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Therapeutic Use of Vegetable Oils as Functional Food in Alleviation of Experimental Depression

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Abstract

This article reports the analysis of phenol content, antioxidant capacity of the oil of black cumin, flax, sesame, mustard and fenugreek seeds, and garlic extract. The oils with strong antioxidant activity were tested for alleviation of experiment depression. Folin-Ciocalteu reagent was used to determine the phenol content and DPPH (2,2-diphenyl-1-picrylhydrazyl) was employed for the antioxidant activity. The phenol content was found to be the highest in black cumin followed by flax and sesame oil (98.21, 71.91±5.98 and 47.6±0.21 mg GAE/100 ml respectively). Free radical scavenging capacity was in the order of flaxseed oil > sesame oil > black cumin oil. Depression was induced by oral dose of reserpine (0.38 mg/kg body weight). The change in depression was evaluated by forced swim and tail suspension tests, fasting blood glucose and plasma malondialdehyde. In alleviation of depression, black cumin, flax, and sesame seed oil showed strongly significant effect.

Keywords: Antioxidant capacity; Depression alleviation; Functional food source; Rat model; Total phenol

Introduction

Black cumin (*Nigella sativ*), Sesame (*Sesamum indicum*), Flaxseed (*Linum usitatissimum*) oils and garlic (*Allium sativum*) extract were considered as functional food source. They are rich phytochemicals or nutraceuticals and are used in the treatment of many ailments [1-3]. Phenol or polyphenols are the major phytochemicals of antioxidant potentials in plant food [1,4]. Phenolic hydroxyl groups react with reactive oxygen and nitrogen species to terminate the cycle of free radical generation [2]. In oils, the polar phenolic fraction is a complex mixture of compounds with a benzene ring bearing one or more hydroxyl groups [3]. This phnenolic compounds partly stabilize the oils to autoxidation. Polyphenols have been reported to associate with 46% reduction in risk for cardiovascular diseases [4] and its recommended intake is 1756.5±695.8 mg/day. Therefore, database on phenols and polyphenols are to be generated to aware the people to intake phenol containing diet.

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Life processes such as energy generation, phagocytosis, detoxification reactions continuously produce, as byproducts, free radicals and reactive oxygen species in the body; defense mechanism scavenge these free radicals. Lifestyle process generates excess free radicals, which cannot scavenge by the defense system in body, thus induces oxidative stress. Antioxidants such as phenolic compounds, ascorbic acid present in green-yellow vegetable and fruit can delay or prevent the oxidation of biomolecules DNA, lipids, proteins or nucleic acids [5,6].

Oxidative stress can produce inflammation, neurodegenerative diseases including depression [7]. The neurological disorder starts a series of neuroendocrine events in the Hypothalamic Pituitary Adrenal (HPA) axis leading to a rise in stress hormone (cortisol in human). Sustained or chronic stress leads to decrease in monoamines which have been linked to make depression [8]. In addition to antidepressant drugs, some plant products including functional oils have been claimed to inhibit oxidative stress [9]. This study aims to assess antioxidant potential of functional oils and investigate alleviation of stress induced depression in rat model.

Materials and Methods

Chemicals

Folin-Ciocalteu reagent, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical, Sodium carbonate, 3,4,5- trihydroxybenzoic acid (Gallic acid), Hexane, Methanol, Hydrochloric acid, Ethyl acetate, Butylated Hydroxyl Toluene (BHT), Reserpine, Clomipramine, Thiobarbituric acid, Trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were analytical grade.

Collection of test sample

Black cumin oil (*Nigella sativa*), Sesame oil (*Sesamum indicum*), Flaxseed oil (*Linum usitatissimum*), Mustard oil (*Brassica nigra*), Fenugreek oil (*Trigonella foenum-graecum*) and Garlic powder (*Allium sativum*) were selected for the study. These plant product have medicinal potential. Triplicate samples were collected for every item, which were then mixed to make three analytes or composite test sample [10].

Preparation of test sample

Preparation of oils: The oils were produced from the oilseeds by cold pressing in a hydraulic press. The temperature recorded in pressing room was 15±2º and 60±10ºC inside the press. Pressing time ranged from 30 minutes to 1 hour, depending on the seeds. The temperature of pressed oils was 38-40ºC, sealed under vacuum in dark bottles and

stored at 20ºC in dark till analysis. The oils were prepared in three independent replications.

