri Venkateswara University, Tirupati and a specimen was kept for herbarium under voucher number 0309.

Extraction

Seeds were powdered and a Soxhlet extractor was used for extraction from 300 g of powdered seeds using hexane and ethyl acetate for 72 hr. The extracts were filtered, dried and stored in containers labeled as HELA (hexane extract of *L. angustifolius*) and EAELA (ethyl acetate extract of *L. angustifolius)*.

Phytochemical screening

Phytochemical screening was performed to check for the presence of alkaloids, flavonoids, glycosides, tannins, phenolic compounds, steroids, coumarins and triterpinoids. According to standard procedures.[8]

Animals

Male Wistar rats weighing between 150-200g were procured from the Raghavendra enterprises, Bangalore, India. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethical Committee (Protocol No: IAEC/XVI/01/RIPER/2020). All animals were maintained under adequate conditions at an ambient temperature of 21±2°C, and were subjected to 12 hr light and dark cycle. They were fed with standard pellet diet and water ad libitum. Animals were kept for 7 days in laboratory for habituation.

Acute oral toxicity

According to Orgnisation for economic and cooperation Development (OECD) guidelines No. 420 male Wistar rats (180-200 g) were chosen randomly. After that animals were administered with fixed dosages of extracts orally (5, 50, 300, and 2000 mg/kg) in a stepwise approach. They were examined for toxic effects for every 8 hr for the first 24 hr after the initial 30 min. Another group of rats was administered with the subsequent dose (50 mg/kg b.wt.) by following the same procedure. Based on the absence of signs of toxicity or mortality within 24 hr, the method was carried out until the maximum dose of 2000 mg/kg b.wt. The rats survived and were observed for any effects once a day for the next 13 days.[9]

Fructose-induced MS

MS was induced in normoglycemic male Wistar rats by serving fructose solution 10% (w/v) in tap water that was prepared every day for eight weeks. Animals were randomly assigned to one of

five groups: Group 1 was designated as the Normal Control (NC) and received a normal pellet diet; Group II received only Fructose Diet (FD); Group III received fructose diet+HELA (200 mg/kg P.O.); and Group IV received fructose diet+EAELA (200 mg/kg P.O. Group V received fructose diet+Metformin (70 mg/kg/P.O.) and group VI administered with fructose diet+Simvastatin (10 mg/kg/P.O.) which serve as controls. At the end of eighth week before the sacrifice blood was collected from retro orbital plexus for the assessment of various biochemical parameters.

Body weight, liver weight, and liver index

All rats were weighed after the experiments were anesthetized with an overdose of ketamine (100 mg/kg b.wt IP). The initial and final body weights were recorded, and the difference between the two weights served as the basis for calculating body weight gain. After the liver was isolated, the wet weights of the liver, peritoneal fat, epididymal fat, and mesenteric fat were determined.[10]

Blood sampling and tissue preparation

Blood samples were taken from the retro-orbital plexus under light ether anesthesia, collected in various tubes to facilitate testing for all parameters. For histologic investigation, thin liver sections from each group were preserved in fixative. Tissue was spun at 4°C in a cooling centrifuge after being homogenized in 10% KCl on the ice using an electric homogenizer. The supernatant was then collected for an investigation into oxidative stress.

Measurement of hematological parameters

The Hemoglobin (Hb) content (gm%) of each animal was measured by Sahli's hemoglobinometer at the end of the 8th week was recorded in gm/% of Hb. The total Red Blood Cells (RBC), total White Blood Cells (WBC), and Packed Cell Volume (PCV) were estimated on fully automated fluorescence flow cytometry 5-part analyzers (Sysmex XS800i, Japan).[11]

Biochemical examinations

Estimation of plasma insulin was estimated by the RIA kit (Sigma-adrich, SA) method according to the modified method.[11] The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated to measure insulin sensitivity using the following formula.[12]

Estimation of metabolic hormones

The hormones levels were measured using ELISA kits (AUTOSPAN, India) following the standard protocols.[11]

