EVALUATION OF *IN VIVO* ANTIOXIDANT ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *LINUM USITATISSIMUM* L. (TUKHM-E-KATAN) AGAINST HIGH FAT DIET INDUCED RATS

Khan Z. J.[a]*, Khan N. A. [a], Naseem I.[b] and Nami S. A. A.[c]

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ABSTRACT

The aim of the present study was to evaluate the *in vivo* antioxidant activity of 50% ethanolic extract of *Linum usitatissimum* against high fat diet induced rats. Animals 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 23 mg/100 gm of body weight of 50% ethanolic extract of *Linum usitatissimum* significantly improved the superoxide dismutase, catalase, glutathione, and lipid peroxidation levels as compared to control group. The present studies revealed that the *in vivo* antioxidant activity of *Linum usitatissimum* was significant, and can be used to protect tissue from oxidative stress. The result showed that the superoxide dismutase, catalase, lipid peroxidase, and glutathione reductase activities significantly declined in group treated with high fat diet than that of normal group. Based on this investigation, it was concluded that the 50% ethanolic extract of *Linum usitatissimum* has good *in vivo* antioxidant activity and can be used in protecting tissue from oxidative stress.

Keywords: *Linum usitatissimum*, alpha tocopherol, superoxide dismutase, antioxidant activity.

INTRODUCTION

The use of plants in the management and treatment of diseases started with the advent of human life. In recent years, considerable research has been done on an array of plants having medicinal values. Therefore, medicinal plants and their therapeutic values are extensively used to cure an array of diseases all over the world. Antioxidants are agents that protect our body against damage by free radicals such as vitamin E, vitamin C,. which are responsible and for combating the diseases caused mainly or partly by oxidative stress. The oxidative stress deregulates a series of cellular functions and leads 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 reactive oxygen species (ROS) and reactive nitrogen species (RNS), and therefore, can enhance the immune defense and lower the risk of many life threatening diseases[1].

Herbal or natural drugs show significant variation in the chemical composition. This can be so drastic as to cause therapy failure or toxicity, so it can be appreciated that different samples of the same natural drug would rather commonly produce significantly different responses. So it is necessary to determine some crucial physicochemical characters of each sample before its pharmacological study to ensure that subsequent study would use same natural drugs. Secondly, the antioxidant activity is basically a chemical activity, so the chemical nature of antioxidant agents is of much greater importance in the elucidation of their pharmacology. Therefore, along with the pharmacological study for antioxidant activity, the test drugs were also subjected to a physicochemical study. The unani drug Tukhm-e-Katan comprises of seeds of a plant *Linum usitatissmum* Linn, a versatile and blue flowering rabi crop belonging to linaceae family, commonly known as flaxseed or linseed[2]. It is one of the most ancient crops cultivated in Egypt. It is also cultivated in India as an oil seed plant. The plant has shown diverse biological and

[a] Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh - 202 002, Uttar Pradesh, India
[b] Department of Biochemistry, Faculty of Life Science, A.M.U., Aligarh - 202 002, Uttar Pradesh, India
[c] Department of Kulliyat, Faculty of Unani Medicine A.M.U., Aligarh - 202 002, Uttar Pradesh, India
* For Correspondence:E-mail: zafarjaved9454@gmail.com

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pharmacological activities. It has been used in Unani medicine and traditional systems of medicine from time immemorial. Its seed and oil are used in various diseases such as asthma, cough, bronchitis, pleurisy, pneumonia, joint pain, renal colic, renal calculi and rheumatic swelling. Tukhm-e-katan is a famous Unani drug used in a number of pathological conditions. Although the entire plant has medicinal value, its seed and oil are more important and have broad medicinal values. Katan is an annual herb of about 0.7 m high with blue flowers and a globular capsule. The seeds are ovate, flattened and obliquely pointed at one end, about 4-6 mm long and 2-2.5 mm broad. The testa is brown, glossy and finely pitted, odouerless, taste mucilaginous and oily. If cruciferous seed are present, a pungent odour and taste may develop on crushing and moistening[3]. The flaxseed plant is native of Egypt, extensively cultivated in India, chiefly in Bengal, Bihar and the United, Provinces[4], cultivated throughout of India and altitudes of 2000 meters above sea level[5]. The seed coat contain mucilage, the surface is studded with fine pits or depression with a ridge just below the apex, having the hilum in the hollow. Seed nuclei or cotyledons are two, large and oily and contained within the external covering, within which is a thin mucous envelope[6]. Linseed oil is fixed oil expressed from linseed. It is clear yellowish brown oil, having characteristic odour, and bland taste. Gradually, it thickens on exposure to air and when spread in a thin film, to a hard transparent varnish[7]. According to Unani, it has Mohallil-e-waram[8,9], Dafe-e-Sua'al, Muqawwi-e-Bah Muqawwi-e-Aaza and Mulayyin, properties[8,10]. Recently, the use of Unani drugs has been increased in various ailments due to the limitations 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 all these facts, the present study was, carried out.