Preparation of garlic powder: Garlic bulbs collected from local market were cleaned with water and air dried. Cloves were separated, cut into small pieces, weighed, dried in the oven, powdered with a grinder and then sieved through a muslin cloth to separate fined particles. This was kept in a zip lock bag and stored in a desiccate at -20ºC.

Preparation of extract

Oil extract: Polar phenolic compounds were extracted with the procedures as described by Gouvinhas et al. [11]. A 4 ml oil sample, 2.5 ml of hexane and 2.5 ml of methanol: water (80:20) mixture were added in a test tube, vortexed and centrifuged for 15 minutes at 1000 rpm in a centrifuge (Hettich, Bayern). The lower phase was carefully removed and taken in a 10 ml volumetric flask. This process of extraction was repeated for three times, and added to the volumetric flask. Triplicate analytes were made for every oil sample.

Garlic extract: A 42.5 ml methanol and 7.5 ml 1N hydrochloric acid were added to 2 g garlic powder in a 250 ml conical flask. It was allowed to soak in the solvent at room temperature for 24 hours with intermittent shaking. The extract was filtered through No.1 Whitman filter paper into a flask. The filtrate was concentrated by rotary evaporator (Heidolph, Germany). After evaporation, methanol was added to concentrate and make the volume 25 ml.

Preparation of standard curve

Standard calibration curve was prepared by dilution the stock solution of Gallic acid (100 µg/ml) with 50% methanol to yield the diluted concentrations of 50, 125, 250, 500 and 1000 µg/ml.

Determination of Total Phenol Content (TPC)

The content of total phenolic compounds in the samples was determined using Folin-Ciocalteu reagent, with gallic acid as the standard [12]. One ml extract (oil and garlic) was mixed with 0.5 ml Folin-Ciocalteu reagent. After 3 minutes, 2 ml 7.5% sodium carbonate solution and 6.5 ml water were added. The mixture was shaken and incubated at 70ºC for 30 minutes. The absorbance of the standards and samples was measured at 750 nm with a double beam UV-Visible spectrophotometer (Evolution™ 300 UV-Vis Spectrophotometer, UK) in relation to a gallic acid standard curve. All measurements were performed in triplicate. The results were expressed as mg of Gallic Acid Equivalents (GAE) per ml.

Determination of antioxidant activity

The antioxidant activity of oils was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical as described elsewhere [13,14], with slight modification. In brief, 10, 20, 40 and 80 µl of each oil and standard (BHT) were taken in test tubes and final volume was made 2 ml with DPPH ethyl acetate solution (93 µM). The mixture was shaken and kept in dark at room temperature for 1 hour. Absorbance was measured at 515 nm in an UV spectrophotometer (Evolution™ 300 UV-Vis Spectrophotometer, UK). BHT was used as standard or positive control (stock-1mg/ml ethyl acetate).

For the garlic, previously prepared methanolic extract of gallic acid for TPC was used as stock solution and modified method of Guptawas employed. Dilution was carried out to obtain the concentration of 100, 200, 300 and 400 µg/ml for both the sample and standard. Then, 2 ml of 0.1 mM methanolic DPPH solution was added to 2 ml extract solution and standard of different concentrations. The mixture was stirred for 15 sec and kept in dark in room temperature for 30 minutes. Absorbance was measured at 517 nm. Ascorbic acid was used as standard or positive control (stock-1mg/ml methanol).

The percentage of DPPH radical scavenging activity for each sample was calculated as-% of inhibition=(Ao-A/Ao)×100

Where, A0 is the absorbance of the blank solution (containing control solution except sample) and A is the absorbance of the DPPH solution containing sample/extract.

Efficient/Effective Concentration fifty" or EC_{50} value (also called IC_{50} value) has been introduced for the interpretation of the results from the DPPH method. This is defined as the concentration of substrate that causes 50% loss or scavenges of the DPPH activity (color). The DPPH radical scavenging activity % was plotted against the sample concentration (µg/ml) to determine the IC50 value.

Animal experimentation

Acclimatization of animal: A total of 30 healthy Wistar Albino female rats weighing between 100-150 gm (initial body weight) and age ranging from 50-60 days was included in this study. Animals were collected from animal house of Pharmacy department, Jahangirnagar University, Savar. Prior to experimentation, rats were acclimatized with basal diet and water in the animal house of Institute of Nutrition and Food Science (INFS), University of Dhaka for 14 days.