Lipid profile parameters

The estimation of lipid parameters was carried out using kits from AUTOSPAN, India, and triglycerides were determined according to the GPO-PAP method. Total cholesterol and HDL cholesterol were calculated using the Fried Ewald formulas and CHOD/POD-Phosphotungstate method: LDL cholesterol was calculated.

$$\text{LDL} = \text{Total cholesterol-(HDL + VLDL)}$$

VLDL cholesterol was calculated.

$$VLDL = \frac{Triglyceride}{5}$$

The following formula was used to calculate the atherogenic index.[13]

$$Atherogenic\ Index = \frac{Total\ Cholesterol - HDL}{HDL}$$

Estimation of oxidative stress in plasma and liver tissue

Collected plasma and extracts of liver tissue homogenates were examined for oxidative stress markers using Superoxide Dismutase (SOD) Catalase (CAT), Reduced Glutathione (GSH), Glutathione Peroxidase (GPx), and lipid peroxidation.[14]

Inflammatory mediators

Inflammatory cytokines in serum were estimated using ELISA kits from Abcam (Cambridge, MA, USA).[15]

Assessment of hepatic lipid regulation enzymes

The liver tissue homogenate prepared were subjected to assessments of the regulation of lipid metabolism in the liver tissue. Using assays such as colorimetric or spectrophotometric methods to measure the activity levels of fatty acid synthase, HMG-CoA reductase, and carnitine palmitoyl transferase..[16]

Liver function markers

Quantitative diagnostic kits (Athenese Bio, India) were used to measure SGOT, SGPT, and alkaline phosphate.[17]

Histopathology

Tissue homogenates of isolated organs were collected and stored separately for analytical purposes. The tissues were neutralized by fixing them in formalin solution for histopathological studies.

Statistical analysis

The experimental data obtained were statistically analyzed by one way ANOVA test employing the trial version of GraphPad Prism, San Diego version (GraphPad Prism version 8.0.2, 263; GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Phytochemical screening

Phytochemical screening of extracts revealed the presence of flavonoids, alkaloids, steroids and tannins (Table 1).

Acute toxicity study

All through the study no animal showed distress but was constantly monitored before, during and after the administration

for the 13 days. At a maximum dose of 2000 mg/kg b.wt as there was no mortality, an optimum dose of 200 mg/kg b.wt was considered for further study.

Physiological parameters

It was observed that food and energy intake was in accordance with that of the normal value and did not alter too much in most of the cases. Only metabolic syndrome induced group showed significant (p<0.001) increase in food and energy intake as expected (Table 2). A change was observed in liver, mesenteric, peritoneal, epididymal fat weight in the induced group comparable to the normal control in all other treatment groups (Table 3).

Inflammatory mediators

Inflammatory mediators exhibited a significant (p<0.01) rise in their levels in disease control which decreased upon administration of standard and extract treatments. HELA showed considerably more effect than EAELA as illustrated in Figures 1, 2 and 3.

Plasma parameters

Induction of disease showed a significant (p<0.001) decrease in the packed cell volume which almost rose to normal in treatment groups. A significant (p<0.1) decrease in RBC count was observed in induced group and it is observed that a significant (p<0.01) raise can be observed in treatment groups when compared with disease control. When compared to normal control the WBC count was significantly (p<0.1) increased in disease control which went down in HELA and EAELA treated groups (Table 4).

There was a non-significant (p>0.05) change in the Blood glucose levels in normal control or disease control during the 2nd or 4th week. In case of disease control there is a significant (p-0.001) increase in blood glucose level indicating disrupted glucose metabolism as observed during the 8th week (Table 5). The same can be observed for treatment groups HELA and EAELA.

Table 6 indicates a significant (p<0.001) increase in HbA1C level in disease control group which decreased significantly (p<0.001) upon administration of HELA and EAELA.

Effect of *L. angustifolius* extracts on metabolic hormones

Figure 4 indicates how disease induction increased the level of leptin which was significantly (p<0.001) lowered by HELA and EAELA administration compared to the disease control. Gherlin levels exhibited a non-significant (p>0.05) increase upon administration of all treatments as compared to disease control. Adiponectin levels were significantly (p<0.1) decreased upon disease induction but administration of EAELA exhibited significant (p<0.01) increase in the levels when compared to standard and HELA treated groups.

