International Journal of Current Biotechnology
ISSN: 2321 - 8371
Journal Homepage : http://ijcb.mainspringer.com

Protective effect of fenugreek seed extract on lipofuscinogenesis and antioxidative profile in reproductive system of aging accelerated male mice

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ARTICLE INFO

Article History:
Received 4 April 2020
Received in revised form 16 April 2020
Accepted 18 April 2020
Available online 30 April 2020

Key words:
Trigonella foenum graecum, D-galactose, free radicals, lipofuscinogenesis, antioxidative enzymes.

ABSTRACT

The free radicals play a central role in aging process and disease progression. Antioxidants are prevents the damage caused by reactive oxygen species (ROS). Fenugreek (*Trigonella foenum graecum*) is a leguminous plant and its seeds used in herbal medicines. The purpose of present study was to demonstrate the effect of fenugreek seed extract (FSE) on lipofuscinogenesis and antioxidative enzymes i.e. SOD (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase) in testis and accessory reproductive organs of D-galactose induced mice. For the present investigation mice (*Mus musculus)* were divided into six groups viz, 1) Control group received 2) D-galactose treated group 3) D-galactose + FSE (50 mg/kg BW) for 20 days. 4) D-galactose FSE (50 mg/kg BW) for 20 days. After the treatment of D-galactose showed significant increase in lipid peroxidation and decreased amount of antioxidative enzymes as compared to control group. After treatment of FSE, the lipofuscinogenesis and fluorescence product were significantly decreased while antioxidative enzymes were significantly increased in testis, Epididymis and Seminal vesicle. So, it may conclude that fenugreek seed extract acts as an antioxidative agent and its treatment effective against the oxidative damage.

INTRODUCTION

Aging refers to a progressive functional decline or gradual changes in physiological changes with age, including decrease in fecundity (Lopez - Otin *et al.*, 2013). The main features during aging are dysfunction of tissues, organs, induce cell aging, including DNA damage, high oxidative stress and other cell stress (Oberdoerffer and Sinclair; Michen *et al*., 2008). Aging result into lower body weight and muscle mass, a lower metabolic rate, longer reaction time, decline in sexual activities and also certain memory functions are decline with age (Hayflick *et al.*,1994).

Free radicals are highly reactive molecules which produce during cellular respiration and process of oxidation in mitochondria. Oxygen species includes Superoxide radicals, hydrogen radicals, hydrogen peroxide free radicals, lipid and organic peroxide radicals (Garinis and Vijg *et al.* ,2009). Reactive nitrogen species like nitric oxide and peroxinitrite anion (Nagendrappa, 2005). ROS affects was exclusively considered toxic to human spermatozoa. Spermatozoa are rich in mitochondria for supplying energy but ROS affects the mitochondrial function (Evenson *et al*., 1982). Spermatozoa more vulnerable to ROS because their plasma membrane and cytoplasm contains large number of Poly Unsaturated Fatty Acids (PUFAs) (Alverez and Storey, 1995). Superoxide anion as well as nitric oxide also shown to promote capacitation and acrosomal reactions (Griveau *et al*., 1995). Thus, excessive generation of ROS in semen by leukocytes and abnormal spermatozoa could be lead to infertility (Sharma and Agarwal, 1996). So, when there is excessive production of ROS or impaired Antioxidants mechanism and oxidative stress occurs which is harmful to reproductive organs.

Human have evolved antioxidant systems (Enzymatic and non-enzymatic), in combination with each other it protects the body against the free radicals damage. The most effective enzymatic Antioxidants in our body are the SOD (Sodium dismutase), CAT (Catalase) and GPx (Glutathione peroxidase) (Mates *et al*., 1999). Non-enzymatic Antioxidants includes low molecular weight compounds, such as vit. E and C, thiol antioxidants, natural flavonoids, melatonin and other compounds (McCall and frei 1999). Recently medicinal properties of plants have been investigated throughout the world, due to its antioxidative activities, no side effect and economic viability (Agarwal and Prabhakaran, 2005). Medicinal plants found to have beneficial effects to scavenge against free radicals which produce during aging.

T. graecum foenum contain the antioxidative property of plant material because it having many active phytochemicals including flavonoids, vitamins, terpenoid, carotenoid, cumarins, saponin and plant sterol etc. (Kaur and Kapoor, 2002; Kaviarasan *et al*., 2004). Ethanolic extract of fenugreek seeds and its alkaloid,

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trigonelline both are promising natural Antioxidants. Fenugreek seeds also prevent cytogenetic and testicular damage in albino rats (Sakr *et al*., 2012). So, due to its protective effect of seeds are widely used in herbal medicines to cure various diseases. The purpose of this study was to demonstrate protective effect of fenugreek seed extract on testis and accessory reproductive organs in D-galactose induced mice.