Ethical permission was taken from the Ethical Committee of the Faculty of Biological Sciences, University of Dhaka

Stress development and experimentation: After acclimatization, the rats were divided into group 1, 2, 3, 4, 5 and 6 each group consisting of five animals; sample size for experimental model was estimated with use of Resource Equation Method [15]. Neurological stress (depression) was developed into the animals of each group by intraperitoneal injection of Reserpine (0.38 mg/kg body weight daily) for consecutive 21 days with concomitant oral administration of 500 µl oil of black cumin, flaxseed, and sesame seeds (having higher phenol and antioxidant activity) to the rats of group 1 (Black cumin oil, BCO), group 2 (Flaxseed oil, FO), and group 3 (Sesame oil, SO) every day for 21 consecutive days. The group 4 animal was given 250 µl antidepressant clomipramine (12.65 mg/kg body weight), and the stressed group 5 and unstressed group 6 rats received only the basal diet for the same time schedule and period.

After 21 days of experimentation on the 22nd day, the Behavioral tests- Tail Suspension Test and Forced Swim Test were performed, and then animals were sacrificed to collect the blood specimen, brains and adrenal glands.

Forced Swim Test: Rats of all groups were systematically subjected to forced swim individually. Rats were placed in a cylinder (40 cm height and 15 cm diameter) containing fresh water (26ºC) upto a height of 30 cm. The total duration of swimming, climbing and immobility in the last 4 minutes of the test were recorded as described by Porsolt et al. [16].

Tail Suspension Test: Rats were individually suspended on the edge of a table, 50 cm above the floor by adhesive tape placed approximately 11 cm from the tip of the tail on day 22. Each animal was visually and acoustically isolated from other animals during the test. The

total period of immobility was recorded manually for five minutes as described by Steru et al. [17] and Nishizawa et al. [18].

Collection of blood specimen: On the 23rd day one drop of blood was taken from each rat by tail bleeding for estimation of fasting blood glucose. Rats were then sacrificed to collect the blood, which was processed to separate serum to be stored in eppendorf tube at -20ºC for biochemical analysis.

Estimation of fasting blood glucose: Fasting blood glucose was estimated with the use of a standard glucometer (Accu Chek, Germany). Normal range of fasting blood glucose level was 2.3-3.7 mmol/L.

Estimation of serum Malondialdehyde (MDA): Serum malondialdehyde content was estimated by thiobarbituric acid assay method [19]. TCA-TBA-HCL reagent (15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 N hydrochloric acid) were used. Five hundred micro litre (500 µl) serum was combined with 1ml of TCA-TBA-HCL, mixed thoroughly and heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 3000 rpm for 10 minutes. The absorbance of the sample was determined at 535 nm against a blank (only reagent without serum). Serum MDA was measured by using the following formula:

MDA concentration: $C=OD/b\mathcal{E}$

Where, extinction coefficient $\mathcal{E}= 1.56\times10^{5}\ M^{-1}\ cm^{-1}$, width of tube b=1 cm, OD=Optical density at 535 nm.

Collection of brain and adrenal gland: Brain and adrenal gland were removed from each of the rats by meticulous dissection and was extracted described by Sadi [20], washed in saline, wiped in tissue paper, weighed by electric balance analyzer and recorded. The specimens were preserved in 10% formalin.

Statistical analysis

All experiments were performed in triplicate and results were presented as the mean±SD. Multiple linear regression, one way analysis of variance and Pearson's correlation were conducted using Microsoft Office Excel 2007 and SPSS version 22.0. For estimation of TPC, Microsoft Office Excel was used for standard curve calibration. For antioxidant activity, IC_{50} value of each sample was estimated by linear regression using Microsoft Office Excel. In experimental animal model, data analysis was made by ANOVA and Post hoc Tukey test.

Results

Total phenol content

Total phenol content was calculated using Gallic acid standard graph. The phenol content ranged from 98.21±7.29 mg GAE/100 ml (black cumin oil) to 4.74±0.08 mg GAE/100 ml (fenugreek oil); the amount was in order of Black cumin oil > Flaxseed oil > Sesame oil > Mustard oil > Fenugreek oil. Garlic contained phenol 7.18 mg GAE/g dry matter (Table 1).

Antioxidant activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is most widely employed test for determination of free radical scavenging capacity of the oils and garlic. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidant to the corresponding hydrazine. The free radical scavenging activity or percent inhibition was measured by calculating the IC_{50} value corresponding to the concentration of oil and garlic extracts. The IC_{50} is scavenging of 50% of DPPH radicals, and it was determined from the curve constructed for inhibition percentage versus concentration used.

