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Research Article

Exploration of Antioxidant Potential of *Moringa oleifera* L. (Sehjana) Fruits/Pods on High Fat Diet Induced Rats

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ABSTRACT

The objective of the present work is to evaluate the in-vivo antioxidant potential of 50% ethanolic extract of *Moringa oleifera* against high fat diet induced rats. Animal were treated with plant extract for 30 days, and high fat diet was given to all groups except plain control through, out the study, and alpha tocopherol acetate (Vit, E) was used as standard. Pre-treatment with 22 mg/100 gm of body weight of 50% ethanolic extract of *Moringa oleifera* improved the Superoxide dismutase, catalase, glutathione, and lipid peroxidation levels significantly as compared to control group. The present studies revealed that *Moringa oleifera* has significant in-vivo antioxidant activity and can be use to protect tissue from oxidative stress. The result showed that the activities of SOD, catalase, lipid peroxidase, and glutathione, in groups treated with high fat diet declined significantly than that of normal group. 50% ethanolic extract of *Moringa oleifera* in the dose of 22 mg/100 gm of body weight, has improved the SOD, catalase, glutathione, and lipid peroxidation levels significantly, as compared to the alpha tocopherol (Vit,E). Based on this study we conclude that 50% ethanolic extract of *Moringa oleifera* possesses in vivo antioxidant activity and can be employed in protecting tissue from oxidative stress.

INTRODUCTION

Moringa oleifera Lam. (Moringaceae) commonly known as Sehjana in Unani Medicine, Drumstick, and horse, radish tree in English. It is medium sized tree about 10-12m height [1, 2]. Sehjana is a famous Indian drug used in a number of pathological conditions although, the entire plant has medicinal value but its fruit, leaves, seeds, have more important and interesting medicinal values. Its different parts are used after little processing as a single drug. The leaves are rich in protein, minerals, vitamins, carotene and antioxidant compounds, and other essential phytochemicals. The seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid >70%) and after refining a notable resistance to oxidative degradation [3]. The seed of pods (fruits) of the *Moringa oleifera* tree are one of the most nutritive and useful parts of this versatile plant.

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The seed of pods are use in a variety of traditional and modern medical treatments and are consumed as food in many areas of the world. The durable, drought-resistant nature of the *moringa* tree makes it a valuable source of nutrition in regions where water is scarce. The fruit is long 20-45 cm, green when young or light brown when ripe, pendulous, triangular, longitudinally ribbed composing of three valves containing a soft white pith and a single row of seeds. Taste is slight bitter without any definite smell [4, 5]. The *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, ß-sitosterol, caffeolquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant act as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic [6], antihypertensive, cholesterol lowering [7], and antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities [1,5], aphrodisiac, anthelmintic, analgesic activities [8,9], Rubefacient, Vesicant [10]. According to Unani literature it possess many actions like Moohallil-e-Waram, Muqawwi-e-Bah, Mushtahi, Qatile Kiram-e-Amaa [11, 12], so medicinanally used in Waja-ul-Mafasil, Waja-ul-Qutu, Zof-e-Ishteha [13, 14]. Antioxidants are agents that protect our body against damage by the free radicals such as Vit E, Vit C, etc. Which are responsible for combating the diseases caused mainly or partly by oxidative stress. An antioxidant is any substance that at low concentration delays the oxidation of proteins, carbohydrates, lipids, and DNA [15]. A free radical is a molecule or a molecular species that contains one or more unpaired electrons and is capable of independent existence. Free radicals are highly reactive, have very short half, life and can generate new radicals by chain reaction and cause damage to biomolecules, cells and tissues. These species may be either oxygen derived (ROS) or nitrogen derived (RNS). They are produce either from normal cell metabolism *in situ* or from external sources (pollution, cigarette, smoke, radiation, medication). An overload of free radicals and their accumulation in the body generates a phenomenon called oxidative stress [16].

This oxidative stress deregulates a series of cellular functions and lead to various pathological conditions like arthritis, asthma, autoimmune diseases, carcinogenesis, cardiovascular diseases, cataract, diabetes, neurodegenerative diseases and ageing. The human body has several mechanisms to counteract oxidative stress mainly by producing antioxidants. Endogenous and exogenous antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS and RNS, and therefore, can enhance the immune defense and lower the risk of many, life threatening diseases [16]. Recently, the use of Unani drugs have been increased in various ailments due to the failure of modern medicine, which could not provide effective treatment for chronic diseases, and adverse effect of chemical drugs, and their increasing cost. Moreover, greater public access to information on traditional medicine has increased interest in alternative treatments. Keeping in view of all these facts, the present study is being, undertaken.

