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Original article

Effects of a medicinal plant *Macrotyloma uniflorum* (Lam.) Verdc.formulation (MUF) on obesity-associated oxidative stress-induced liver injury

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ABSTRACT

Obesity is a global health burden due to lifestyle modifications that have a strong association with a high incidence of diseases, such as dyslipidemia, glucose intolerance, nonalcoholic fatty liver diseases, diabetes, hypertension, coronary heart disease and cancer. The aim of the present study is to investigate the protective effects of a *Macrotyloma uniflurom* formulation (MUF) against high-fat diet (HFD)-induced oxidative stress and inflammation in obese rats. Male albino Wistar rats were fed a high-fat diet for 6 weeks to facilitate fat-induced oxidative stress and were simultaneously treated with MUF (400 mg/kg b.w.) through oral gavage from the third week onwards during the treatment phase. At the end of the experimental period, hepatic and oxidative stress markers were examined. The mRNA expression levels of inflammatory marker genes [Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6)] were also determined by reverse transcriptase-polymerase chain reaction in liver tissue. Hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase) and lipid peroxidation markers (Thiobarbituric acid reactive substances and LOOH) were significantly increased in HFD-fed rats, and administration of MUF resulted in remarkable suppression of these markers. Administration of MUF to HFD rats enhanced the activity of enzymatic (superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic (vitamin E, vitamin C and glutathione) antioxidants compared to HFD-fed rats. An anti-inflammatory effect of MUF was demonstrated by attenuating gene expression of TNF-α and IL-6. Therefore, the results of this study indicate that MUF could be a strong herbal therapeutic alternative for the protection of the liver as well as prevention and treatment of high-fat-induced oxidative stress and inflammation.

1. Introduction

Currently, approximately 1.9 billion adults worldwide are overweight and approximately 600 million of them are clinically obese due to the lifestyle changes (Centre, 2015). Obesity occurs through an imbalance between food consumption and energy expenditure, which culminates in excessive accumulation of fat in adipose tissue, which cause enlargement of adipose tissue cells, increase in adipose fat pad weight, and increase in adipose cell number (Ronkainen et al., 2015).

Obesity induced by a high fat diet has been considered to be one of the most popular models among researchers due to its ability to mimic the usual pattern of obesity in humans (Buettner et al., 2007), and it is believed to be a reliable tool for studying obesity because test subjects will readily gain weight when they are fed high-fat diets (HFD) (Gajda, 2009). It is well established that excessive consumption of a HFD leads to overweight and ultimately

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leads to obesity as well as promotes low-grade chronic inflammation related to adipocyte expansion and dysfunction, which is deeply tied to the pathogenesis of metabolic syndrome and other chronic diseases. Low-grade inflammatory states are commonly associated with increased levels of certain biomarkers, especially pro-inflammatory cytokines, such as TNF-α, and IL-6 (Hotamisligil, 2006). Adipocytokines play a primary role in obesity by binding to receptors in the liver and other tissues. The liver is bombarded by free fatty acids (FFA) that evacuate adipose tissue into portal blood. This process can directly cause inflammation within liver cells, which then release further pro-inflammatory cytokines and lead to more hepatocyte injury as well as affecting the integrity of liver cells (Guicciardi et al., 2013). Leptin, which is an adipocyte-derived hormone, is elevated in obese individuals and can provoke oxidative stress. It plays a main role in arbitrating a pro-inflammatory state in obesity (Castro et al., 2017), and Korda et al. (2008) stated that this physiological relationship may help to explain the relationship between obesity, inflammation and oxidative stress.

Overconsumption of a high fat diet promotes lipid metabolism, particularly mitochondrial β-oxidation of fatty acids, which also excite intracellular pathways, produce reactive oxygen species (ROS), and promote oxidative stress through multiple biochemical mechanisms, including superoxide generation from NADPH oxidases, oxidative phosphorylation, glyceraldehyde autoxidation, and protein kinase C (PKC) activation as well as the polyol and hexosamine pathways (Sies et al., 2005; Serra et al., 2012). Lipid peroxidation, such as thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide levels, are markers of the oxidative damage of ROS (Olusi, 2002; Uzun et al., 2007). In addition, oxidative damage is aggravated by the diminished activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) that inadequately neutralize the free radicals (Blokhina et al., 2002) that are formed by a high fat diet.

