Pravara Med Rev; September 2021, 13(03) , 13 – 20
DOI: 10.36848/PMR/2020/44100.51005

Original article

Antioxidant activity of Murraya koenigii leaves methanolic and aqueous extracts on oxidative stress in high fat-fructose fed rats

Rohan S. Phatak[1*], Chitra C. Khanwelkar[2], Somnath M. Matule[3], Anup S. Hendre[4], Kailas D. Datkhile[3]

[1]Ph. D Scholar of Pharmacology, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
[2]Professor and Head of Pharmacology, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
[3]Assistant Professor of Pharmacology, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
[4]Assistant Professor of Biochemistry, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
[5]Senior Research Officer of Molecular Biology and Genetics, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
*Corresponding Author: Dr. Rohan Sharadanand Phatak, Email: phatak.rohan1983@gmail.com

Abstract:

Background: High fructose-fat diet (HFFD) induces increased body weight, hyperlipidemia, alterations in metabolism and elevated oxidative stress. Evaluation of antioxidant effects of *Murraya koenigii* on HFFD induced oxidative stress in rats was very less reported. To study the influence of *Murraya koenigii* leaves against the effect of HFFD on the antioxidant status of rats.

Material and Methods: Thirty six rats of either sex were randomly divided into six groups of six animals each. HFFD was fed p. o to all rats from Groups I, II, IV, V and VI except Group III throughout the period of 14 weeks. Group III rats received normal diet and water *ad libitum* only. Group I, II, IV and V were treated respectively with aqueous extract of *Murraya koenigii* leaves (AEMK-200 mg/kg/day, p. o), methanolic extract of *Murraya koenigii* leaves (MEMK-200 mg/kg/day, p. o), metformin (MET-50 mg/kg/day, p. o) and atorvastatin (ATO-10 mg/kg/day, p. o). On the last day of experimental study, blood was collected by retro-orbital puncture method. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-s-transferase (GST), glutathione peroxidase (GPx) were assessed in the serum. Oxidative stress biomarker, thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC) were estimated in the serum.

Results: Both of aqueous and methanolic extracts of *Murraya koenigii* leaves treatment have improved CAT, GST, GPx, TBARS and TAC ($p < 0.001$) significantly in HFFD-fed rats compared to hyperlipidemic rats of Group VI.

Conclusion: The current study suggests that *Murraya koenigii* leaves methanolic and aqueous extracts have showed *in-vivo* antioxidant activity against HFFD induced oxidative stress and exhibited the protective role.

Keywords: High fructose-fat diet, Oxidative stress, *Murraya koenigii*

Introduction:

In the last two-three decades, it is observed that use of fructose as a sweetener in the food and confectionary industries is increased. Sucrose and high-fructose corn syrup are cheap available sweeteners which are extensively used by food manufacturers [1]. Moreover, the oxidative stress is usually linked to obesity and as risk factor for diabetes therefore adipose tissue secretes adipokines which leads to the production of reactive oxygen species (ROS) that cause insulin resistance (IR). All of these factors may contribute to metabolic syndrome (MetS) [2].

High fat-fructose diet (HFFD) induces mitochondrial dysfunction by impairing mitochondrial respiration in the phosphorylating state and inhibiting complex IV activity [3] and increasing the supply of electrons to the electron transport chain through upregulating the tricarboxylic acid cycle [3].

In HFFD model, fructose in contrast with glucose does not stimulate insulin secretion so it substitutes fat. Oxidative stress in the metabolism induced by fructose leads to excessive phosphorylation of this carbohydrate at the expense of ATP, causing phosphate deficiency, AMP accumulation, and increased synthesis of both uric acid and triglycerides [3]. Lipid peroxidation as a biomarker of oxidative stress has been implicated in non-alcoholic steatohepatitis (NASH) [4]. Hypolipidemic effects of this plant have been reported in our previous study [5].

