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Effects of Fermented Ginger Rhizome (*Zingiber officinale*) and Fenu Greek (*Trigonella foenum-graceum*) Supplements on Oxidative stress and Lipid Peroxidation Biomarkers in Poloxamer-407 Induced -Hyperlipidemic Wistar Rats

*** Tanko Y.[1], Kabiru A.[1], Abdulrazak A.[3], Mohammed K.A.[3], Salisu A.I.[2], Jimoh A.[1], Gidado N.M.[1], Sada N.M. [3]**

Department of Human Physiology, [1]Ahmadu Bello University, Zaria, Nigeria. [2]Bayero University, Kano, Nigeria.[3]Kaduna State University, Kaduna, Nigeria.

Summary: This research was aimed at investigating the Effects of Fermented Ginger Rhizome (*Zingiber officinale*) and Fenu Greek (*Trigonella foenum-graceum*) on Oxidative stress and Lipid Peroxidation Biomarkers in Poloxamer 407Induced-Hyperlipidemic Wistar Rats. Hyperlipidaemia was induced with poloxamer P407 (1.5 g/kg b.w. *i.p*.) The Animals were grouped into six of five animals each group. Group 1 normal control, Group 2 served as the hyperlipidemic control, Group 3 administered 0.26 g/kg cholestyramine, Group 4 fed on Fenugreek 25% supplement. Group 5 fed on 25% fermented ginger supplement, while group 6 were fed on 25% ginger and fenu greek combined respectively. All treatments were given for a period of four week. Serum antioxidant activities such as catalase (CAT), glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Malondialdehyde were evaluated. As regards to the catalase activity there was a significant decrease in the groups' fed on 25% fenugreek and 25% fermented ginger supplements respectively. However, co-fed with both supplements significantly increase the catalase activity as compared with the hyperlipidaemic control untreated. Comparism with the positive control cholestyramine, there was also a significant increase. Also in relation to the SOD activity there was a significant increase in the activity as compared with the hyperlipidemic control. Furthermore, the Gpx activity there was a significant increase in the as compared with the hyperlipidemic control. oxidative stress biomarker activities SOD) there was significant increase ($p<0.05$) when compared with hyperlipidemic control. There was a significant ($p<0.05$) decrease in the Malondialdehyde levels in the groups fed with the supplement when compared with hyperlipidemic control. In conclusion supplements of Fenugreek and Ginger improved antioxidant status and reduced Malondialdehyde in Poloxamer-407 Induced-Hyperlipidemic Wistar Rats.

Keywords*:* Ginger Rhizome (*Zingiber officinale*), Fenugreek (*Trigonella foenum-Graceum*), Oxidative stress, Poloxamer-407

**Address for correspondence*: yusuftanko@abu.edu.ng. Phone number: +2348037054274

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INTRODUCTION

Hyperlipidemia is a heterogeneous disorder involving multiple etiologies. It is commonly characterized by an increased flux of free fatty acids (FFAs), raised triglycerides, low density lipoprotein cholesterol (LDL-C) and Apo lipoprotein B (apoB) levels and reduced plasma high-density lipoprotein cholesterol (HDL-C) concentration as a consequence of metabolic effects, or dietary and lifestyle habits (Micallef and Garg 2009). This condition is known to increase the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen through various mechanisms. These ROS are capable of damaging many biological macromolecules such as DNA, RNA, protein and lipids (Fang *et al*., 2002) and have been implicated in the pathophysiology of heart failure, ischemic heart disease (Prasad *et al*., 1996), hepatic injury (Scott *et al*., 2000; Montilla *et al*., 2006) and chronic renal damage and failure (Baker *et al*., 1985) in animals and humans. Hence, it is possible that the oxidative stress in hyperlipidemia may damage organs such as heart, liver and kidney. Despite the fact that there are drugs available clinically for treating hyperlipidemia, the consumption of functional foods/dietary supplements in lowering/ controlling serum cholesterol levels and risk of cardiovascular diseases has gained enormous global acceptance over the years by the general public (Kwok *et al*., 2010) This medical condition or problem is divided into two subtypes: primary hyperlipidemia and secondary hyperlipidemia. Hyperlipidemia can be induced by dietary means (e.g. chronic feeding of a high fat diet) (Gawronska-Szklrz *et al*., 1994; Sullivan *et al*., 1993;

Xu *et al*., 2009) or by treatment with compounds such as Triton (Holt and Dominguez, 1980; Levine and Saltzman, 2007) or poloxamer 407 (P407) (Johnston and palmer, 1993). Poloxamer407, a non-ionic synthetic copolymer surfactant, provides an attractive means of inducing hyperlipidemia because of its rapid onset and seeming lack of overt toxicity; within 24 hours of its intraperitoneal (i.p.) injection a profound hyperlipidemia state is achieved. It has been used to induce experimental hyperlipidemia in several rodent species including rat (Johnston and palmer, 1993).