Epidemiological evidence has supported the role that dietary antioxidants play in the prevention of several chronic diseases, such as cancer, cardiovascular disease, and diabetes (Zhang et al., 2015). Numerous reports have demonstrated that antioxidants may be a regulator of obesity in mice or rats with high fat-diets (Abdali et al., 2015). In the present study, an attempt was made to evaluate the antioxidant activity of seed and leaf formulations of MUF, which is listed in indigenous medicine as having a high therapeutic value and is even now being used to treat various diseases.

M. uniflorum, which belongs to the family *Fabaceae*, is a potential grain legume that has excellent nutritional and remedial properties with better climate resilience to adapt to harsh environmental conditions. It has been employed in ethnomedicine for treating hemorrhoids, tumors, bronchitis, cardiopathy, nephrolithiasis, urolithiasis, splenomegaly, strangury, hiccups, ophthalmopathy, verminosis, and a vitiated condition of *vata*, kidney stones, inflammation and the liver problems (Kirtikar and Basu, 2004). Furthermore, the study aimed to explore the effects of seed and leaf formulations of *M. uniflurom* on hepatic marker enzymes, antioxidant status and pro-inflammatory markers in the liver of obese rats fed a high fat diet.

2. Materials and methods

2.1. Collection and extraction of plant material

M. uniflorum was collected from Lalgudi and identified by a taxonomist at the Department of Botany, St. Joseph college, (Voucher number VB001) Tamil Nadu, India. Leaves and seeds were separately shade-dried and pulverized to a coarse powder, and then, equal proportions of seed and leaf powders of *M. uniflorum* were mixed properly. The seed and leaf powder (100 g) was suspended in 300 ml of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40 ± 5 °C. The concentrated extract was exhaustively defatted by refluxing with n-hexane (18 h twice). The extract was kept in a deep freezer until use.

2.2. Animals

Healthy adult male albino Wistar rats that were bred and reared in the Central Animal House, Department of Animal Science, SASTRA University, Tamil Nadu, India were used for the experiment. Weight-matched animals (180–200 g) were selected and housed in polypropylene cages lined with husks and kept in semi-natural light/dark conditions (12 h light/12 h dark). Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, SASTRA University (Reg. No. 376/SASTRA/IAEC/RPP).

2.3. Diet

The standard diet consisted of a balanced diet containing 21.1% protein, 5.1% fat, 60.0% carbohydrate, 3.9% fiber, 7.9% minerals and 2.0% vitamins. The 40% HFD was prepared by mixing beef tallow (34.1%) with a standard pellet diet every day. All measures were taken to ensure uniform mixing of the additives of the diet before kneading the mixture using water.

2.4. Experimental design

Test animals were fed standard diets before the study. Then, animals were assigned to one of four groups with six rats in each group:

Group 1 received a standard pellet diet for 12 weeks;
Group 2 received a MUF (400 mg/kg, b.w.) for the last 4 weeks;
Group 3 received a HFD for 12 weeks;
Group 4 received a HFD for the first 8 weeks, then oral administration of MUF (400 mg/kg, b.w.) along with HFD for the next 4 weeks.
Group 5 received HFD for the first 8 weeks, then oral administration of orlistat (10 mg/kg, b.w.) along with a HFD for the next 4 weeks.

MUF (400 mg/kg, b.w.) was administered as a suspension with 0.5% dimethyl sulfoxide vehicle and drinking water directly into the stomach using a gastric tube in the morning for the last 4 weeks of the experiment.

2.5. Biochemical estimations

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using the methods of Reitman and Frankel (1957). Alkaline phosphatase (ALP) and gamma-glutamyltranspeptidase (GGT) were assessed using the methods of Kind and King (1954) as well as Rosalki and Rau (1972), respectively. TBARS and lipid hydroperoxides (LOOH) were estimated using the methods of Niehaus and Samuelsson (1968) and Jiang et al. (1992), respectively. The non-enzymatic antioxidants glutathione (GSH), vitamin C and vitamin E were estimated using the methods of Ellman (1959), Roe and Kuether (1943) and Baker et al. (1980), respectively. The activities of SOD, CAT, and GPx were measured using the methods of Kakkar et al. (1984), Sinha (1972), and Rotruck et al. (1973), respectively.