Sample, mg GAE/100 ml oil)*	Total Phenol Content
Sesame oil	47.6±0.21
Black cumin oil	98.21±7.29
Flaxseed oil	71.91±5.98
Mustard oil	20.99±1.33
Fenugreek oil	4.74±0.08
Garlic	7.18±1.04

Table 1: Total Phenol Content (TPC) of selected sample.

*value expressed in mean± standard deviation of n=3 of independent samples

Table 2 shows that the DPPH free radical scavenging activity of the sample was increased with increasing concentration. Flaxseed oil gave the strongest antioxidant capacity at IC_{50} value 30.8 µg/ml, and garlic showed the lost capacity. IC_{50} value of the oils and garlic was in order of Flaxseed oil > Sesame oil > Black cumin oil > Mustard oil > Fenugreek oil > Garlic. BHT (Butylated hydroxyl toluene) was used as standard control. Antioxidant activity was estimated from graph made by plotting % inhibition versus the concentration, which was used to calculate IC_{50} values. It was indicated that the antioxidant activity of oils was higher than the antioxidant BHT (IC50 value 83.94 µg/ml). Bahrami et al. [21], reported the IC_{50} value for sesame oil and flaxseed oil were 26 µg/ml and 22 µg/ml respectively.

Sample	% Inhibition at Different Concentration				
	5µg/ml	10µg/ml	20µg/ml	40µg/ml	IC_{50} value (µg/ml)
Sesame oil	38.43±1.89	45.32±0.63	49.17±0.41	49.86±0.24	34.53
Black cumin oil	24.24±4.96	32.64±0.41	42.7±0.24	47.52±0.41	40.15
Mustard oil	20.25±1.49	32.78±1.04	42.42±2.28	46.42±0.63	40.78
Flaxseed oil	10.19±0.63	39.94±4.14	50.55±0.64	52.75±0.63	30.8
Fenugreek oil	3.03±1.26	20.39±0.86	36.5±1.19	42.01±1.26	43.28
Garlic	100µg/ml	200µg/ml	300µg/ml	400µg/ml	
	8.88±0.62	19.21±3.93	39.26±0.83	49.38±0.62	397.16

Table 2: DPPH free radical scavenging activity and IC_{50} value of oil and garlic sample.

*values are expressed as mean±SD (n=3).

For garlic, ascorbic acid was used as positive control (Figures 1 and 2). Figure 3 shows the antioxidant activity of garlic and ascorbic acid. It was seen that the radical scavenging activity of ascorbic acid (IC_{50}155.96 µg/ml) was much higher than that of garlic (IC_{50}397.16). IC_{50} value for garlic oil was reported 500 µg/ml by Lawrence & Lawrence [22].

Figure 3 showed a negative correlation between the phenol content and IC_{50} value. As the higher phenol content results in higher antioxidant activity, which, therefore, resulted in lower IC_{50} value. Pearson correlation coefficient (r-0.476, p=0.418) done was found to be insignificant. However, Gouvinhas et al. [11], reported correlation of the antioxidant activity of plant materials with their phenolic content.

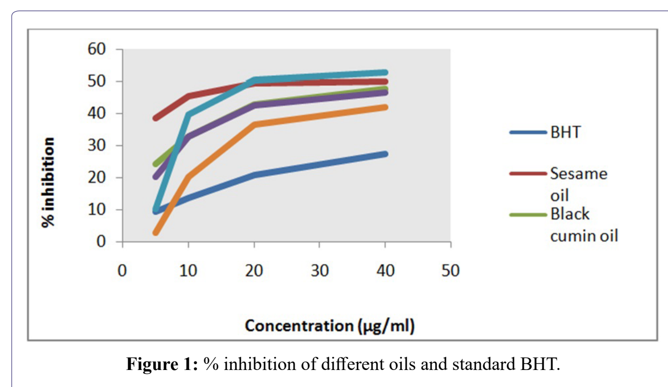

Figure 1: % inhibition of different oils and standard BHT.