MATERIALS AND METHODS

Preparation of fenugreek seed extract:
Fenugreek seeds were collected from the local market of Kolhapur and subjected to various treatments for investigation of antioxidants potential. Extraction was carried out by the soxhelt method (Lim Cheung, 2002). Dry fenugreek seeds were cleaned and ground into fine powder using a grinding machine. Ethanol was used for extraction (Bukari *et al*., 2008; Choudhary *et al*., 2001; Basch *et al*., 2003). The extract was filtered and evaporated to dryness under reduced pressure 60ºC by a rotary evaporator. Extract was placed in dark bottle and stored at -8ºC until further analysis.

Animals:
Six month old Swiss albino mice (*Mus musculus*) weighing about 50-55 gm were used for the present study. Animals were housed in departmental animal house approved by the [CPCSEA/233]. Animals were kept under a 12:12 hr L: D cycle and fed Amrut mice feed [Pranav Agro Industries, Sangli, India] and water was *ad libitum*. The record of their age and body weight was maintained. Animals were divided into four groups.

1) Control group:
The six month male mice were given subcutaneous injection of 0.5 ml distilled water/ day/ animal for 20 days.

2) D-galactose treated group:
Male mice were given subcutaneously injection of 0.5 ml of 5% D-Galactose / day/Kg of the animal for 20 days to induce aging.

3) D+ FSE group:
Male mice were subcutaneously injected with 0.5 ml of 5% D-Galactose/ day/ animal along with fenugreek seed extract 50 mg/kg body weight of animal/day for 20 days (very little volume of alcohol 0.01ml was used to dissolve fenugreek seed extract and volume raised to 100ml with 5% D-galactose).

4) D àFSE group:
Male mice were injected with 0.5 ml of 5% D-Galactose for 20 days and then for next 20 days they were injected subcutaneously fenugreek seed extract prepared as above.

After completion of the dose, the animals were killed by cervical dislocation. Testes, epididymis and seminal vesicle were dissected out, weighed and they were homogenized in respective homogenization medium and used for lipid peroxidation, fluorescence product and antioxidative enzymes estimation.

Determination of Lipid Peroxidation (Will, 1966)
Tissue homogenate were prepared in chilled mortar and pestle using 75mM potassium phosphate buffer pH 7.0. The lipid peroxidation was estimated by measurement of malondialdehyde (MDA) an end product of fatty acid peroxidation and reacts with TBA to form coloured complex that has maximum absorbance at 532 nm by using calorimeter.

Measurement of Fluorescence Product (Dillard and Chapel 1971)
The lipofuscin granules from SM and SL glands were extracted using chloroform: Methanol mixture (2:1 v/v). The fluorescence was measured by using quinine sulphate as a standard by using photoflurometer.

Estimation of antioxidative enzymes: Measurement of total SOD activity was performed according to Beauchamp and Fridovich by calculating percentage of formazon dye formation. The catalase mediated decomposition of H_2O_2 was estimated directly at 240 nm with a modified method of Luck. Glutathione peroxidase activity was assayed spectrophotometrically by using Beers and Sizer method. Glutathione oxidation was recorded at 240 nm in presence of sodium azide.

Statistical analysis: All values were expressed as mean ± S.D. The statistical analysis was performed using one way Analysis of variance (ANOVA) followed by Tukey's Post Hoc Test.

RESULTS

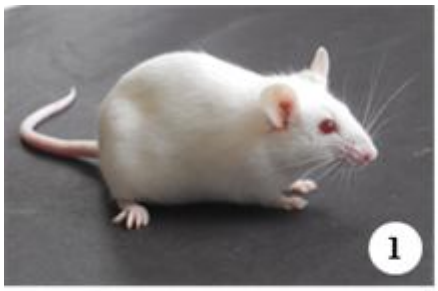
1) Lipid peroxidation and fluorescence product: Effect of fenugreek seed extract (FSE) on total lipid peroxidation (n mol MDA/mg tissue) and fluorescence product in testes, epididymis and seminal vesicle of D-galactose induced aged mice is illustrated in table no. 1 & 2). The total lipid peroxidation and fluorescence product in testes, epididymis and seminal vesicle of control group mice was drastically increased in D-galactose accelerated aging mice group. The increase was significant as compared to control group (1:2, $P<0.01$). The total lipid peroxidation in D + F group was significantly reduced as compared to D-galactose accelerated aged mice group (2:3, $P<0.01$). The total lipid peroxidation in testes from D '!F group mice was reduced and decrease was significant as compared to D-galactose accelerated aged mice group (2:4, $P<0.01$).