2.6. Extraction of RNA and semi-quantitative RT-PCR analysis

Total RNA was extracted from liver tissue using the AxyPrep™ Multisource total RNA miniprep kit (Axygen Biosciences, CA, USA) according to the manufacturer's protocol. A one-step reverse transcription polymerase chain reaction (RT-PCR) method was employed in this study (QIAGEN Onestep RT-PCR kit, Qiagen, USA) according to the manufacturer's protocol. Ten microliters of total RNA template (1–2 μg) was mixed with 5× RT-PCR buffer (10 μL), dNTP mix (400 μmol/L; 2 μL), 2 μL of each primer (0.6 μmol/L), one step RT-PCR enzyme mix (2 μL of a mixture of omniscript, sensiscript reverse transcriptases, and Hot Star Taq DNA polymerase), and RNase-free water (22 μL). RT-PCR was performed at 50 °C for 30 min and at 95 °C for 15 min for reverse transcription followed by 40 cycles of PCR reactions consisting of 94 °C (1 min) for denaturation at various primer-specific annealing temperatures for 1 min and 72 °C (8 min) for final extension. The primer sequences were synthesized as follows: TNF-α,5′-GGC TCC CTC TCA TCA GTT CCA-3′(forward), 5′-CGC TTG GTG GTT TGC TAC GA-3′(reverse); IL-6, 5′-TGC CTT CTT GGG ACT GAT GTT G-3′ (forward), 5′-TGG TCT GTT GTG GGT GGTATC C-3′(reverse). glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5′-ACC ACG AGA AAT ATG ACA ACT CCC-3′(forward), 5′-CCA AAG TTG TCA TGG ATG ACC-3′ (reverse). The levels of mRNA were normalized relative to the amount of GAPDH mRNA. The PCR products were subjected to electrophoresis in 1.5% agarose gels containing ethidium bromide visualized under UV illumination and photographed using a gel documentation system (Bio Rad, CA, USA).

2.7. Statistical analysis

All quantitative measurements were expressed as the means ± SD for control and experimental animals. The data were analyzed using a paired sample *t* Test on SPSS (Statistical Package for the Social Sciences) Version 20. The results were considered statistically significant if the *p* value was less than 0.05.

3. Results

The activities of serum liver marker enzymes, including AST, ALT, ALP, and GGT, were tested in control and obese rats fed HFD, and the results are compared in Table 1. The increased activities of AST, ALT, ALP, and GGT were observed in HFD-fed obese rats compared to the control groups. Oral administration of MUF and orlistat significantly reduced the obesity-induced hepatic marker enzymes AST, ALT, ALP, and GGT to normalize the liver functions. Similarly, compared to controls, lipid peroxidation (TBARS) was higher in the plasma, liver and adipose tissues of obese rats (Table 2). MUF and orlistat treatments of obese rats caused a remarkable reduction in lipid peroxidation in the plasma, liver and adipose tissues. On the other hand, LOOH synthesis was also drastically elevated in the plasma, liver and adipose tissues of HFD-obese rats, whereas it was reduced due to the effects supplementation with MUF and orlistat (Table 3).

The relative susceptibility of the liver tissues, plasma and erythrocytes to oxidation challenge is reported in Tables 4. The HFD resulted in a significant decrease in the antioxidant enzymes, such as SOD, CAT and GPx, in the erythrocytes, the liver and adipose tissues ($p < 0.001$) in obese rats. Compared to controls, obese animals showed lower SOD activity in erythrocytes, the liver and adipose tissues, and the herbal powder MUF as well as orlistat alleviated the oxidative stress damage in obese rats by increasing SOD activity in erythrocytes, the liver and adipose tissues. However, MUF and orlistat treatment induced an increase in CAT activity in erythrocytes, the liver and adipose tissues in obese rats to recover from the oxidative stress. Obese rats had lower activity of GPx

Table 1
Effect of *M. uniflorum* formulation on serum liver marker enzyme of control and HFD-fed rats.