Table 1: Phytochemical screening of extracts of *L. angustifolius*.

Sl. No.	Phytoconstituents	HELA	EAELA
1	Alkaloids	+++	++
2	Glycosides	+++	+++
3	Tannins	++	+
4	Flavonoids	+++	++
5	Phenolic compounds	++	++
6	Steroids	+	+
7	Coumarins	++	+
8	Terpenoids	++	++

Note: '+' indicates present.

Table 2: Effect of *L. angustifolius* extracts on physiological parameters (n=6).

Group	Initial body weight (g)	Final body weight(g)	Food intake (g/day)	Water intake (ml/day)	Energy intake (kj/day)	Energy efficiency (kj/g)
I	182.9±4.06	224.9±5.19	18.9±0.15	21.1±0.92	312.0±4.72	0.19±0.008
II	176.7±1.68^{nS}	282.8±2.52***	23.8±0.44***	18.5±0.13*	428.5±1.44***	0.38±0.005***
III	176.9±0.76^{nS}	180.1±2.83$^{###}$	19.3±0.40$^{###}$	19.1±0.18^{nS}	318.3±9.55$^{###}$	0.22±0.01$^{###}$
IV	176.4±4.60^{nS}	196.4±3.32$^{###}$	21.4±0.28$^{##}$	18.0±0.18^{nS}	338.5±7.41$^{###}$	0.26±0.007$^{###}$
V	170.8±2.35^{nS}	234.8±7.21$^{###}$	20.1±0.84$^{##}$	18.7±0.49^{nS}	325.5±1.41$^{###}$	0.25±0.009$^{###}$
VI	174.5±0.99	177.7±0.96$^{###}$	18.7±0.36$^{###}$	17.3±0.32^{nS}	312.8±9.25$^{###}$	0.19±0.005$^{###}$

All values are expressed as Mean+SEM, n=6 **p<0.01 compared to normal control, ### p<0.001 compared with fructose diet.

Lipid profile

Compared to normal control disease control showed increase in total cholesterol level. (Table 7) Total cholesterol considerably decreased upon administration of HELA and EAELA and HDL increased upon the administration of HELA and EAELA. Though not as significant as that of standard Simvastatin the treatment groups showed considerable improvement in all the lipid values. Atherogenic Index of HELA treated group was comparable to the levels of standard treated group (p<0.001) (Table 7).

Plasma oxidative stress parameters

Disease induction significantly (p<0.01) upregulated the production of stress inducing enzymes and neurotransmitters which lead to decline in SOD, CAT, and GSH and reduced GSH levels which upon administration of treatment group showed significant (p<0.001) increase in comparison with standard simvastatin. Lipid peroxidation was elevated significantly (p<0.001) upon disease induction declined upon the administration of HELA (Table 8).

Table 3: Effect of *L. angustifolius* extracts on liver wet weight, peritoneal fat wet weight, epididymal fat wet weight, mesenteric fat weight.

Group	Liver wet weight (g/100 g of body weight)	Peritoneal fat wet weight (g/100 g of body weight)	Epidedymal fat wet weight (g/100 g of body weight)	Mesenteric fat weight. (g/100 g of body weight)
I	2.5±0.09	0.5±0.02	0.9±0.02	0.6±0.03
II	3.6±0.13***	1.8±0.2***	2.2±0.07***	1.4±0.02***
III	2.6±0.06###	0.6±0.03###	0.9±0.05###	0.7±0.05###
IV	2.9±0.04###	1.4±0.19ns	1.8±0.13#	0.8±0.09###
V	2.5±0.09###	1.8±0.06ns	1.7±0.12##	1.0±0.03##
VI	2.6±0.02###	0.5±0.02###	0.9±0.007###	0.7±0.06###

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, ### p<0.001 compared with fructose diet, ## p<0.01 compared with Fructose Diet, # p<0.1 compared with Fructose Diet.

Table 4: Effect of *L. angustifolius* extracts on hematological parameters.