The beneficial effects of *Murraya koenigii* leaves on HFFD induced oxidative stress in rats have very less reported in the recent literature. So the present study was aimed to assess the antioxidant activity of *Murraya koenigii* leaves in HFFD rat model.

Material and Methods:

Drug and chemicals

Metformin and atorvastatin as pure powder purchased from Sigma-Aldrich Ltd, India. Fructose obtained from Loba-chemie, India. Vanaspati ghee, egg yolk and coconut oil were procured from the local market. All reagents and chemicals used were of analytical grade and stored in a refrigerator at 4°C. The reagents were equilibrated at room temperature for 30 min before analysis.

Collection and authentication of plant material

Leaves of *Murraya koenigii* were collected and authenticated by Department of Botany, M. S. Shinde Mahavidyalaya, Tisangi, Kolhapur, India. The plant specimen voucher no: V03 (Ref: MHST/2016-17/28) of the plant was deposited in the herbarium.

Preparation of *Murraya koenigii* leaves extracts

Fresh leaves were washed under tap water thoroughly; dried under shade and powdered. Methanolic (MEMK) and aqueous (AEMK) extracts of *Murraya koenigii* leaves were prepared by soxhletation method. About 50 g of shade dried leaves powder of *Murraya koenigii* was defatted by petroleum ether; dried it then packed in a cloth bag and placed in the thistle of Soxhlet apparatus. Aqueous and methanolic extracts of *Murraya koenigii* leaves were prepared by water and methanol in Soxhlet apparatus respectively. Collected aqueous and methanolic extracts were concentrated in the vacuum rotary evaporator and dried in hot air oven. Percentage yield of MEMK and AEMK were calculated with respect to the total quantity of powder used for the extraction.

Acute toxicity study of extracts

AEMK and MEMK were performed in the acute toxicity test as per OECD-423 guidelines [6] for

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

fixing the therapeutic dose. The dose of 2000 mg/kg b w of AEMK and MEMK taken as a starting dose and were orally administrated to two rats. LD_{50} was determined and $1/10^{th}$ of LD_{50} was taken as therapeutic dose for the activity.

Animal ethics approval

Experiment study protocol was compiled with the guidelines of committee for the CPCSEA for animal experimentation in laboratory and Institutional Animal Ethics Committee, Krishna Institute of Medical Sciences, Karad (Reg. No. 255/PO/2000/bc/CPCSEA) approved the study.

Experimental design

Healthy Wistar rats of either sex weighing between 100-120 g were used in the study. Animals were maintained under standard husbandry conditions at room temperature with light: dark cycle for an acclimatization period of 7 days. Thirty six healthy Wistar rats of either sex were randomly divided into six groups (six animals per group) and they had free access to water and animal diet throughout the study period. Hyperlipidemia in rats was induced through HFFD model using 10% fructose added in drinking water bottle and high fat (vanaspati ghee: coconut oil in ratio of 3:1) mixed with an egg yolk in dough of animal food diet [5]. HFFD was fed to rats of Group I to VI (except Group III) throughout the study period of 14 weeks. Drugs or extracts in given doses were given per oral (p. o) treatment.

Group I-AEMK: Rats received HFFD and administrated AEMK-200 mg/kg/day, p. o

Group II-MEMK: Rats received HFFD and administrated MEMK-200 mg/kg/day, p. o

Group III-NC: Rats received normal diet and served as normal control

Group IV-MET: Rats received HFFD and administrated Metformin-50 mg/kg/day, p. o

Group V-ATO: Rats received HFFD and administrated Atorvastatin-10 mg/kg/day, p. o

Group VI-HC: Rats received HFFD and served as hyperlipidemia control

Blood collection and preparation of serum

On the end day of experimental study, all the animals were anesthetized, blood was collected by retro-orbital puncture method, centrifuged at 10000 rpm for 10 min and collected serum for analyzing *in-vivo* antioxidant parameters. All animals were sacrificed at the end of study.