MATERIAL AND METHODS

Collection of Plant

The pods/ fruits of Sehjana (*Moringa oleifera* Lam.) were collect directly from the herbal garden of department of Ilmul Advia AMU, Aligarh, and are properly identified according to the botanical, Unani and Ayurvedic literature and then confirmed in pharmacognosy section of department of Ilmul Advia. A herbarium sample of the test drugs were prepared and submitted to mawalid-e-salasa museum of the department after identification for further reference, Sehjana voucher no, SC- 0185/15.

Preparation of Extracts

The pods of *Moringa* was cleaned from the earthy material, washed with double distilled water and shade dried to powdered in electrical grinder with slow and light movement to avoid sticking of the drug material with the grinder and there after the drug was passed through the sieve no. 80 to confirm its fineness and uniformity of particle size. And the powder was packed in to soxhlet apparatus and extracted with 50% ethanol (64.5 – 65.5°C) The extract was filtered and concentrated by evaporation on water bath, the yield percentage was calculated with reference to crude drug and was found to be 31% for Sehjana.

Preliminary Physicochemical and Phytochemical Screening

The physicochemical study of the test drugs included the study for organoleptic characters, ash value, moisture content, pH value, loss of weight on drying, successive extractive value, alcohol and water soluble, matter, bulk density.

The Preliminary phytochemical screening was carried out with different extract of *Moringa oleifera* pods for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [17].

Safety Study

The powder of pods/fruits of *Moringa oleifera* was studied to evaluate the presence of microbial load, pesticides residue, aflatoxins and heavy metals at Delhi Test House, Azadpur, Delhi-110033.

Drugs

The test drugs were obtained as describe above, sample of *Moringa oleifera* was found to be the standard in light of our physicochemical studies, therefore, they used for pharmacological studies. α-tocopherol acetate, used as the standard drug, was obtained from Loba chemical. The hydro-alcohlic (50% alcohol and 50% water) extract of drug was use for the study. The yield percentage was calculated with reference to dried drug. The extract of test drugs was dissolved in distilled water. The dose was obtained by multiplying the Unani clinical dose with appropriate conversion factor of 7 for rats [18], and found to be 15 mg/kg BW for α-tocopherol acetate, and 22 mg/100gm BW for Sehjana. A feeding canula was use to administer the suspension, which was homogenized by shaking well for two minutes.

Diet

Animals during the acclimation period were fed the commercially available rat chow (Ashirwad diet). Afterwards a special high lipid diet/atherogenic diet [19], containing butter (5g), bread slice (1), wheat flour 3 tea spoon), milk powder (1.5 tea spoon), cholesterol powder (60 mg/kg) and coconut oil (1 ml) was also given during experimentation period along with the normal diet to all the animals except those in plain control. The high lipid diet was given every day in the morning to the animals and when they consumed it they were allowed free access to the normal diet.

Animals

Male albino rats (Wister strain) of same age, weighing 150-200 gm, were purchase from the central animal house of Indian veterinary research institute Bareilly, Up, India. The animals were housed in sufficiently, large cages and treated under humane and hygienic condition with maintained at uniform temperature 25 ± 2°C and 12 h day: night cycle according to departmental ethical committee for animal experimentation. and were fed on standard diet (Ashirwad industries, Chandigarh, India) and tape water, *ad libitum*, The animal are deprived of food for 12 hours before the administration of treatment, water was provided throughout the study. The animals in all the, group were administered with the treatment by oral route once a day for 30 days. Before starting the experiment, permission from the Institutional Animal Ethics Committee was obtained, animal experimentations were permitted by Ministry of environment and forests, government of India under registration no, 714/02/a CPCSEA. It was issue by committee for the purpose, of control and supervision of experiments on animals (CPCSEA) dated 15th September 2016 and approved by the Institutional Animal Ethics Committee (IAEC) of department of biochemistry, Faculty of Life Science, Aligarh Muslim University, Aligarh, India.