MATERIAL AND METHODS

Collection of plant

The seeds of *Linum usitatissimum* (Tukhm-e-Katan) were procured from local market of 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, Katan, voucher no. SC- 0184/15.

Preparation of extracts

The seeds of *L. usitatissimum* was cleaned from the earthy material, shade dried and powdered, in electrical grinder with slow and light movement to avoid sticking of the drug material with the grinder. Thereafter, the drug was passed through sieve no. 80 to confirm its fineness and uniformity of particle size, and the powder was packed into Soxhlet apparatus and extracted with 50% ethanol (64.5 – 65.5^{0}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 14% for Tukhm-e-Katan,

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 and bulk density. The preliminary phytochemical screening was carried out with different extracts of *L. usitatissimum* seed for the detection of various phytochemicals, tests for common phytochemicals were carried out by standard methods[11].

Safety study

The powder of seeds of *L. usitatissimum* 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 described above. Sample of *L. usitatissmum* was found to be the standard in the light of our physicochemical studies, therefore, they were 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 used for the study. The yield percentage was calculated with reference to the 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[12], and found to be 15 mg/kg bw for α-tocopherol acetate, and 23 mg/100gm bw for *L. usitatissmum*. A feeding canula was used to administer the suspension which was homogenized by shaking well for two minutes.

Diet

Animals during the acclimatization period were fed the commercially available rat chow (Ashirwad diet).

Table I: Effect of hydro-alcoholic extract of Tukhm-e-Katan (*Linum usitatissimum*) 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 $a^{***}b^{*}$
Tukhm-e-Katan	1.78±0.04 a, b, c^{***}	3.75±0.02 a, b, c^{***}	2.65±0.04 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.*

Table II: Effect of hydro-alcoholic extract of Tukhm-e-Katan *(Linum usitatissimum)* 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 $a^{***}b^{***}$
Tukhm-e-Katan	1.83±0.038 a, b, c^{***}	4.37±0.070 a, b^{***}	2.64±0.041 a, b, c^{***}

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

Table III: Effect of hydro-alcoholic extract of Tukhm-e-Katan *(Linum usitatissimum)* 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^{***}
Katan	4.08±0.034 a, b, c^{***}	6.67 ±0.371 a, b, c^{***}	5.24 ±0.060 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.*

Table IV: Effect of hydro-alcohlic extract of Tukhm-e-Katan (*Linum usitatissimum*) 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)***
Katan	1.69±0.13 a, b, c,***	3.03±0.16 a, b, c^{***}	3.69±0.09 a, b, c^{***}

Table V: Effect of test drugs 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	86.59±2.45 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 Katan	91.35±4.35 a^{***} y^{**} z^{*}	169.96 ±1.18 a^{***} b^{**}	32.45 ±0.84 a,b,c^{***}	20.28 ±2.60 a^{***} b,c^{*}	34.17 ±0.43 b^{***}	1.79 ±0.29 a^{**} $b^{*}c^{*}$	0.719