Assessment of antioxidant parameters

1. Superoxide Dismutase (SOD)

It was determined by method of Martin *et al*. (1987) [7]. Copper-zinc superoxide dismutase (Cu-Zn SOD or SOD1) activity was measured by hematoxylin autoxidation method. Ten percent of serum in ice-cold 50mM phosphate buffer containing 0.1mM EDTA, pH 7.4. The homogenate was centrifuged at 12,000 g for 15 min and the supernatant collected. Inhibition of haematoxylin autooxidation by the cell free supernatant was measured at 560 nm. Two unit enzyme activity is 50% inhibition of the rate of autooxidation of haematoxylin in 1 min/mg protein. The enzyme activity was expressed as units/min/mg of tissue protein.

2. Catalase (CAT)

It was analyzed by method of Beers and Sizer (1952) [8]. To 1.9 ml of phosphate buffer (pH 7.0), 1.0 ml of 20mM H_2O_2 was added and then the reaction was initiated by the addition of 0.1 ml of serum. Decrease in absorbance was monitored at 1 min intervals for 5 min at 240 nm and activity was calculated using a molar absorbance coefficient of H_2O_2 as 43.6 M^{-1} cm^{-1}. The activity was expressed as mmoles of H_2O_2 decomposed/min/mg protein.

3. Reduced Glutathione (GSH)

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

Total reduced glutathione content was measured by the method of Ellman (1959) [9]. Ten percent of serum (0.1 ml) was deproteinized with 3.5 ml of 5% trichloroacetic and centrifuged at 4000 rpm for 5 min. To 0.5 ml of supernatant, 3.0 ml 0.2 M phosphate buffer (pH 8.0) and 0.5 ml of Ellman's reagent were added and the yellow color developed was measured at 412 nm. A series of standards (4–20 µg) were treated in a similar manner along with a blank and values were expressed as µg of GSH/mg protein.

4. Glutathione-s-transferase (GST)

It was measured by monitoring the increase in the absorbance at 340 nm using 1-Chloro-2, 4-Dinitrobenzene (CDNB) as a substrate by the method of Habig *et al*. (1974) [10]. To 1.7 ml phosphate buffer (pH 6.5), 0.2 ml of GSH and 0.04 ml of serum was added and the reaction was initiated by the addition of 0.06 ml CDNB. The change in absorbance was recorded at 1 min intervals at 340 nm for 5 min and the activity was calculated using extinction coefficient of CDNB-GSH conjugate as $9.6mM^{-1}cm^{-1}$ and expressed as m moles of CDNB-GSH conjugate formed/min/mg protein.

5. Glutathione Peroxidase (GPx)

It was assessed by method of Rostruck *et al*. (1973) [11] allowed a known amount of the enzyme preparation to react with H_2O_2 in the presence of GSH for specific time period and remaining GSH was measured by following the method of Ellman (1959) [9] as described earlier. To 0.5 ml 0.4 M phosphate buffer (pH 7.0), 0.2 ml of 10% serum, 0.2 ml of GSH and 0.1 ml of H_2O_2 were added and incubated at room temperature (25±2°C) for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 ml of TCA, centrifuged at 4000 rpm for 5 min and GSH content in 0.5 ml of supernatant was estimated. The activity was expressed as µg of GSH consumed/min/mg protein.

6. Total Antioxidant Capacity (TAC)

Total antioxidant capacity was estimated in terms of Ferric Reducing Ability of Plasma (FRAP) by method of Benzie and Strain (1996) [12]. To 0.04 ml of serum were allowed to react with 2 ml of working FRAP solution containing acetate buffer (pH 3.6), 10 mM of 2, 4, 6-Tripyridyl-S-Triazine (TPTZ) in 40 mM HCl, and 20 mM of $FeCl_3 \cdot 6H_2O$ in the ratio of 10: 1: 1 at 37°C. Fe^{+2}-TPTZ complex was measured at 593 nm, and time scanning was done at 30-second intervals for 4 minutes. The activity was expressed as U/mg protein.