Experimental Design

Rats were divided randomly into four group of six animals each and treated for 30 days as follows. Group-I animals served as plain control, treated with vehicle (vehicle control). Group-II served as control group treated with high fat diet and normal diet throughout the study. Group –III served as a standard group, and was administered alpha tocopherol acetate in a dose of 15 mg/kg bw. Group-IV was treated with daily dose of 22 gm/100 gm, bw. of ethanolic extract of *Moringa oleifera* for 30 days. High fat diet was given in all groups except in plain control group. Six hr after the last treatment, on day 31 the rats were anaesthetized by chloroform and sacrificed, all efforts were made to minimize suffering, blood, liver, and brain was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 rpm for 10 min at 4°C. The supernatant obtained was use for the estimation of catalase, lipid peroxidase, and other enzymes. Further, the homogenate was centrifuge at 1000 rpm for 20 min at 4°C and the supernatant was used for biochemical estimation.

Collection and preparation of biological samples

After sacrificing the animals, the blood was collected and centrifuged at 2500 x g for 10 minutes at 4ºC and the separated serum was collected carefully. The liver and brain were, also removed immediately washed with chilled normal saline and preserved in ice. A 10% W/V homogenate was prepared in chilled 0.15M KCL for Lipid Peroxidation, and Superoxide Dismutase; in 0.1M Chilled Tris Hcl buffer (pH 8.2) for Glutathione Reductase, and 50mM phosphate buffer for Catalase. The parameter estimated in serum, liver and brain were Lipid peroxidation, Superoxide Dismutase, Catalase, and Glutathione Reductase.

Statistical Analysis

The concentration of each parameter in various animal groups (Gp I- IV) were statistically compared for determining significance of difference by one- way ANOVA test followed by pair-wise comparison of various groups by LSD. P value of 0.05 or less was consider significant. The analysis was carried out by using the software of the website, www.myassay.com. Values are presented as mean ± standard deviation for groups of six animals.

Biochemical Estimation

Estimation of superoxide dismutase (SOD)

The activity of superoxide dismutase was estimated by Elisa reader with the help of commercially, available Detectx Superoxide Dismutase (SOD) colorimetric activity kit. (K028-H1) Arbor Assays USA.

Principle

The substrate is added followed by Xanthine oxidase reagent and incubated at room temperature for 20 minutes. The Xanthine oxidase generates superoxide in the presence of oxygen, which convert a colorless substrate in the detection reagent into a yellow colored product. The colored product is read at 450 nm. Increasing levels of SOD in the samples causes a decrease in superoxide concentration and reduction in yellow product. The activity of the SOD in the sample is calculated after making a suitable correction for any dilution, and expressed in terms of unit of SOD activity per mL.

Estimation of Catalase.

The catalase activity was determined by elisa reader with the help of commercially, available Detectx Catalase colorimetric activity kit. (K033-H1) Arbor Assays USA.

Principle

Samples are diluted in the provided assay, buffer and added to the wells of a half area clear plate. Hydrogen peroxide is added to each well and the plate incubated at room temperature for 30 minute. The supplied substrate is added, followed by diluted horseradish peroxidise and incubated at room temperature for 15 minutes. The HRP reacts with the substrate in the presence of hydrogen peroxide to convert the colorless substrate into a pink-colored product. The colored product is read at 560 nm. Increasing levels of catalase in the samples causes a decrease in H_2O_2 concentration and reduction in pink product, and expressed in terms of units of Catalase activity per mL.

Estimation of Lipid Peroxidation (TBARS)

Lipid peroxidation was estimated by Elisa reader with the help of commercially, available Quantichrom ™ TBARS Assay kit (DTBA-100), Bioassay Systems USA.

Principle

Bioassay system' TBARS assay is based on the reaction of TBARS with thiobarbitturic acid (TBA) to form a pink colored product. The color intensity at 535 nm or fluorescence intensity at (λex/em =560 nm / 585 nm) is directly proportional to TBARS concentration in the sample, and expressed in terms of μM MDA (μM=μmole/liter=nmole/ml).

Estimation of Glutathione Reductase (GR)

The Glutathione reductase activity was estimated by ELISA reader with the help of commercially, available Quantichrom™ Glutathione Reductase Kit (ECGR-100), Bioassay Systems USA.

Principle

Bioassay system' non-radioactive, colorimetric GR assay is designed to accurately measure GR activity in biological samples with a method that utilizes Ellaman's method in which DTNB reacts with the GSH generated from the reduction of GSSG by the GR in a sample to form a yellow product (TNB^{2-}). The rate of changes in the optical density, measured at 412 nm, is directly proportional to GR activity in the sample, and expressed in terms of unit per mL, The 1 Unit (U) of GR will catalyze the conversion of 1 μmole of GSSG to 2 μmole GSH per min at pH 7.6.