Group	RBC ($x10^6/mm^3$)	WBC ($x10^6/mm^3$)	Hb (g/dL)	Packed cell volume ($x10^6/mm^3$)
I	7.36±0.33	7.76±0.21	11.7±0.59	40.4±1.12
II	4.43±0.14**	9.56±0.10**	8.53±0.37***	31.1±0.99***
III	6.71±0.13 ####	8.14±0.38ns	11.1±0.24##	29.9±0.36ns
IV	6.17±0.15 ###	8.61±0.23ns	9.03±0.75ns	36.8±1.04###
V	6.84±0.21####	7.89±0.24##	11.8±0.38###	38.1±0.46###
VI	7.18±0.20####	8.18±0.09####	12.9±0.17###	39.0±0.14###

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, **p<0.1 compared to normal control, #### p<0.001 compared with fructose diet, ### p<0.01 compared with fructose diet, ## p<0.1 compared with fructose diet.

Table 5: Effect of *L. angustifolius* extracts on blood glucose.

Group	2nd week	4th week	8th week
I	89.5±1.48	98.7±5.66	91.25±6.02
II	178.8±1.86**	179±2.91***	276.7±7.82***
III	140.5±3.92ns	141±2.44###	129.1±4.06###
IV	133.8±1.68 ###	131±1.94###	86.74±6.14###
V	127±1.34###	104±8.12###	81.7±1.81###
VI	138.7±2.03###	144±1.40###	115.3±5.34###

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, **p<0.1 compared to normal control, ### p<0.001 compared with fructose diet, ## p<0.01 compared with fructose diet, # p<0.1 compared with fructose diet.

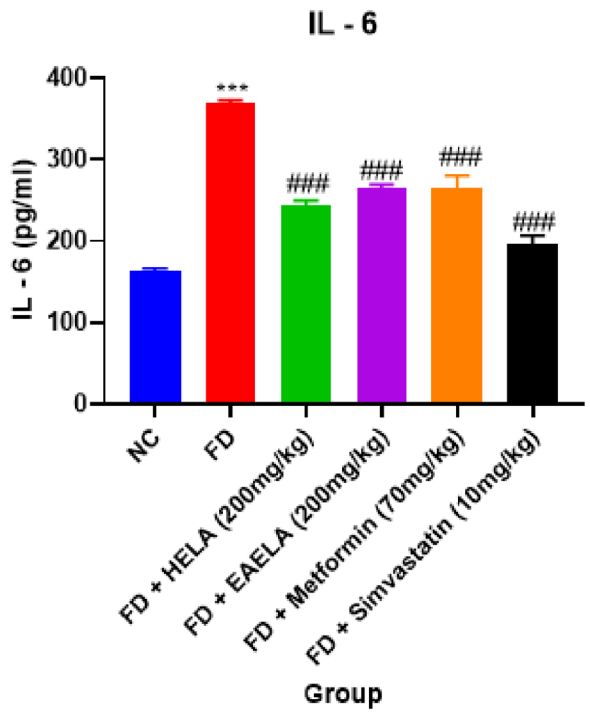

Figure 1: Effect of *L. angustifolius* extracts on inflammatory mediator IL-6.

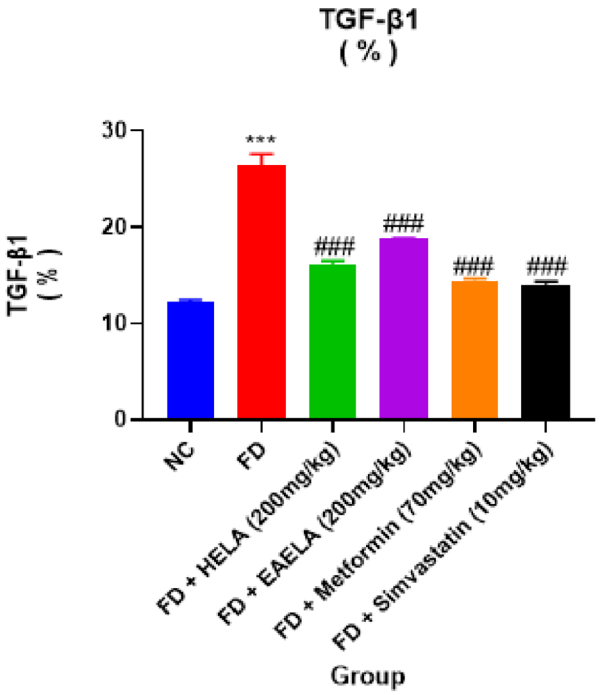

Figure 3: Effect of *L. angustifolius* extracts on inflammatory mediator TGF-β1.