7. Thiobarbituric Acid Reactive Substances (TBARS)

It was determined by Okhawa *et al*. (1979) [13]. Serum was quickly placed in ice-cold phosphate buffer saline (PBS). Lipid peroxidation (LPO) was initiated by adding 100 µl of 15 mM ferrous sulfate solution to 0.1 ml of serum. After 30 min of incubation at room temperature, 0.1 ml of serum was taken in a tube containing 0.1 ml sodium dodecyl sulfate (8.1% w/v), 0.75 ml of 20% acetic acid and 0.75 ml of 0.8% thiobarbituric acid aqueous solution and heated on water bath at 95º C for 60 min. The volume was made up to 2.5 ml, to which 2.5 ml of butanol: pyridine (15:1) was added. The reaction mixture was centrifuged at 4000 rpm for 10 min. Butanol layer was read at absorbance 532 nm spectrophotometrically. Malondialdehyde (MDA) production was measured in the activity and expressed as nmoles of MDA/mg protein.

Statistical Analysis:

Data were expressed as the mean ± S.E.M (n=6). Statistical analysis was done using analysis of One Way Analysis of Variance (ANOVA) followed by

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

Tukey's test and all values were considered significant at $p < 0.05$.

Results:

Assessment of antioxidant parameters

Our findings of the present study has indicated that HFFD reduced the antioxidant enzyme levels i.e; SOD, CAT, GSH, GST, GPx and non-enzymatic TAC in Group VI. Increased MDA level was observed in Group VI. *Murraya koenigii* leaves aqueous and methanolic extracts treatment attenuated the levels of CAT, GST, GPx, TBARS and TAC ($p < 0.001$) significantly in HFFD-fed rats of Group I and Group II respectively. Moreover, metformin and atorvastatin have shown an improvement ($p < 0.001$) significantly in HFFD-fed rats of Group IV and Group V respectively. Apart from all antioxidant parameters, no significant results were found in the antioxidant enzymes SOD and GSH (Table 1).

Table 1: Effect of *Murraya koenigii* leaves extracts on various antioxidant parameters in HFFD fed rats

Group		SOD	CAT	GSH	GST	GPx	TAC	TBARS
I-AEMK		0.234 ± 0.036	3.746 ± 0.020***	2.033 ± 0.388	3.761 ± 0.139***	2.995 ± 0.40***	1.266 ± 0.108***	0.364 ± 0.096***
II-MEMK		0.236 ± 0.041	3.713 ± 0.035***	1.679 ± 0.305	3.555 ± 0.114***	2.290 ± 0.246**	1.340 ± 0.080***	0.458 ± 0.090***
III-NC		0.326 ± 0.106	3.806 ± 0.071	2.071 ± 0.509	3.484 ± 0.031	2.039 ± 0.306	1.527 ± 0.078	0.403 ± 0.036
IV-MET		0.127 ± 0.020	3.669 ± 0.020***	1.093 ± 0.211	3.716 ± 0.049***	0.899 ± 0.101	1.227 ± 0.157***	0.510 ± 0.210***
V-ATO		0.260 ± 0.056	3.789 ± 0.039***	1.941 ± 0.380	3.629 ± 0.063***	2.184 ± 0.395**	1.376 ± 0.08***	1.033 ± 0.251***
VI-HC		0.081 ± 0.014	1.721 ± 0.188	0.894 ± 0.012	0.886 ± 0.018	0.693 ± 0.02	0.515 ± 0.05	2.626 ± 0.173
ANOVA	**F**	92.632	2.224	193.63	9.684	12.978	29.596	29.596
	p	**<0.0001**	0.0778	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Values are expressed in Mean ± SEM, Number of animals = 6, SOD-Superoxide dismutase, CAT- Catalase, GSH-Reduced Glutathione, GST- Glutathione-s-transferase, GPx - Glutathione Peroxidase, TAC- Total antioxidant capacity, TBARS- Thiobarbituric Acid Reactive Substances, Unit of SOD, CAT, GSH, GST, GPx and TAC expressed in U/mg protein and unit of TBARS expressed in nmol of MDA/mg protein, NS- Not significant, *** - *p<0.001*, **- *p <0.01*, * - *p <0.05* (Comparison with VI), ATO-Atorvastatin (10 mg/kg/day), MET-Metformin (50 mg/kg/day), AEMK-Aqueous extract of *Murraya koenigii* leaves (200 mg/kg/day), MEMK-Methanolic extract of *Murraya koenigii* leaves (200 mg/kg/day)