Estimation of Lipid Profile

Estimation of Cholesterol

Cholesterol was estimated by photocolorimeter with the help of commercially available cholesterol (SR) kit based on CHOD / PAP method, (Erba Mannheim Germany).

Principle

The estimation of cholesterol involves the following enzyme catalyzed reactions.

$$\text{Cholesterol ester} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en3-one} + H_2O_2$$

$$2H_2O_2 + 4AAP + \text{Phenol} \xrightarrow{\text{POD}} 4H_2O + \text{Quinoneimine}$$

Estimation of Triglyceride

Triglyceride was estimated by photocolorimeter with the help of commercially available triglyceride (SR) kit based on GPO / PAP method, (Erba Mannheim Germany).

Principle

$$\text{Triglyceride} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow[\text{Mg}+2]{\text{GK}} \text{Glcerol-3-Phosphate} + ADP$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} DAP + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5\text{-DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2H_2O$$

$$\text{Triglyceride (mg/dL)} = \frac{\text{Abs.of Test}}{\text{Abs.of Standard}} \times \text{concentration of standard (mg/dL)}$$

Estimation of HDL Cholesterol

HDL cholesterol was estimated by photocolorimeter with the help of commercially available HDL cholesterol PPT. set based on phosphotungstic acid method, (Erba Mannheim Germany).

Principle

chylomicrons, LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remain unaffected in the supernat and is estimated using ERBA cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \underset{\text{(Supernatent)}}{\text{HDL}} + \underset{\text{(Precipitate).}}{\text{(LDL+VLDL+Chylomicrons)}}$$

$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Abs .of Test}}{\text{Abs .of Std .}} \times \text{concentration of standard (mg/dL)} \times \text{dilution factor}$$

$$= \frac{\text{Abs .of Test}}{\text{Abs .of Standard}} \times 25 \times 3$$

$$= \frac{\text{Abs .of Test}}{\text{Abs .of Standard}} \times 75$$

Estimation of LDL and VLDL

The values of LDL were calculated by following formulae.

LDL = Total cholesterol – HDL – VLDL (Friedewald formulae).

VLDL = Triglyceride /5 (Friedewald WI, 1972).

The atherogenic index of plasma was calculated by the formula:

AIP = [TGL/HDL]
While HDL/LDL ratio by dividing the value of HDL with that of LDL.

RESULTS

The present study determines a comprehensive range of physicochemical characters of the drug according to the parameters used in pharmacopeia, which may serve as the standard for ensuring optimum efficacy and safety of various samples of the drug.

Phytochemicals Investigation

It was founds that different extract of Sehjana (Moringa) contained alkaloids, phenol, carbohydrate, protein, amino acids, steroaids, glycosides and tannins.

Safety Study

The result of the study demonstrated that heavy metals (Arsenic, Mercury, Cadmium) was not found to be present, only Lead 7.4 mg/kg present, and microbial load count (Bacterial count 560 and yeast and mould 40) were found which is within permissible limit as per WHO guidelines. While aflatoxins, pesticides was, found to be absent in the crude drug sample, indicating that the drug is free from toxicity.

In-Vivo Antioxidant Activity

The present study was undertaken to assess the *in-vivo* antioxidant potential of 50% ethanolic extract of *Moringa oleifera* against high fat diet induced rats, in serum, liver, and brain homogenate of control and experimental groups of rats. The results showed that the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) in the liver, brain and serum of control and experimental groups of rats was significantly lower in the high fat diet control group as compared to the plain control group. In the standard group, and *Moringa oliefera* treated group the activity of these enzymes was significantly, increased in comparison to that in the control group (Table 1 to 3).

Table 1: Effect of 50% ethanolic extract of Sehjana (*Moringa oleifera*) on the activity of Superoxide dismutase (SOD) in high fat diet induced rats, in serum, liver and brain.

Superoxide dismutase (SOD) (U/mL)*			
Groups	**Serum**	**Liver**	**Brain**
Plain control	1.05±0.02	2.81±0.06	1.71±0.31
Control	0.69±0.05 b***	1.79±0.02 b***	1.08±0.00 b**
Standard	1.10±0.07 a***b*	2.95±0.03 a*** b*	2.17±0.02 a***b*
Sehjana	1.63±0.11 a, b, c***	3.28±0.04 a, b, c***	2.40±0.03 a*** b**

(n=6) Values are in Mean ±SEM. Where* P<0.05 and **P<0.01 *** p<0.001. a = Against control, b = Against plain control, c = Against standard. * The results are expressed in term of unit of SOD activity per mL.