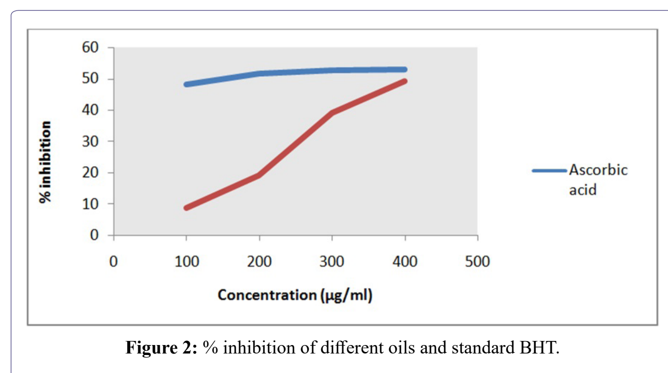

Figure 2: % inhibition of different oils and standard BHT.

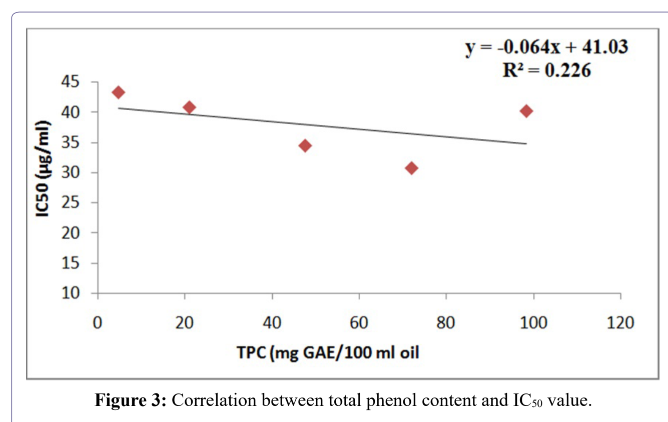

Figure 3: Correlation between total phenol content and IC_{50} value.

Effect of oil on depression

In the assessment of the effect of plant seed oils on neurological stressed (depression), behavioral tests, biochemical parameter, weight of brain and adrenal gland and body weight were measured.

Behavioral test (TST, FST): In tail suspension test, the time period (in second) of immobility was found significantly lower as compared to the negative control and was similar or less than that of the clomipramine (Table 3). Sesame and flaxseed oils showed stronger activity. In forced swimming test, climbing time was found to be higher than those of the clomipramine as well as negative control or basal diet; it was significantly higher in sesame oil and flaxseed oil. Swimming time was significantly less in sesame oil, but in immobility, sesame oil was also significantly active. One way analysis of variance showed the effects of the oils in tail suspension and forced swim tests were found to be significant.

Effect on brain, adrenal gland and body weight: The brain weight of flaxseed oil was significantly (p=0.023) higher than the negative control. However, mean brain weight altered by seed oil of black cumin, flaxseed, and sesame was almost similar to clomipramine (Table 4). On the other hand, these oils reduced the weight of adrenal gland significantly ($p<0.05$), but it was almost similar to clomipramine group. One way analysis of variance showed variation in weight of brain, adrenal gland, rat body was found to be significant.

Group	TST Immobility (s)	Forced Swim Test (s)		
		Climbing (s)	Swimming (s)	Immobility (s)
Black cumin oil (A)	18± 2.24	121.4±3.21	82.2±3.42	36.4±1.14
Flaxseed oil (B)	15.6± 1.14	125.4±3.65	81.6±4.98	33±1.58
Sesame oil (C)	14± 1.58	130.2±3.11	78.4±1.82	31.4±3.51
Positive control (D)	14.4± 1.14	121.2±4.82	83.6±2.88	35.2±2.86
Negative control (E)	20±1.76	116.4±4.34	86.4±4.22	37.2±1.3
Normal control (F)	13.8± 1.92	129.8±3.11	88±2.24	22.2±3.7
ANOVA	P=11.62 F=0.001	P=10.33 F=0.0001	P=5.07 F=0.003	P=28.99 F=0.0001
Tukey test	D vs A, B, C: 0.022, 0.85, 1.00	D vs A, B, C: 1.00, 0.51, 0.01	D vs A, B, C: 0.97, 0.94, 0.20	D vs A, B, C: 0.98, 0.75, 0.22

Table 3: Antidepressant activity of black cumin, flaxseed and sesame oils.