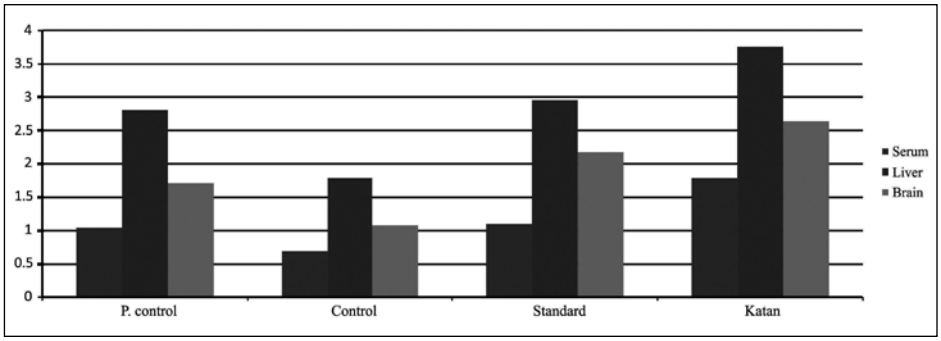

Fig. 1: Activity of Superoxide Dismutase (SOD) in serum, liver and brain (U/mL)

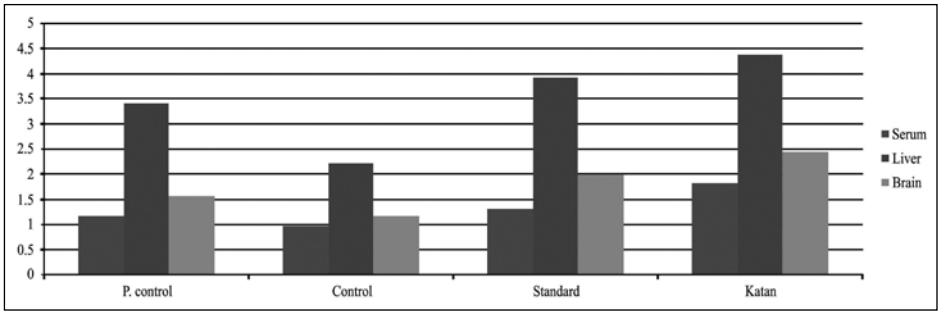

Fig. 2: Catalase Activity in serum, liver and brain (U/mL)

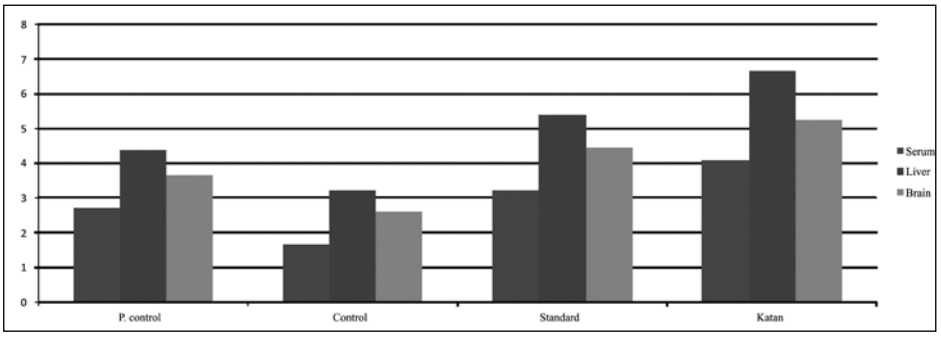

Fig. 3: Glutathione Reductase (GR) activity in serum, liver and brain (U/mL)

Afterwards a special high lipid diet/atherogenic diet[13], 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 (Wistar strain) of same age, weighing 150-200 g, 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 tap water, *ad libitum*, The animal were deprived of food for 12 hours before the administration

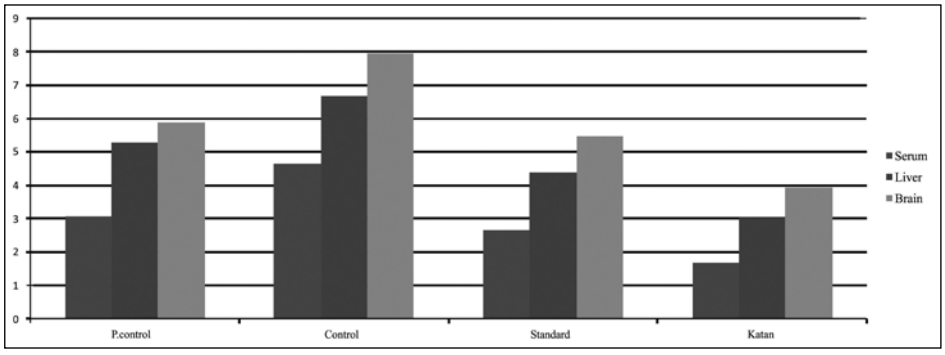

Fig. 4: Lipid Peroxidation (TBARS) in serum, liver and brain (µM MDA)