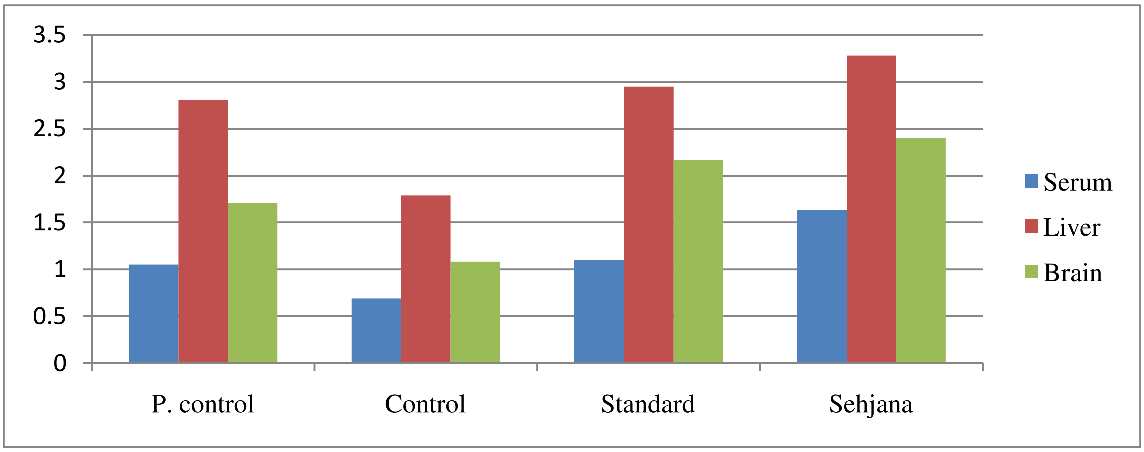

Figure 1: Activity of superoxide dismutase (SOD) in serum, liver and brain (U/mL).

Table 2: Effect of 50% ethanolic extract of Sehjana (*Moringa oleifera*) on the activity of catalase in high fat diet induced rats, in serum, liver and brain.

Catalase (U/mL)*			
Groups	**Serum**	**Liver**	**Brain**
Plain control	1.18±0.019	3.41±0.037	1.57±0.029
Control	0.97±0.020 a***	2.22±0.059 a***	1.16±0.025 a***
Standard	1.32±0.031 a*** b**	3.92±0.039 a***b***	2.00±0.013 a***b***
Sehjana	1.68±0.034 a, b, c***	4.06±0.027 a, b***	2.44±0.055 a, b, c***

* The results are expressed in term of units of Catalase activity per mL.

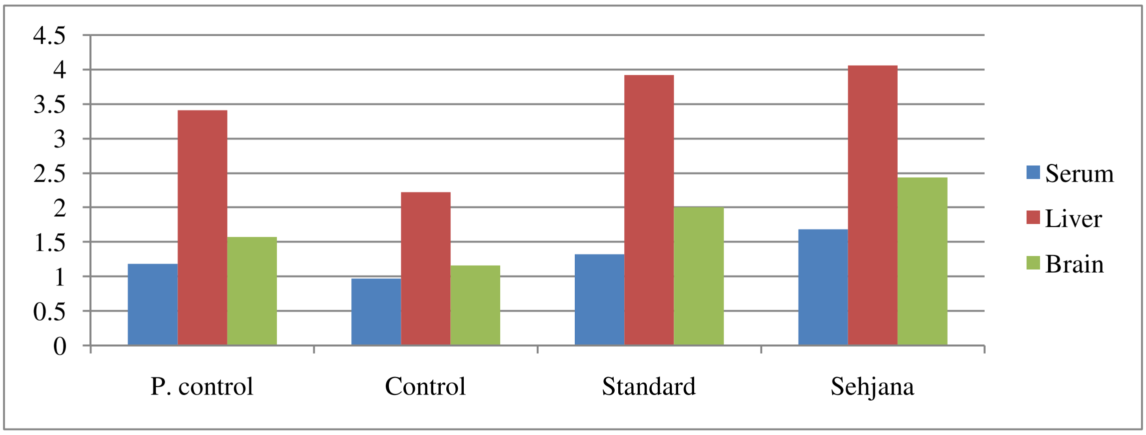

Figure 2: Catalase activity in serum, liver and brain (U/mL)

Table 3: Effect of 50% ethanolic extract of Sehjana (*Moringa oleifera*) on the activity of Glutathione Reductase (GR) in high fat diet induced rats, in serum, liver and brain.