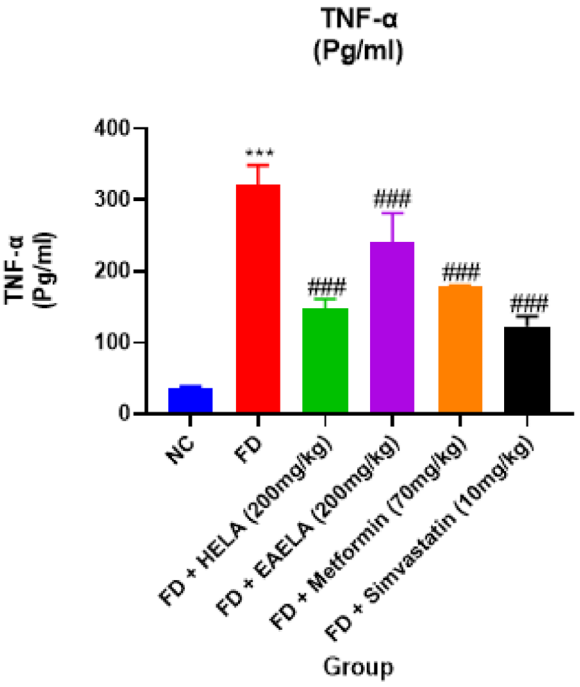

Figure 2: Effect of *L. angustifolius* extracts on inflammatory mediator TNF-α.

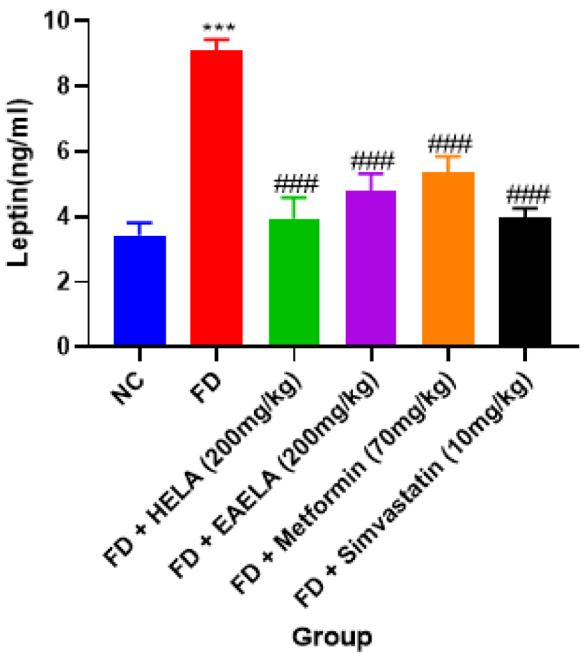

Figure 4: Effect of *L. angustifolius* extracts on leptin.

Effect of *L. angustifolius* extracts on liver tissue, function markers and lipid regulation enzymes

Table 9 shows a significant (p<0.01) increase in the SOD, CAT and lipid peroxidation levels upon disease induction due to increased neurotransmitter and glandular secretions. HELA and EAELA administration resulted in declination of these levels significantly (p<0.001). Reduced glutathione levels have also significantly (p<0.001) increased upon the administration of HELA and EAELA in comparison with standard (Table 9).

Fatty acid synthase levels increased significantly ($p<0.1$) upon disease induction and they considerably($p<0.1$) decreased with the administration of HELA and EAELA. HELA and standard groups exhibited similar effects ($p<0.1$). HMG CoA reductase and carnitine palmityl transferase levels were significantly decreased when compared to standard (Table 10).