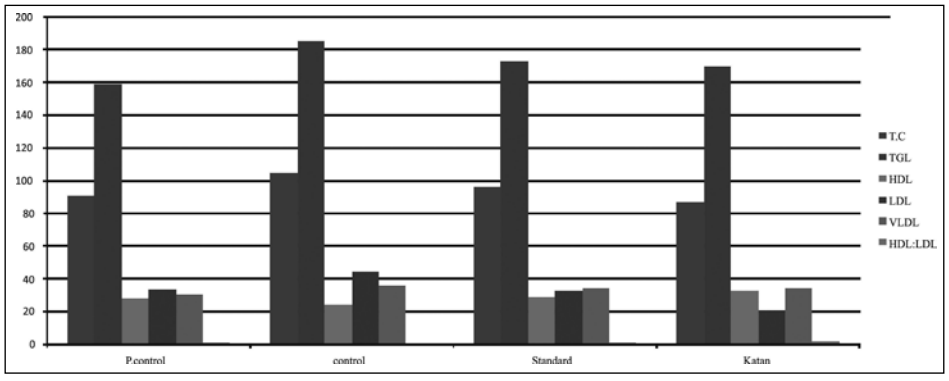

Fig. 5: Effects of test drugs on Lipid Profile

of treatment, water was provided throughout the study. The animals in all the groups were administered with the treatment by oral route once a day for 30 days. Before starting the study, ethical clearance was taken from the Institutional Animal Ethics Committee, animal experimentations were permitted by Ministry of Environment and Forests, Government of India under registration no. 714/02/aCPCSEA. It was issued 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

The animals were divided randomly into four groups 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 received α-tocopherol acetate (15 mg/kg bw) orally and served as the standard group, Group-IV was treated with daily dose of 23 g/100 g, body weight, of ethanolic extract of *L. usitatissmum* for 30 days. High fat diet was given to in all groups except to in plain control group. Six hours after the last treatment, on day 31 the rats were anaesthetized by chloroform and sacrificed, all efforts being made to minimize suffering. Sacrificed animals blood, liver, and brain were rapidly excised, rinsed in ice-cold saline, and a 10% w/V homogenate was prepared using 0.15M KCl, centrifuged at 800 rpm for 10 min at 4^{0}C. The supernatant obtained was used for the estimation of catalase, lipid peroxidase, and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4^{0}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 4^{0}C 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 parameters estimated in serum, liver and brain were lipid peroxidation, superoxide dismutase, catalases, and glutathione reductase.

Statistical analysis

The concentration of each parameter in various animal groups (Groups 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 considered significant. The analysis was carried out by using the software of the website, www.myassay.com. The data 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; xanthine oxidase reagent is added subsequently and incubated for 20 minutes at 20 to 25 ^{0}C temperature. Superoxide is generated by the xanthine oxidase in presence of oxygen, and this converts a colorless substrate in the detection reagent into a yellow colored product which is read at 450 nm. Increasing SOD levels in the samples cause a decrease in superoxide concentration and subsequently reduction in yellow product. The activity of the SOD in the sample is expressed in terms of unit of SOD activity per mL and calculated after making a suitable correction for any dilution.

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 added to the wells of a half area clear plate after dilution in provided assay buffer. Hydrogen peroxide (H_2O_2) is added to each well and the plate is incubated at 20 to 25 ^{0}C temperature for 30 minutes. The supplied substrate is added; diluted horseradish peroxidase is added subsequently and incubated at 20 to 25 ^{0}C temperature for 15 minutes. The colorless substrate converts into a pink-colored product, which is read at 560 nm, when the HRP reacts with the substrate in the presence of hydrogen peroxide. Increasing levels of catalase in the samples causes a decrease in H_2O_2 concentration and consequently reduction in pink product, and is 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, in this assay the Thiobarbituric acid reactive substances (TBARS) is reacted with thiobarbituric acid (TBA) and forms a pink colored product. The fluorescence intensity at (λex/em =560 nm / 585 nm) or color intensity at 535 nm is directly proportional to thiobarbituric acid reactive substances (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™ Glutathion Reductase Kit (ECGR-100), Bioassay Systems USA.