Glutathione reductase (U/L)*			
Groups	**Serum**	**Liver**	**Brain**
Plain control	2.73 ±0.061	4.37 ±0.153	3.64±0.066
Control	1.66±0.061 a***	3.22 ±0.147 a***	2.60±0.148 a***
Standard	3.21±0.053 a*** b***	5.38 ±0.188 a***b***	4.44 ±0.232 a*** b***
Sehjana	4.25±0.096 a, b, c***	6.66 ±0.384 a, b, c***	5.26 ±0.088 a, b, c***

* The 1 Unit (U) of GR will catalyze the conversion of 1 µmole of GSSG to 2 µmole GSH per min at pH 7.6.

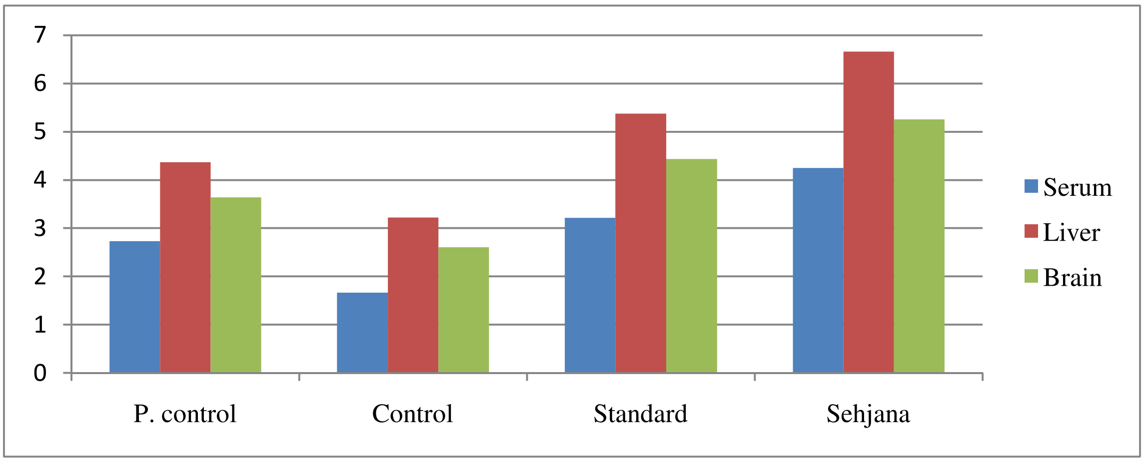

Figure 3: Glutathione reductase (GR) activity in serum, liver and brain (U/mL).

Table 4: Effect of 50% ethanolic extract of Sehjana (*Moringa oleifera*) on the activity of Lipid Peroxidation in high fat diet induced rats, in serum, liver and brain.

Lipid peroxidation (TBARS) µM MDA (µM=µmole/liter=nmole/ml)			
Groups	**Serum**	**Liver**	**Brain**
Plain control	3.05±0.03	5.28±0.05	5.87±0.06
Control	4.63±0.06 b***	6.68±0.11b***	7.96±0.03 b***
Standard	2.65±0.12 a*** b**	4.38±0.08 (a, b)***	5.47±0.09 (a, b)***
Sehjana	1.81±0.07 a, b, c,***	3.10±0.03 a, b, c***	3.93±0.03 a, b, c***