Groups	AST ($IU^{@}$/L)	ALT ($IU^{@}$/L)	ALP (IU^{*}/L)	GGT ($IU^{\#}$/L)
Control	75.83 ± 2.43	28.46 ± 2.47	85.34 ± 2.14	21.88 ± 0.23
MUF (400 mg/kg BW)	74.21 ± 2.98$^{\#}$	26.31 ± 1.71$^{\#}$	84.92 ± 2.51$^{\#}$	21.99 ± 0.22$^{\#}$
HFD	96.72 ± 2.06**	42.90 ± 3.79**	104.45 ± 6.47**	34.48 ± 0.29*
HFD + MUF (400 mg/kg BW)	82.69 ± 5.32**	35.74 ± 1.78**	90.31 ± 4.02**	23.86 ± 0.24*
HFD + Orlistat	79.15 ± 2.24**	32.89 ± 1.45**	87.42 ± 3.68**	22.05 ± 0.27*

$IU^{@}$-μmol of pyruvate liberated per hour; IU^{*} - μmol of phenol liberated per minute; $IU^{\#}$ - μmol of p-nitroanilideliberated per hour.

Table 2
Effect of *M. uniflorum* formulation on TBARS level of control and HFD-fed rats.

Groups	TBARS	TBARS (mmol/100 g wet tissue)	
	Plasma (mmol/dL)	Liver	Adipose Tissue
Control	0.15 ± 0.01	0.80 ± 0.06	1.66 ± 0.09
MUF (400 mg/kg BW)	0.15 ± 0.01$^{\#}$	0.79 ± 0.05$^{\#}$	1.63 ± 0.10$^{\#}$
HFD	0.39 ± 0.04$_{\#}$	2.22 ± 0.18**	3.74 ± 0.27**
HFD + MUF (400 mg/kg BW)	0.17 ± 0.01$_{\#}$	0.92 ± 0.05**	1.92 ± 0.18**
HFD + Orlistat	0.16 ± 0.01$_{\#}$	0.87 ± 0.05**	1.87 ± 0.15**

Table 3
Effect of *M. uniflorum* formulation on LOOH levels of control and HFD-fed rats.

Groups	LOOH	LOOH (mmol/100 g wet tissue)	
	Plasma (mmol/dL)	Liver	Adipose Tissue
Control	8.40 ± 0.67	72.19 ± 5.13	60.61 ± 1.98
MUF (400 mg/kg BW)	7.92 ± 0.63$^{\#}$	71.70 ± 5.00$^{\#}$	61.32 ± 1.62$^{\#}$
HFD	18.36 ± 0.99**	112.14 ± 9.1**	158.33 ± 7.60**
HFD + MUF (400 mg/kg BW)	10.05 ± 0.73**	78.32 ± 5.19**	66.76 ± 1.32**
HFD + Orlistat	9.77 ± 0.75**	75.37 ± 5.96**	64.19 ± 1.14**

Table 4
Effect of *M. uniflorum* formulation on SOD, CAT and GPx activity of control and HFD-fed rats.