Principle

Bioassay System' glutathione reductase (GR) colorimetric assay is designed to measure the accurate activity of GR in biological samples with a method that utilizes Ellman'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 optical density change rate is measured at 412 nm, and is directly proportional to GR activity in the sample, and expressed in terms of unit per mL, The 1 Unit (U) of Glutathione reductase 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 following enzyme catalyzed reactions are involves in estimation of cholesterol.

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

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

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

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

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

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

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

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

Triglyceride (mg/dL) = $\frac{\text{Abs.of Test}}{\text{Abs.of Standard}} \times$ 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 remains unaffected in the supernatant 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].

HDL/LDL ratio was calculated 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. In phytochemical investigations, it was found that different extracts of Tukhm-e-Katan (*Linum usitatissimum*) contained alkaloids, phenol, flavonoid, carbohydrate, protein, sterols, glycosides, saponin and tannins. In safety study, it was found that heavy metals (arsenic, mercury, cadmium) were not found to be present, only lead was present, and microbial load count (Bacterial count 400 and yeast and mould 20) were found to be is within permissible limits as per WHO guidelines, while aflatoxins, pesticides and specific pathogens were 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 *Linum usitatissimum* 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 experimental and control 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 *L.usitatissimum* treated group the activity of these enzymes was significantly increased in comparison to that in the control group. (Table I to III).

Table IV shows the activities of lipid peroxidation (TBARS) in the liver, brain and serum of experimental and control 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 *L. usitatisimum* treated group, the activity of (TBARS) was significantly lower in comparison to that in the control group (Table IV).

Table V shows the activities of lipid profile in serum of experimental and control 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 *L. usitatissimum* treated group the activity of lipid profile was significantly lower in comparison to that in the control group (Table V).

The tested drug exhibits high, efficacious antioxidant activity. It is shown to be more effective than the standard antioxidant agents, have been found to be effective in three biological samples, namely liver and brain homogenates and serum.

DISCUSSION

The present study was design to evaluate the potential activity of 50% ethanolic, extract of *L. usitatissimum* on antioxidant status in high fat diet induced rats. The above findings show that *L. usitatissimum* produce a striking increase in the activity of SOD in all the three 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[14],

$$2O_2^{-\bullet} + 2H^+ + SOD \rightarrow H_2O_2 + O_2$$

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[15]. Therefore, the striking increase in SOD activity indicates that the test drug opposes the oxidative damage at an early and crucial point by preventing the generation of one of the most reactive and dangerous oxidative groups. 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[16]. The test drugs produced a striking increase in catalase activity along with that in SOD activity, show that it very effectively prevents 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 protecting 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 indicates 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[17,18]. 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[19,20]. 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 Vitamin E (α-tocopherol acetate), indicates the integrity and validity of our experimental procedure by showing the expected protective effect of Vitamin E against lipid peroxidation. So the increase in lipid peroxidation and decrease 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 reactive oxygen species (ROS) because of antioxidant enzymes they can alter oxygen metabolism. By the increase of adipose tissue, the antioxidant enzymes activity such as superoxide dismutase, and catalase was found to be significantly diminished[21]. Finally, high reactive oxygen species (ROS) production and the decrease in antioxidant capacity leads to various abnormalities especially, endothelial dysfunction. A study showed that a diet high in fat and carbohydrates induces a significant increase in oxidative stress and inflammation in person with obesity[22]. Therefore, a significant reduction in antioxidant enzymes activity such as superoxide dismutase, catalase, and glutathione level was observed in almost all tissue of high fat induced rats as 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 show that high fat diet induced decrease antioxidant capacity in different organs. In the present study, the significant decrease in MDA concentration and significant increase in other antioxidant parameters shown in the standard group, administered with standard antioxidant agent Vitamin E (α-tocopherol acetate), indicates the integrity and validity of our experimental procedure by showing the expected protective effects of Vitamin E against all antioxidant parameters. The present study showed that the 50% ethanolic extract of *L. usitatissimum* exerts its antioxidant effect mainly by ROS generation blockade due to increased superoxide dismutase, catalase and glutathion reductase activity. However, lipid peroxidation also plays an important role in its antioxidant activity. Based on this study, we conclude that ethanolic extract of *L.usitatissimum* has significant antioxidant activities as compared with alpha tocopherol acetate (standard).

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