Induction of disease increased the liver function markers levels which correlate with the lipid metabolism (Table 11).

DISCUSSION

The use of nutraceuticals as a therapeutic option for metabolic syndrome is widespread. A preliminary phytochemical screening showed the presence of flavonoids, alkaloids, steroids, tannins, and flavonoids in the seeds.[9]

Administration of HELA and EAELA seed extracts body weight, food intake, and the amount of water consumed. The extracts

Table 6: Effect of *L. angustifolius* extract on HbA1C, plasma insulin and HOMA-IR.

Group	HbA1C	Plasma Insulin (µIU/ml)	HOMA - IR
I	2.34±0.08	14.0±0.40	72.0±1.44
II	9.05±0.19***	4.40±0.24***	135±1.74***
III	4.07±0.21###	12.3±0.25###	80.0±2.95###
IV	5.21±0.21###	11.4±0.46###	89.7±1.03###
V	3.38±0.17###	12.8±0.46###	76.3±0.95###
VI	4.58±0.40##	10.4±0.31###	91.5±1.39###

All values are expressed as Mean+SEM, ***$p<0.01$ compared to normal control, ### $p<0.001$ and ## $p<0.01$ compared with Fructose Diet.

Table 7: Effect of *L. angustifolius* extracts on lipid profile.

Group	Triglyceride s (mg/dL)	Total cholesterol (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	Atherogenic index (%)
I	69.2±0.73	90.1±1.15	38.7±1.61	14.9±0.66	28.2±0.27	2.6±0.18
II	160±2.25***	179.5±5.29***	143.9±1.66***	38.0±0.38***	17.2±0.66***	9.5±0.17***
III	70.9±0.74###	116.1±4.62###	47.0±3.12###	17.8±0.24###	25.8±0.89###	3.8±0.38###
IV	84.9±1.48###	139.3±2.12###	74.0±1.80 ###	25.4±1.52###	19.15±0.59nS	6.3±0.71###
V	125±5.18##	181.7±4.40ns	139.8±1.50ns	34.4±1.08ns	17.5±0.52ns	9.1±0.36ns
VI	70.8±1.07###	84.8±0.59###	43.7±1.20###	15.5±0.59###	27.9±0.52###	2.5±0.20###

All values are expressed as Mean+SEM, ***$p<0.01$ compared to normal control, **$p<0.1$ compared to normal control, ### $p<0.001$ compared with Fructose Diet and ns is non-significant.

Table 8: Effect of *L. angustifolius* extracts on plasma oxidative stress parameters.

Group	SOD (U/mg protein)	CAT (µmol of H_2O_2 decomposed/min/mg protein)	Reduced GSH (µg/g tissue)	GSH peroxidase (mmol/l)	Lipid peroxidation (µmol MDA/mg protein)
I	29.5±1.42	64.0±0.64	75.8±2.40	150±2.1	15.7±0.42
II	12.5±0.43***	20.15±0.78***	30.9±2.37***	70.5±5.5***	66.8±0.75***
III	26.7±0.72###	54.57±1.71###	66.4±1.22###	129±1.9###	19.6±1.41###
IV	20.8±0.95##	48.18±1.84###	39.7±0.71##	105±1.75 ###	25.2±1.50###
V	28.3±0.37###	38.17±0.44###	54.8±0.96###	100±1.83 ###	19.1±0.66###
VI	28.7±2.55###	45.67±1.47###	63.8±1.54###	131±2.10###	18.2±1.02###

All values are expressed as Mean+SEM, ***$p<0.01$ compared to normal control, ### $p<0.001$ compared with fructose diet.

Table 9: Effect of *L. angustifolius* extracts on antioxidant enzymes and glutathione levels in liver tissue.