Groups	Erythrocyte	Liver	Adipose
SOD ($U^{@}$/mg protein)			
Control	7.09 ± 0.52	8.50 ± 0.64	14.99 ± 1.19
MUF (400 mg/kg BW)	7.22 ± 0.51$^{\#}$	8.63 ± 0.63$^{\#}$	15.14 ± 1.63$^{\#}$
HFD	3.15 ± 0.22**	4.17 ± 0.34**	8.32 ± 0.64**
HFD + MUF (400 mg/kg BW)	6.16 ± 0.45*	7.08 ± 0.41*	12.18 ± 1.03*
HFD + Orlistat	6.71 ± 0.50*	7.90 ± 0.45*	13.23 ± 0.96*
CAT ($U^{\#}$/mg protein)			
Control	160.61 ± 8.99	70.08 ± 4.80	31.50 ± 2.92
MUF (400 mg/kg BW)	161.03 ± 8.93$^{\#}$	71.93 ± 4.20$^{\#}$	32.15 ± 2.41$^{\#}$
HFD	105.39 ± 6.30**	58.21 ± 3.00**	15.28 ± 1.17**
HFD + MUF (400 mg/kg BW)	153.30 ± 7.44**	67.34 ± 3.45**	27.14 ± 2.06**
HFD + Orlistat	157.92 ± 7.64**	68.94 ± 3.52**	29.96 ± 1.39**
GPx (U^{*}/mg protein)			
Control	15.91 ± 1.55	7.58 ± 0.50	8.52 ± 0.65
MUF (400 mg/kg BW)	16.86 ± 1.54$^{\#}$	7.92 ± 0.62$^{\#}$	8.93 ± 0.63$^{\#}$
HFD	6.83 ± 0.57**	4.69 ± 0.38**	3.20 ± 0.24**
HFD + MUF (400 mg/kg BW)	13.88 ± 1.08*	6.30 ± 0.54*	6.93 ± 0.54*
HFD + Orlistat	14.33 ± 1.17**	7.11 ± 0.58**	7.47 ± 0.64**

$U^{@}$-enzyme concentration required to inhibit the NBTto 50%in one minute; $U^{\#}$- μmol of H_2O_2 consumed per minute/mg protein; U^{*} - μg of GSH utilized per minute/mg protein; Values are means SD of six rats in each group;
* Significant different at $p < 0.05$.
** Highly significant different at $p < 0.001$.
$^{\#}$ Non-significant different at $p > 0.05$

compared to controls that was enhanced by supplementation of MUF and orlistat.

In addition, MUF and orlistat administration resulted in a significant increase in non-enzymatic antioxidants in erythrocytes and the liver as well as adipose tissues of HFD-fed obese rats (Fig. 1). HFD-induced obesity caused decreases in GSH, vitamin C and vitamin E in plasma ($p < 0.05$) compared to normal control rats. Treatment with MUF or orlistat prevented these changes in HFD-fed obese rats and improved the levels of GSH. Similarly, the liver and adipose tissues of obese rats recovered from oxidative stress by elevating vitamin C and vitamin E during supplementation with MUF and orlistat. Thus, expression of the TNF-α and IL-6 genes was up-regulated in HFD-fed rats (Fig. 2), and treatment with MUF significantly down-regulated the expression of these genes.

4. Discussion

Feeding a high-fat diet to rats was found to be an appropriate model of the putative effects of dietary fat in humans (Lopez et al., 2003). Rat models are therefore useful tools for inducing obesity because they will readily gain weight when they are fed HFD (Diemen et al., 2006). The liver has a fundamental role in the metabolism, toxicity and elimination of endogenous and exogenous components, and although antioxidant enzymes have a major role in protecting the tissues from free radicals, understanding the influence of obesity on the metabolic capacity of the liver would be beneficial (Abdali et al., 2015). AST, ALT, ALP and GGT are reliable markers of liver function. ALT is a cytoplasmic enzyme that is found in very high concentrations in the liver. AST is present in the cytoplasm as well as the mitochondria and is less precise than ALT as a marker of hepatic damage. Serum ALP and GGT membrane-bound enzymes are released inequitably depending on the pathological condition. ALP is excreted by the liver *via* bile, and therefore, when the liver is affected, the serum enzyme level increases due to defective excretion. In the present study, significant increases in the activities of serum hepatic marker enzymes were observed in HFD-fed rats, which are consistent with previous reports (Abbas and Sakr, 2013). Elevation of the AST, ALT, and ALP activities in the serum is the result of liver cell destruction or changes in membrane permeability (Chhavi et al., 2014). Reduced antioxidant status is also involved in hepatic injury since reactive free radicals act as potential mediators of tissue damage.