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

Discussion:

SOD is a family of metallo-enzyme catalyzes the dismutation of the highly reactive superoxide anion which converts into O_2 and H_2O_2 [14]. Diminished activity of SOD indicates oxidative stress by producing ROS thus leads to oxidative damage and thereby reducing the possibility of superoxide anion reacting with nitric oxide to form reactive peroxynitrite [15]. CAT scavenges H_2O_2 produced by SOD by catalyzing in the decomposition of H_2O_2 into H_2O and O_2 [14]. Decreased level of CAT causes the elevation of MDA level [16] which induces to the accumulation of superoxide and hydrogen peroxide radicals [17] and in turn to accumulation in ROS. Increased ROS leads to the deleterious effects of oxidative damage. *Murraya koenigii* leaves extracts have elevated the diminished activity of CAT and also reduced ROS induced oxidative damage. GSH is tripeptide of L-cysteine, L-glutamic acid and glycinecysteinyl moiety, non-enzymatic antioxidant and its related enzymes like GST and GPx [17-18]. GSH, GST and GPx quench free radical reactive species like H_2O_2, superoxide radicals and thereby provide the protection from binding to protein thiols of membrane and substrate for GPx [18]. Distorted levels of GSH, GPx and GST are related with an enhanced MDA. TAC was expressed in terms of FRAP. Increased FRAP values has suggested that natural plant extracts that supplementation of *Murraya koenigii* leaves extracts ameliorated the activity of all antioxidant enzymes and non-enzymatic antioxidants by owing to free radical scavenging properties of bioactive and cytoprotective compounds [19-20]. TBARS levels were measured as a marker of LPO and MDA production. ROS reacts with all biological substrates lead to LPO. Increased LPO impairs cell membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptor [21]. In our study, the findings of TBARS are altered by treatment of *Murraya koenigii* leaves extracts are in conformity with the study by Khan *et al*. (1997) [22].

Conclusion:

The current study suggests that *Murraya koenigii* leaves methanolic and aqueous extracts have showed *in-vivo* antioxidant activity against HFFD induced oxidative stress and provides the protection.

Recommendations:

Curry leaves supplementation is useful for enhancing the protection from oxidative stress. It has no side-effects and safe to eat.

Study limitations:

This study has undertaken in the animal study only and needs more elaboration in the clinical study.

Acknowledgement:

Authors express their hearty gratitude to Honorable Chancellor, Dr. Suresh Bhosale Sir for providing the necessary facilities and financial support to the Ph. D research project.

References:

1. Bellamkonda R, Karuna R, Sasi Bhusana Rao B, Haritha K, Manjunatha B, Silpa S, Saralakumari D. Beneficiary effect of *Commiphora mukul* ethanolic extract against high fructose diet induced abnormalities in carbohydrate and lipid metabolism in Wistar rats. *J Tradit Complement Med* 2017; 8(1):203-211.