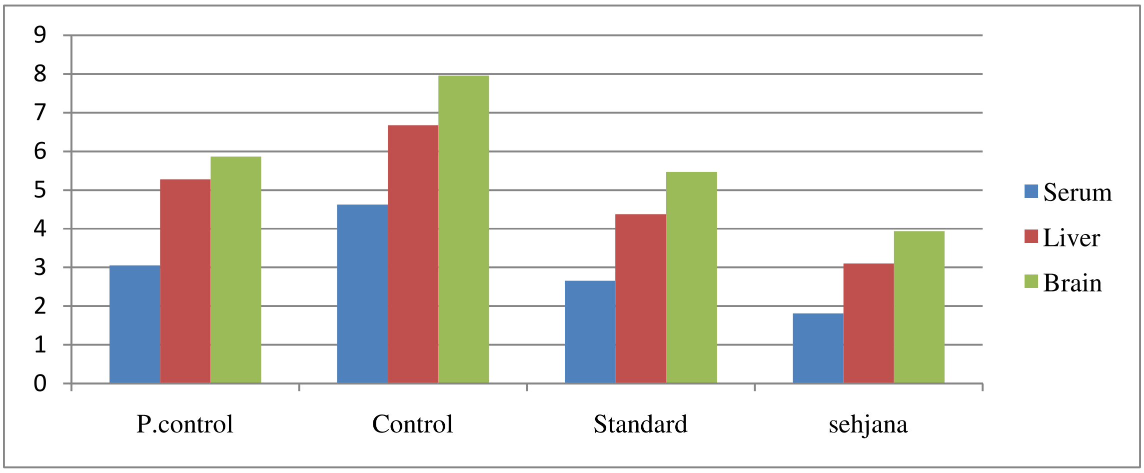

Figure 4: Lipid peroxidation (TBARS) in serum, liver and brain (µM MDA).

Table 5: Effect of *Moringa oliefera* on Lipid Profile in high fat diet induced Rats (Mean±SE).

Lipid profile (mg/dL)							
Group	**Total Cholesterol mg/dl**	**Triglyceride (mg/dl)**	**HDL (mg/dl**	**LDL (mg/dl**	**VLDL (mg/dl)**	**HDL: LDL**	**Atherogenic Index of Plasma**
Group I P. control	90.87±0.98	159.18±2.69	28.2±0.77	33.74±1.20	30.75±0.56	0.83±0.02	0.751
Group II Control	104.74±0.66 a***	185.67±2.45 a***	24.11±1.13 a***	44.59±1.19 a*	36.07±0.79 a***	0.53±0.01	0.886
Group III Standard	96.12±1.67 a*	173.18±2.47 a*** b***	28.73±0.55 a*** b***	32.62±1.87 a**	34.63±0.40 b***	0.89±0.05	0.780
Group IV Sehjana	91.35±4.35a*** y** z*	171.77±2.84 a*** b**	31.28±1.05 a,b,c***	23.35±5.19 a*** b,c*	34.38±0.53 b***	1.59±0.23 a** b*c*	0.739

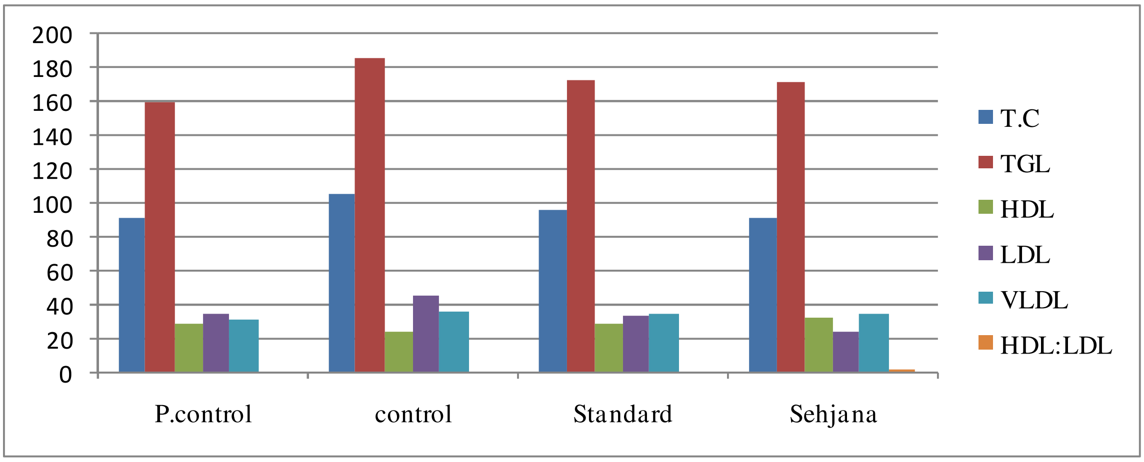

Figure 5: Effects of test drugs on Lipid profile.

Table 4 shows the activities of lipid peroxidation (TBARS) in the liver, brain and serum of control and experimental groups of rats. The activity of lipid peroxidation (TBARS) in liver, brain and serum was significantly elevated in the high fat diet control group as compared to the plain control group. In the standard group, and *Moringa oliefera* treated group the activity of (TBARS) was significantly lower in comparison to that in the control group (Table 4).

Table 5 shows the activities of lipid Profile in serum of control and experimental groups of rats. The activity of lipid profile in serum was significantly elevated in the high fat diet control group as compared to the plain control group. In the standard group, and *Moringa oliefera* treated group the activity of lipid profile was significantly lower in comparison to that in the control group (Table 5).