2) Antioxidant enzymes: Effect of fenugreek seed extract (FSE) on antioxidative enzymes i.e. SOD (Superoxide dismutase), CAT (Catalase), GpX (Glutathione peroxidase) (unit/mg protein/hr) in testis, epididymis and seminal vesicle of D-galactose induced aged mice is illustrated in table no. 3, 4 and 5. The SOD, CAT, GPX activity in testis, epididymis and seminal vesicle from control group mice was significantly decreased in D-galactose treated aging induced mice (1:2, $P<0.01$). In testis, epididymis and seminal vesicle from D + F group mice, the all antioxidative enzyme activity was slightly increased as compared to D- galactose treated aging induced mice group and it was increased significantly as compared to D-galactose treated aging induced group (2:3, $P<0.01$). The antioxidative enzyme activity in testis, epididymis and seminal vesicle from curative group was significantly increased in curative group as compared to D- galactose treated aging induced mice (2:4, $P<0.01$). The enzyme activity in D '!F group was significantly increased as compared to the D + F group (2:4, $P<0.01$).

DISCUSSION

Free radicals direct cause to normal metabolism of cell and leads to variety of diseases resulting from antioxidative defence. D-galactose is physiological nutrient and a reducing sugar reacts with free amines of amino acid in proteins (Yang *et al.*, 2006). The effect of oxidative stress on male reproduction was studied by many investigators. Sulistyoningrum (2017) reported that D-Galactose disrupt the development of sperm and damage testicular development in rats. Long term exposure of male animals with D-Galactose revealed the decreased testicular, epididymal weights, altered sperm morphology, testicular atrophy and thus infertility (Shaikh *et al*., 2015).

In present study antioxidative enzymes SOD, CAT, and GPx are decreased in D-Galactose treated group, because D-Galactose is aging inducing agent that causes free radical formation leading to increased Advance Glycation End Product (AGEs) which accelerates natural aging



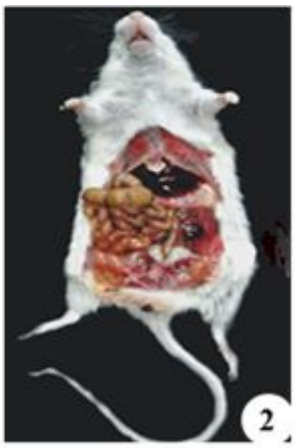


Fig. 1: Swiss Albino male mice
Fig. 2: Dissected Reproductive System of male mice

Table No-1: Effect fenugreek seed extract on total lipid peroxidation (lipid peroxidation in n mol MDA / mg wet tissue) of testis, epididymis and seminal vesicle during aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sl. No.	Treatment (n=5)	Lipid peroxidation (Testis)	Lipid peroxidation (epididymis)	Lipid peroxidation (seminal vesicle)	Statistical significance
1	Control	12.3421±4.2842	8.5755±2.689	8.5755±2.689	1:2 P ? 0.01 2:3 P < 0.05 2:4 P < 0.01
2	Dg-treated	55.0653±5.4847	36.4877±4.8125	38.4877±4.8125	
3	D + F	39.0218±4.3933	21.2267±3.6148	25.2267±3.6148	
4	D → F	29.7569±4.3688	19.0275±3.5996	22.0275±3.5996	

Table No- 2: Effect fenugreek seed extract on fluorescence product (fluorescence product in ìg /mg tissue) of testis, epididymis and seminal vesicle during aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sl. No	Treatment (n=5)	Fluorescence product (Testis)	Fluorescence product (epididymis)	Fluorescence product (seminal vesicle)	Statistical significance
1	Control	0.001314±0.00006	0.001658±0.00061	0.001858±0.00061	1:2 P < 0.01 2:3 P < 0.01 2:4 P <0.01
2	Dg-treated	0.01541±0.000777	0.007545±0.00527	0.009545±0.00527	
3	D + F	0.01071±0.00099	0.006776±0.00022	0.007776±0.00022	
4	D →F	0.008602±0.00102	0.004537±0.00112	0.005837±0.00011	