Groups	Brain Weight (g)	Adrenal Gland Weight (mg)	Body Weight (%)
Black cumin oil (A)	1.498±0.0397	11.2±1.3038	10.51±2.6791
Flaxseed oil (B)	1.527±0.0828	9.8±1.9235	13.03±3.6909
Sesame oil (C)	1.491±0.107	10.4±1.1402	12.29±2.2412
Positive control (D)	1.48±0.0772	10.2±2.1679	9.16±2.1563
Negative control (E)	1.36±0.0497	15.2±1.6432	5.77±1.6874
Normal control (F)	1.56±0.0806	10.6±2.0736	17.03±2.9569
ANOVA	F=3.99 P=0.009	F=6.51 P=0.001	F=10.34 P=0.0001
Tukey test	D vs A, B, C: 1.00, 0.92, 1.00	D vs A, B, C: 0.94, 1.00, 1.00	D vs A, B, C: 0.96, 0.23, 0.44

Table 4: Change in brain and adrenal gland weight and % body weight change in stress rats.

Effect on stress biomarker: No statistically change was obtained in fasting blood glucose level by the oil extracts, but apparently it was lower than the negative control and was almost similar to clomipramine group (Table 5). In stress marker Malondialdehyde (MDA) analysis, the oil extracts strongly and significantly ($p<0.0001$) reduced the MDA level (Figure 4).

Discussion

Phenol content

Phenols are strong natural antioxidants which prevent oxidative stress [23] and have a wide spectrum of medicinal values such as anti-inflammatory, anti-microbial, anti-aging, anti-cancers to skin cell renewal [24]. Determination of phenol content with Folin-Ciocalteu reagent using gallic acid standard was based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products. A very few studies reported the phenol content

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Groups	Malonaldehyde (nmol/ml)	Fasting Blood Glucose (mmol/L)
Black cumin oil (A)	1.026±0.0818	3.02±.2775
Flaxseed oil (B)	0.733±0.1895	2.82±.3962
Sesame oil (C)	0.75±0.1767	2.78±.2490
Positive control (D)	0.822±0.2732	3.14±.2793
Negative control (E)	1.772±0.1821	3.3±.1414
Norman control (F)	0.354±0.1318	3.22±.6221
ANOVA	P=34.07 F=0.0001	P=1.74 F=0.16
Tukey test	D vs A, B, C: 0.50, 0.98, 0.97	D vs A, B, C: 1.00, 0.73, 0.62

Table 5: Change in malondialdehyde and fasting blood glucose in stress rats.

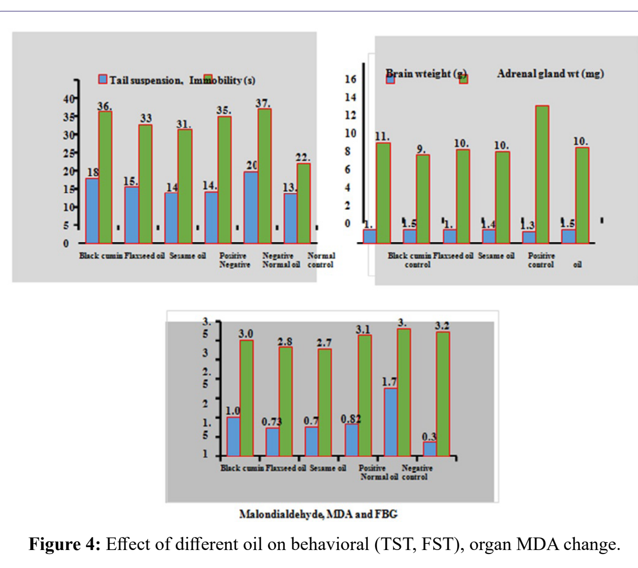

Figure 4: Effect of different oil on behavioral (TST, FST), organ MDA change.

in oils [11,25]. The phenol content obtained in the present study was found to be higher as reported others [11,25], but somewhat similar as obtained elsewhere [26-28]. This variation in phenol content might be due to climatic conditions, agronomic practices, plant species or cultivars and tissues and processing [29,30] and the method of extraction and separation of hydrophilic portion of oils.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is the most suitable for the determination of the antioxidant activity of cold-pressed oils [28], is employed for evaluation of free radical scavenging activity by antioxidants. Some studies reported antioxidant activity of oils and garlic [11,25,31]. The result of this study was not comparable to others, which might be because of as discussed above [29,30].

Depression (neurological stressed)

Oil extracts significantly reduced the depression induced by stress of clomipramine. These oil of black seeds, sesame and flaxseed contain omega fatty acids-linolenic, α-linolenic acids, which are strong antioxidant [26,27,32]. These antioxidant constituents in the oils reduces the oxidative stress, consequently alleviate the depression.

Conclusion

The oils obtained from black seed, flaxseed and sesame seed contained rich amount of phenol, which have rich antioxidant potential, which, in turn, showed alleviation of stress induced depression in experimental rat model.

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