2. Rivera-Ramírez F, Escalona-Cardoso GN, Garduño-Siciliano L, Galaviz-Hernández C, Paniagua-Castro N. Antiobesity and hypoglycaemic effects of aqueous extract of *Ibervillea sonorae* in mice fed a high-fat diet with fructose. *J Biomed Biotechnol* 2011; 2011:968984.
3. García-Berumen CI, Ortiz-Avila O, Vargas-Vargas MA, Del Rosario-Tamayo BA, Guajardo-López C, Saavedra-Molina A, *et al*. The severity of rat liver injury by fructose and high fat depends on the degree of respiratory dysfunction and oxidative stress induced in mitochondria. *Lipids Health Dis* 2019; 18(1):78.
4. Sena CM, Cipriano MA, Botelho MF, Seiça RM. Lipoic Acid prevents high-fat diet-induced hepatic steatosis in Goto Kakizaki rats by reducing oxidative stress through Nrf2 activation. *Int J Mol Sci* 2018; 19(9). pii: E2706.
5. Phatak RS, Khanwelkar CC, Matule SM, Datkhile KD, Hendre AS. Antihyperlipidemic activity of *Murraya koenigii* leaves methanolic and aqueous extracts on serum lipid profile of high fat-fructose fed rats. *Pharmacogn J* 2019; 11(4): 836-841.
6. OECD/OCDE. Acute oral toxicity–acute toxic class method. *OECD Guidel Test Chem* 2001; 423:1-14.
7. Martin JP, Daily M, Sugarman E. Negative and positive assays of superoxide dismutase based on hematoxyline autoxidation. *Arch Biochem Biophys* 1987; 255: 329-326.
8. Beers Jr, RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952.195: 133–140.
9. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
10. Habig WH, Pabst MJ, Jakoby WB. Glutathione–S-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130-39.
11. Rostruck JJ, Pope AL, Ganther HE, Swanson AB, Hofeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973; 179:588-90.
12. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Biochem* 1996; 239 (1): 70-76.
13. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-58.
14. Sharma V, Gupta R and Sharma S. Preventive Effects of *Tinospora cordifolia* extract against Aflatoxin-b1 induced oxidative stress in Swiss albino mice. *Asian J Pharm Clin Res* 2011; 4(4): 149-155.
15. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; 17(1):24-38.
16. Selmi S, Jallouli M, Gharbi N and Marzouki L. Hepatoprotective and renoprotective effects of Lavender (*Lavandula stoechas*) essential oils against malathion-induced oxidative stress in young male mice. *J Med Food* 2015; 18(10): 1103-11.
17. Palanivel MG, Rajkapoor B, Kumar RS, Einstein JW, Kumar EP, Kumar MR. Hepatoprotective and anti-oxidant effect of *Pisonia aculeata* against CCl_4-induced hepatic damage in rats. *Sci Pharm* 2008; 76: 203-15.
18. Phatak RS, Matule SM. Reno-hepatoprotective effects of *Murraya koenigii* leaves chloroform extract (MKCE) against lead-induced oxidative stress in mice. *Int J Pharm Sci Res* 2017; 8(10): 4106-4112.
19. Desai RR, Khanwelkar CC, Gidamudi S, Phatak RS, Jadhav S, Thorat V, Salve P. Antidiabetic effect of *Murraya koenigii* leaves aqueous extract on blood sugar levels in alloxanized diabetic rats. *Pravara Med Rev* 2019; 11(2): 19-24.
20. Phatak RS, Khanwelkar CC, Datkhile KD, Durgawale PP. Effect of *Murraya koenigii* leaves extract on gluconeogenesis and glycogenolysis in isolated rat hepatocytes culture. *Asian J Pharm Clin Res* 2018; 11(11): 264-266.
21. Onoja SO, Omeh YN, Ezeja MI, Chukwu MN. Evaluation of the in vitro and in vivo antioxidant potentials of Aframomum melegueta methanolic seed extract. *J Trop Med* 2014; 2014:159343.

22. Khan BA, Abraham A, Leelamma S. Anti-oxidant effects of curry leaf, Murraya koenigii and mustard seeds, Brassica juncea in rats fed with high fat diet. *Indian J Exp Biol* 1997; 35(2):148-50.

Date of Publication: 30 September 2021
Author Declaration: Source of support: Nil , Conflict of interest: Nil
Plagiarism Checked: Plagramme