Table No-3: Effect fenugreek seed extract on superoxide dismutase (SOD) activity (Enzyme activity expressed in unit/mg protein/hr) of testis, epididymis and seminal vesicle during aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sl. No.	Treatment (n=5)	SOD activity (Testis)	SOD activity (Epididymis)	SOD activity (Seminal vesicle)	Statistical significance
1	Control	51.699±2.9613	33.583±1.8132	38.583±1.8132	1:2 P < 0.01
2	Dg-treated	22.2199±1.6597	14.8899±0.9705	18.8899±0.9705	2:3 P < 0.01
3	D + F	35.5101±2.7694	20.6715±0.7094	24.6715±0.7094	2:4 P < 0.01
4	D → F	41.1739±2.4748	22.9156±1.3129	31.9156±1.3129	

Table No-4: Effect fenugreek seed extract on catalase (CAT) activity (Enzyme activity expressed in unit/mg protein) of testis, epididymis and seminal vesicle during aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sl. No.	Treatment (n=5)	CAT activity (Testis)	CAT activity (Epididymis)	CAT activity (Seminal vesicle)	Statistical significance
1	Control	10.712±1.0794	5.2707±0.4657	7.2707±0.6257	1:2 P < 0.01
2	Dg-treated	2.2838±0.2659	1.5225±0.3244	1.5225±0.2844	2:3 P < 0.01
3	D + F	4.1313±0.5301	2.4136±0.2308	2.4136±0.2207	2:4 P < 0.01
4	D →F	7.7978±0.4552	3.1349±0.556	5.1349±0.512	

Table No-5: Effect fenugreek seed extract on Glutathione peroxidase (GPx) activity (Enzyme activity expressed in unit/mg protein) of testis, epididymis and seminal vesicle during aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sl. No.	Treatment (n=5)	GPx activity (Testis)	GPx activity (Epididymis)	GPx activity (Seminal vesicle)	Statistical significance
1	Control	11.7512±0.7907	5.957±0.404	7.896±0.404	1:2 P < 0.01
2	Dg-treated	2.1669±0.2074	1.478±0.2738	1.583±0.2738	2:3 P < 0.05
3	D + F	4.2222±0.3541	2.3590±0.2894	2.4129±0.2894	2:4 P < 0.01
4	D → F	8.21±0.493	5.3309±0.519	5.3622±0.519	

process. (Song *et al.,*1999). Results obtained in this work showed that D-Galactose treated group containing oxidative stress caused an increase in lipid peroxidation and marked decrease in testicular CAT and SOD activity. Fenugreek is one of the oldest medicinal plants, its aqueous extracts of seeds and leaves of fenugreek have been shown to possess many beneficial effects in therapy of diseases. Fenugreek seeds exhibit hypoglycemic, hypolipidamic, anti androgenic, anti-aging, antifertility as well as good source of healing properties (Abou El-Soud *et al*., 2007).

The present study showed that after the administration of fenugreek seeds extract improved the histological changes induced by D-Galactose and suppress the oxidative stress as indicated by decrease of lipid peroxidation and increase activity of antioxidative enzymes in the D+ F and D '! F groups. This was may be due to free radical capacity of phenolic compounds of fenugreek seeds. In agreement with this results, Sakr *et al.,* (2012) reported that fenugreek seeds extract ameliorate Adriamycin induced testicular toxicity and oxidative stress in mice. Fenugreek seeds have been documented for their multiple pharmacological activities including antioxidation (Ahmadiani *et al.,* 2001), fenugreek seed polyphenols prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2 in vitro in human erythrocyte (Kaviarasan *et al.*, 2004). Moreover, it was demonstrated that the supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats (Ravikumar and Anuradha, 1999). It was also showed that the polyphenolic extract of fenugreek seeds has an antioxidative activity in vitro (Kaviarasan *et al.,* 2007). The antioxidant activity of fenugreek was attributed to the presence of flavonoids and polyphenols (Deshmukh *et al*., 2014a, b). Supplementation and treatment of antioxidants may help in prevention and removal of free radicals (Walvekar *et al*., 2013a, b; Deshmukh *et al*., 2015). Thus the effect of fenugreek against testicular toxicity of D-Galactose may be due to the antioxidant activity of these constituents. The satisfactory results observed in D '! FSE group as compared to D + FSE group it indicates fenugreek administration after injury is more beneficial than co-treatment.

CONCLUSION

The present study investigated that the fenugreek seed extract which protects the antioxidative enzymes in male testis, Epididymis and Seminal vesicle against oxidative stress induced by D-galactose. Antioxidative property of fenugreek helpful in scavenging free radicals and normalizing the level of MDA level in reproductive organs. Fenugreek seed extract is important to manage pathological condition of those diseases caused by reactive oxygen species. Therefore, our results suggest a great potential of fenugreek seeds for reducing oxidative damage and various other detrimental factors associated with aging.

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