Numerous reports have mentioned that high dietary fat intake-promoted inflammation associated with increased levels of leptin is strongly connected to pro-inflammatory cytokine elevation (e.g., TNF-α and IL-6) (Huang et al., 2015). Ding et al. (2010) reported that the HFD and bacteria interaction increased TNF-α mRNA production and intestinal inflammation in the mice. Increased TNF-α is high and significantly connected with the progression of obesity and progression of insulin resistance by activating NF-kB and other inflammatory pathways. Circulating cytokines, such as TNF-α or IL-6, have been shown to be elevated in obese humans (Kern et al., 2001; Syrenicz et al., 2006), and this trend can be reversed with weight loss. Although the mechanisms underlying obesity-associated inflammation is not fully understood, several studies have suggested that inflammation may derive from the accumulation of activated macrophages within the adipose tissue, the liver, and enlarged adipocytes in obese animals and humans (Park et al., 2010). According to these results, it has been observed that the HFD increased mRNA expression of these cytokines (IL-6 and TNF-α) and was associated with increased hepatic damage. In the liver injury, leptin has a proinflammatory role and is considered to be an essential mediator of liver fibrosis. Adiponectin acts as an antagonist of adipogenesis and plays an effective role in regulating lipid and glucose metabolism in insulin sensitive organs in both animals and humans (Harp, 2004). A low concentration of circulating adiponectin has been demonstrated in diet-induced and genetic models of obesity (Abdali et al., 2014). Accordingly, in this study, increased levels of leptin and decreased adiponectin levels were observed. It has been reported that depletion of adiponectin levels is correlated with body fat mass and insulin resistance (Yoon-Young et al., 2011). In addition, ROS diminishes the levels of adiponectin, which suggests that treatment with antioxidants or ROS inhibitors could refurbish the regulation of adipokines (Furukawa et al., 2004). Therefore, supplementation with antioxidants could reduce the risk of complications related to obesity and ROS (Fernandez-Sanchez et al., 2011). In this case, the administration of MUF to HFD rats significantly altered the levels of liver marker enzymes, reducing inflammation, possibly because of the antioxidant potential of MUF.