Group	SOD (U/min/gm tissue)	CAT (U/min/gm tissue)	Reduced GSH (µg/mg protein)	Lipid peroxidation (µ mol MDA/ mg protein)
I	178.0±2.98	19.4±0.59	26.2±0.58	13.8±0.46
II	80.2±4.70***	9.0±0.58***	13.05±0.51***	62.7±4.65***
III	299.6±5.45###	17.2±0.63###	22.4±1.06###	22.5±1.42###
IV	245.8±2.95###	14.3±0.47###	17.2±0.47###	22.1±1.22###
V	194.0±8.70###	16.5±0.41###	16.7±0.42###	28.7±2.04###
VI	268.2±7.59###	16.8±0.34###	19.4±0.87###	18.9±1.34###

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, ### p<0.001 compared with Fructose Diet

Table 10: Effect of *L. angustifolius* extracts on activity of hepatic lipid regulation enzymes.

Group	Fatty acid Synthase (mol/min/ml protein)	HMG -CoA reductase (mol/min/ml protein)	Carnitine palmityl transferase (mol/min/ml protein)
I	0.10±0.02	0.25±0.03	0.4±0.03
II	0.25±0.03**	0.6±0.03**	0.05±0.01***
III	0.12±0.01##	0.3±0.03#	0.4±0.06###
IV	0.20±0.01ns	0.4±0.09ns	0.3±0.03##
V	0.20±0.02ns	0.5±0.08ns	0.2±0.07ns
VI	0.12±0.02##	0.2±0.03##	0.3±0.03##

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, **p<0.1 compared to normal control, ### p<0.001 compared with fructose diet, ## p<0.01 compared with fructose diet, # p<0.1 compared with fructose diet

Table 11: Effect of *L. angustifolius* extracts on liver function markers.

Group	SGPT (U/l)	SGOT (U/l)	ALP (U/l)
I	71.1±2.03	80.7±1.21	136±1.62
II	212.8±2.84***	256.0±13.55***	348±10.2***
III	115.9±5.77###	108.2±2.86###	147±3.44###
IV	133.6±3.16###	158.9±0.95###	160±1.44###
V	141.3±1.66###	185.9±1.13###	186±2.37###
VI	109.2±0.94###	109.5±1.93###	163±1.65###

All values are expressed as Mean+SEM, ***p<0.01 compared to normal control, ### p<0.001 compared with fructose diet ***p<0.01 compared to normal control, ### p<0.001 compared with fructose diet.

reduced obesity by reducing the oxidized LDL, and influencing lipolysis in adipose tissue there by reducing free fat acids and associated triglycerides. Administration of HELA and EAELA showed decreased expression of IL-6 when compared with disease control, and the expression of TGF-β1 was also down regulated due to administration of HELA and EAELA when compared with disease control. Expression of TNF-α was also reduced when compared with disease control.[15]

The RBC count was higher in the EAELA and HELA-administered groups compared to the disease control group. WBC count was unaffected by the administration of HELA and EAELA. Groups administered with HELA exhibited more hemoglobin levels when compared with disease controls. In the EAELA-treated group packed cell volume was higher when compared with disease control.[11] These can be attributed to the up-regulation of enzymes upon the administration of extracts in Treatment groups.

Administration of fructose diet to rats resulted in a substantial increase in blood glucose levels up to 8 weeks. With respect to blood glucose levels HELA-treated group's result were non-significant till 2nd week while the EAELA-treated group showed significant decrease in blood glucose levels when compared with disease control from 2nd week itself. This might be due to the homeostatic control mechanisms operating in the initial period of disease progression. However, with the progression of disease beyond control the blood glucose levels were significantly

elevated indicating the development of metabolic syndrome with the continuous input of the high fat diet. Modulatory property on glucose levels was observed superiorly in EAELA over HELA almost similar to metformin by the end of the 8th week. HELA and EAELA showed decreased HbA1C when compared with disease control and were almost similar to standard metformin. Both HELA and EAELA restored insulin levels when compared with the standard metformin and Normal control. The decrease in blood glucose level can be attributed to the action of extracts at the transcriptional level leading to the expression of glucose transporters and also reversed pancreatic dysfunction and metabolic hormone imbalance under the influence of excess ROS by the antioxidant nature of phenol phyto constituents present in them. Administration of HELA and EAELA showed a greater reduction in HOMA-IR levels when compared with the disease control group.[12]