The test drugs exhibits high efficacious antioxidant activity. They are shown to be more effective than the standard antioxidant agents, the test drugs shown to be comprehensive antioxidant agents as they have been found to be effective in three biological samples, namely liver and brain homogenate and serum.

DISCUSSION

The present study was evaluate the potential effects of 50% ethanolic extract of *Moringa oliefera* on antioxidant status in high fat diet induced rats. The above findings show that *Moringa oliefera* produce a striking increase in the activity of SOD in all the 3 samples studied viz liver, brain and the serum, which is greater than the SOD activity in the standard group and even that in the plain control group. Superoxides dismutase is one of the most early and fundamental means of combating the excess ROS, by converting the superoxide ion into the relatively, less reactive oxygen and hydrogen peroxides and thus form a crucial part of the cellular antioxidant defense mechanism[19], $2O_2^{-\bullet} + 2H^+ + SOD \rightarrow H_2O_2 + O_2$ the superoxide ion is itself reactive and may cause oxidative damage, but its harmfulness lies mainly in its being the progenitor of the more reactive and dangerous hydroxyl radical [20]. Therefore, the striking increase in SOD activity indicates that the test drugs oppose the oxidative damage at an early and crucial point by preventing the generation of one of the most reactive and dangerous oxidative group. Catalase is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase is involved in the detoxification of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. The catalase completes the task initiated by SOD by converting less reactive H_2O_2, produced by SOD, into two molecules of water and harmless molecular oxygen [21]. The test drugs produce a striking increase in catalase activity along with that in SOD activity, show that they very effectively prevent the generation of ROS and hence, the antioxidant activity is likely to be strong, comprehensive and complete, due to effective prevention and blockade of ROS generation will protect all biomolecules. Thus, the present study shows that MDA concentration is significantly, increased in the high fat diet control group. Malondialdehyde (MDA) is one of the many products of lipid peroxidation caused by reactive oxygen species (ROS), therefore, the increase in MDA concentration indicate an increase in Lipid peroxidation. Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation, lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues [22, 23]. During lipid oxidation, malanoaldehyde (MDA) can react with the free amino group of proteins, phospholipids, and nucleic acids damaging their structure and functions. Increased levels of lipid oxidation products are associated with diabetes and atherosclerosis [24, 25]. Oxidative stress, i.e. induced generation of ROS that cannot be fully antagonized, by physiological antioxidants, results in oxidative damage to all biomolecules, however, lipid damage is the most important and takes the form of lipid peroxidation. The significant decrease in MDA concentration shown in the standard group, administered with standard antioxidant agent Vit E (α-tocopherol acetate), indicate the integrity and validity of our experimental procedure by showing the expected protective effect of Vit E against Lipid peroxidation. So the increase in lipid peroxidation and decreases in SOD, Catalase and GR concentration shows that the high fat diet induced in rats successfully, causes oxidative stress and damage. Therefore, lipid rich diets also capable of generating ROS because of antioxidant enzymes they can alter oxygen metabolism. Upon the increase of adipose tissue, the activity of antioxidant enzymes, such as SOD, CAT and glutathione peroxides (GPx), was found to be significantly diminished [26], finally, high ROS production and the decrease in antioxidant capacity, leads to various abnormalities especially, endothelial dysfunction, in a study [27], showed that a diet high in fat and carbohydrates induces a significant increase in oxidative stress and inflammation in person with obesity. Therefore, we observed a significant reduction in antioxidant enzymes such as SOD, CAT, activity and glutathione level in almost all tissue of high fat induced rats compared with non-fat animals. On the other hand non-enzymatic oxidative stress parameters lipid peroxidation marker MDA level increased in these tissues. Several studies have been shown that high fat diet induced decrease antioxidant capacity in different organs. In present study the significant decrease in MDA concentration and significantly increase in other antioxidant parameters shown in the standard group, administered with standard antioxidant agent Vit E (α-tocopherol acetate), indicates the integrity and validity of our experimental procedure by showing the expected protective effects of Vit E against all antioxidant parameters. In the present study showed that the 50% ethanolic extract of *Moringa oliefera* shown to exert its antioxidant effect mainly by ROS generation blockade due to increased SOD and catalase and Glutathion Reductase activity. However, Lipid peroxidation also plays an important role in its antioxidant activity.

CONCLUSION

Based on this study we conclude that ethanolic extract of *Moringa oliefera* have significant antioxidant, activities as compared with alpha to copherol acetate (Standard).

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