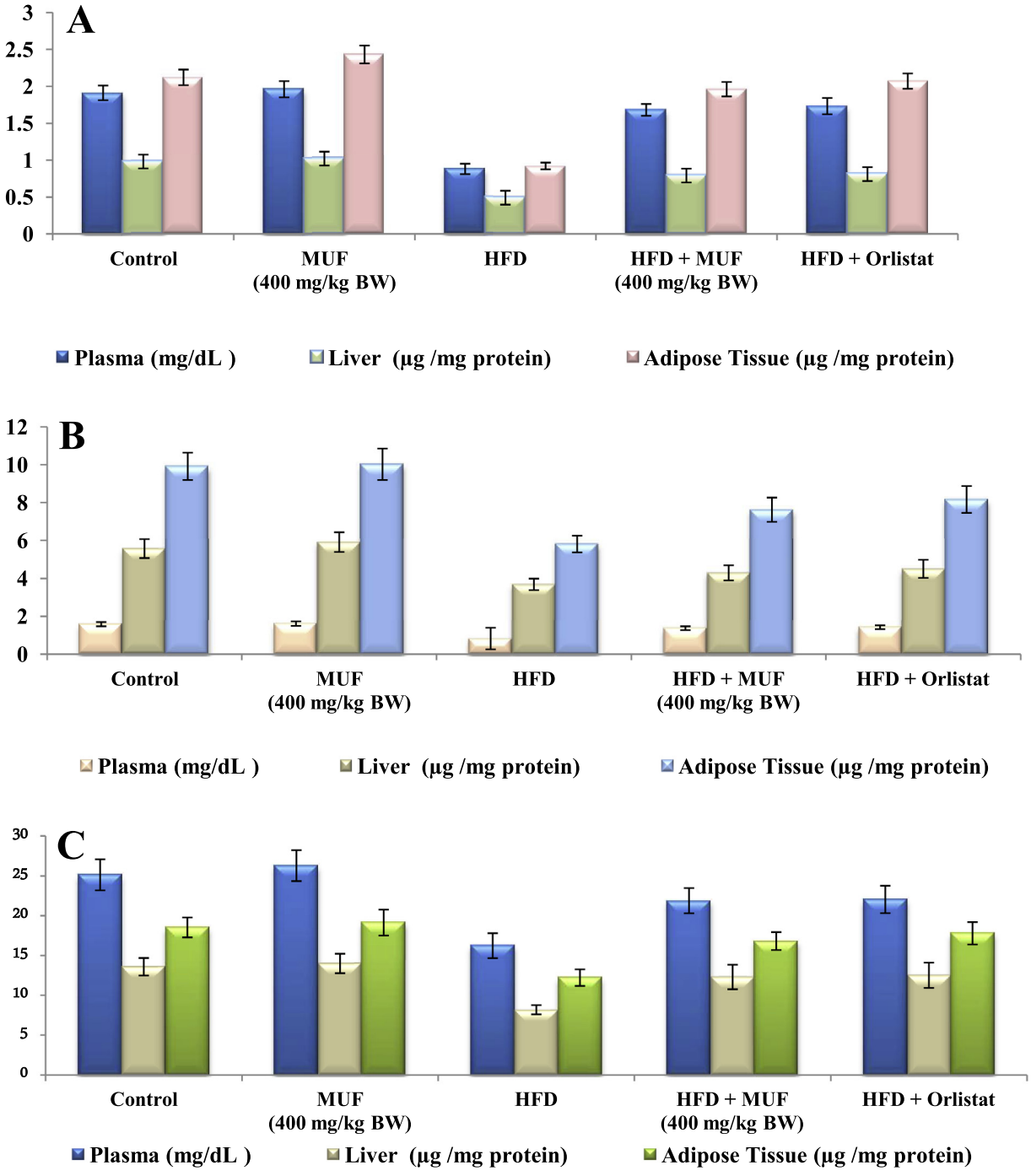
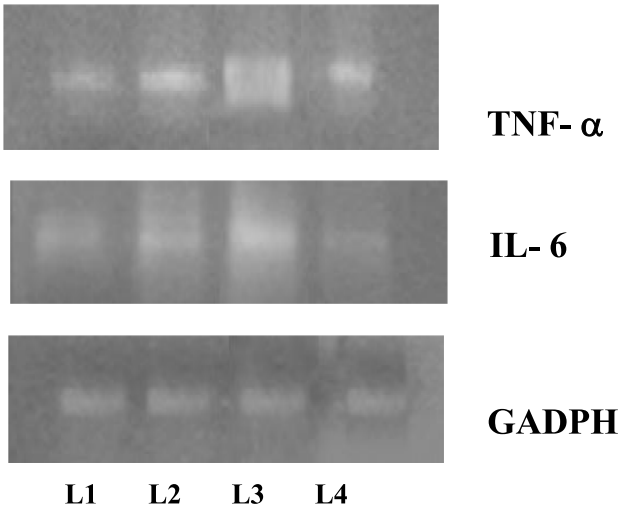


Fig. 1. (A–C) Effect of *M. uniflorum* formulation on Vitamin C (A), E (B) and GSH (C) contents in control and HFD-fed rats.

The imbalance between the oxidation and antioxidation may increase the generation of $OH^{\cdot}$, which is a powerful oxidant for many compounds. The increased free radical production might lead to lipid peroxidation of polyunsaturated fatty acids in the cell membrane, which has been implicated in pathological conditions, such as increased cell membrane rigidity, decreased cellular deformability and lipid fluidity. A high fat diet leads to significant modifications in the antioxidant defense mechanism against lipid peroxidation. It has been reported that lipid peroxidation products, disseminate from damaged tissue and therefore, can be evaluated in plasma. An increased concentration of the end products of lipid peroxidation is the most frequently quoted evidence for the involvement of toxic radicals in some diseases, including obesity (Rahman, 2007).

For example, SOD scavenges the superoxide anion to form hydrogen peroxide, which diminishes the toxic effects of this radical. CAT removes H_2O_2 by breaking it down directly to O_2. GPx is involved in the reduction of peroxides that can damage fatty acids, which prevents lipid peroxidation as well as damage to membrane phospholipids and the formation of TBARS (Ayala et al., 2014). In