The tested extracts have quantitative differences with respect to the reversal of various parameters towards basal or comparable with the standards employed i.e., Metformin and Simvastatin. HELA and EAELA administration affected metabolic hormones such as leptin, gherlin, and adiponectin. With regard to disease control, there was no difference in ghrelin level after administration of HELA and EAELA.[11] HELA and EAELA treatment groups had lower triglyceride levels. The overall cholesterol levels in the HELA and EAELA treatment groups were low as compared to the disease control group. Comparing the disease control group to the HELA and EAELA, LDL levels were also diminished. Compared to the disease control group, the HELA and EAELA-treated groups displayed lower levels of VLDL. Administration of HELA resulted in upregulation of HDL significantly when compared to disease control. However, EAELA treatment didn't result in a significant increase in HDL. The extracts might have reduced cholesterol levels either by inhibiting the synthesis of cholesterol or by adsorption of cholesterol, the exact mechanism need to be elucidated. Administration of HELA and EAELA showed a decrease in atherogenic index when compared with the disease control group indicating a great antihyperlipidemic potential.[14]

When compared to the disease control group, plasma SOD levels were higher in the HELA and EAELA-treated groups. Plasma catalase levels were decreased in HELA and EAELA-treated groups when compared with the disease control group. GSH level in the HELA and an EAELA-treated group was higher. GSH peroxidase protects the tissue from oxidative damage and in HELA and EAELA treated groups it was higher when compared with the disease control group. When compared to the control group, lipid peroxidation was reduced in the HELA and EAELA-treated groups.[15,16] GSH, catalase and SOD are prominent *in vivo* antioxidants that quench free radicles. The extracts might have up-regulated the expression of antioxidant enzymes there by reduced the ROS towards normal and regard the progression of disease and further more the basal level of ROS served their physiological role by influencing the secretion of various endogenous compounds like neurotransmitters and glandular secretions. The significant modulatory effect on intracellular antioxidants and free radicals shows the antioxidant potential of *L. angustifolius* and suppressing oxidative stress is a crucial mechanism that can be helpful in retarding the progression of MS.

CONCLUSION

The present study suggests that *L. angustifolius* extracts can be used as a nutraceutical for the treatment of MS. The plant showed an anti-inflammatory effect by decreasing IL-6, TGF β-1, and TNF-α level. Decreasing LDL levels confirms its anti-hyperlipidemic properties. As it decreases total blood glucose levels, it could be used for treating type-2 diabetes. Effects on SOD, glutathione, catalase, glutathione peroxidase, and lipid peroxidation confirm its antioxidant activity. *L. angustifolius* increases RBC levels and has no impact on WBC levels, which indicates a positive effect on hematological markers. It contains a high nutritional content, making it a good nutraceutical for reducing the progression of MS since it exhibits a vast range of biological activities. Moreover, daily supplementation may result in better outcomes due to its nutritional value, but further research is needed to confirm the current indications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

OECD: Organization for Economic and Cooperation Development; **IAEC:** Institutional Animal Ethical Committee; **KCl:** Potassium Chloride; **ip:** Intraperitoneal; **p.o.:** per os; **SEM:** Standard error of Mean.

ETHICAL APPROVAL

All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethical Committee for Protocol No: IAEC/XVI/01/RIPER/2020.

SUMMARY

The hexane and ethyl acetate extracts of seeds of *Lupinus angustifolius* were screened for phytochemical constituents. Their nutraceutical ability in the treatment of Metabolic Syndrome was tested through anti-hyperlipidemic activity, antioxidant activity, antidiabetic activity, haematopoietic and anti-inflammatory activity. The extracts show very significant effect at test dose in *in vivo* animal models.

CONTRIBUTION DETAILS

Ch. Malathi Suvarna, Design, literature survey, experimental studies, data analysis, statistical analysis, manuscript preparation and editing. Subhakar Raju R, Concept, design, data analysis, manuscript review. Narender Malothu, Literature survey, data analysis, manuscript preparation, editing and review. Chakravarthi Guntupalli, Concept, Manuscript review.

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