L1: Control; L2: MUF; L3: HFD; L4: HFD + MUF

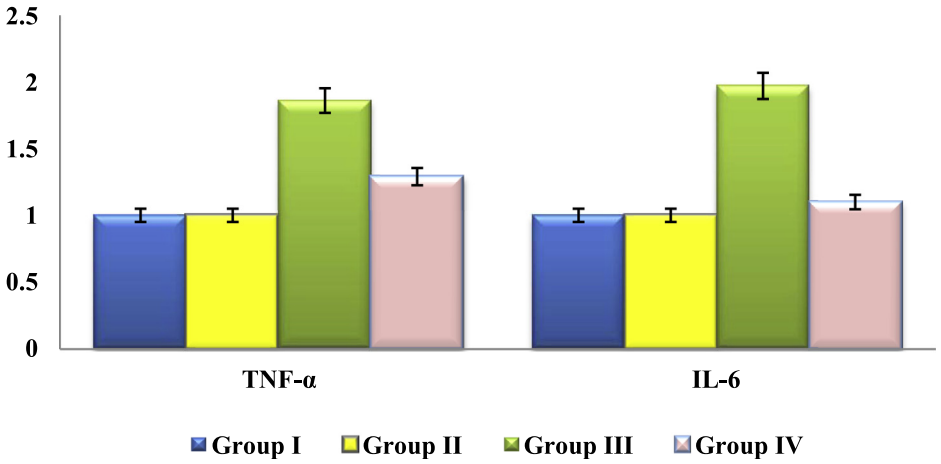


Fig. 2. Effect of *M. uniflorum* formulation on inflammatory markers gene expression in the liver of HFD-fed rats using RT-PCR.

this study, SOD, CAT and GPx were decreased in HFD rats compared to controls, which may be an important factor for limiting the antioxidant capacity due to increased TBARS and LOOH.

Apart from enzymatic antioxidants, non-enzymatic antioxidants, such as vitamins C and E as well as GSH, are excellent for protecting cells from oxidative threats. In our study, decreased levels of GSH, vitamin C and vitamin E in plasma and tissue were observed in HFD-fed rats. The depletion of GSH may be due to enhanced oxidation or its consumption by electrophilic compounds, such as lipoperoxidation aldehydes (Wolin, 2000). In addition, the decreased GSH concentration in erythrocytes from hyperlipidemia has been found to be moderately related to a high level of cholesterol (Ponce-Canchihuamán et al., 2010). Diminished vitamin C levels may be due to greater utilization for trapping ROS or could be due to declines in the GSH concentration because GSH is involved in the process of vitamin C recycling. Reduced vitamin E levels may due to improved utilization for scavenging oxyradicals generated by high levels of glucose or could be due to a reduced concentration of vitamin C because there is well known synergism between vitamin E and vitamin C (Adaramoye et al., 2008).

Potential antioxidant therapy should, therefore, include natural free radical scavenging enzymes or agents that can augment the activity of antioxidants. The radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols/flavonoids, which would contribute to their electron/hydrogen donating ability. Phenolic compounds disrupt the propagation stage of lipid autoxidation chain reactions through radical scavenging or metal chelating activity that converts hydroperoxides or metal prooxidants into stable compounds. (Mathew et al., 2015) have reported that high molecular weight phenolics have a strong ability to scavenge free radicals as well as a high efficiency based on their molecular weight, number of aromatic rings and hydroxyl group substitution compared to specific functional group substitution. The significant increase in the activity of these enzymes that occurred following the administration of MUF along with HFD indicates that compounds present in the MUF fraction, particularly phenolic compounds, such as Kaempferol and p-coumaric acid, can protect tissues from lipid peroxidation due to their antioxidant abilities.

5. Conclusion

The present study suggests that MUF possesses antioxidant and anti-inflammatory potential, which protects the body against the

adverse effects of obesity induced by a HFD. The results of this study demonstrated that a HFD elevates the liver enzymes and depletes antioxidants. However, supplementation with MUF reverses all of those parameters, which suggests that it has antioxidant potential. The scavenging of these oxidants is thought to be an effective measure to decline the level of oxidative stress. Therefore, the present work was designed to provide scientific evidence of the consumption of MUF in the management of obesity-associated oxidative stress.

Conflicts of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

VB, RLR and AVA designed the experiments. VB, AVA, RLR and RR conducted the experiments. AVA, RR, EFA and AAA wrote and revised the article in addition to conducting statistical analyses. All of the authors approved the final version of this manuscript.

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