Ginger (*Zingiberofficinale Roscoe*) belongs to the family *Zingiberaceae* and genus *Zingiber*. The family zingiberaceae is represented by about 46 genera, distributed through the tropics and subtropics. The genus includes about 85 species of aromatic herbs from East Asia and tropical Australia (Bhatt *et al*., 2013). The plant is an aromatic herb and its taxonomic position is as follows (Gupta and Sharma, 2014): The English botanist William Roscoe (1753-1831) gave the plant the name *Zingiber*, derived from a Sanskrit word *singabera* which means horn-shaped due to the protrusions on the rhizome. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds (Grzanna *et al.,* 2005). Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present (Langner 1998; Shukla Y and Singh, 2007).

Fenugreek (*Trigonella foenum-graecum*L.) is widely distributed throughout the world and belongs to the family Fabacecae (Leela and Shafeekh, 2008). The yields can be significant increase in quantity and quality through the suitable management of cultivation, irrigation and harvesting. Fenugreek is a medicinal plant that has therapeutic value to certain disease. This plant is useful for blood and glucose decreasing effect in diabetic and non-diabetic patients and has antioxidant and antibacterial activity (Thomas *et al*, 2011). The plant contains active constituents such as alkaloids, flavonoids, steroids, saponins etc (Paridar *et al*, 2011; Vaidya *et al*, 2013). It is an old medicinal plant. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects; Anti-inflammatory effects (Prasad *et al.*, 2014). Recent research has identified fenugreek as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones (Acharya *et al*, 2007). A large number of allopathic hypolipidemic drugs are currently available in the market, but these lag behind the desired properties such as efficacy and safety on long term use, cost and simplicity of administration. Some of these factors do not fulfill conditions for patient's compliance (David and Tooth, 2004) Consumption of such synthetic drug have been reported to cause hyperuricemia, diarrhea, nausea, myositis, gastric irritation, dry skin and abnormal liver function (Dhaliya et al., 2013). Poloxamer-407 is most avidly taken up by the liver (Li et al., 1996). Poloxamer-407 it has a marked effect on the ultrastructure of the liver sinusoidal endothelial cells (Cogger et al., 2006). Liver sinusoidal endothelial cells are very thin cells that are perforated with pores called fenestrations that are thought to facilitate the transfer of substrates such as lipoproteins from blood to hepatocyte. The proposed mechanism for Poloxamer-407 induced hyperlipidaemic is related to the reduction in the transfer of lipoproteins from the blood to the hepatocytes.

The aim of this study is to determine the effects of Fermented ginger rhizome (*Zingiber officinale*) and Fenu greek (*Trigonella foenum-graceum*) supplements on oxidative stress and Lipid peroxidation biomarkers in Poloxamer-407 Induced -Hyperlipidemic Wistar Rats

MATERIALS AND METHODS

Animals

Thirty (30) Wistar rats of both sexes (120-150 g) were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University Zaria, Nigeria. The rats were maintained on standard laboratory animal feed and water *ad libitum,* This research was conducted in accordance with the internationally accepted principle for laboratory animal use and care in Ahmadu Bello University guidelines.

Drug used

Cholestyramine and Poloxamer 407were purchased from Sigma Chemical Company St. Louis U.S.A.

Collection and Preparation of ginger and fenugreek

The Fenu-Greek seed and fresh rhizomes of ginger were purchased from Terminus Jos, Plateau State, and Sabon Gari market in Zaria Nigeria respectively. The Fenu Greek seeds and ginger rhizomes where taken to the herbarium unit of the Department of Botany, Faculty of life Science, Ahmadu Bello University, Zaria, where they were identified by a Taxonomist Malam Namadi Sanusi, and the voucher numbers 2261 for Ginger and 12034 for Fenugreek were deposited respectively. The ginger was fermented for 2 days, and then air dried under the shade and grounded into a fine powder using mortar and pestle. Also, the fenugreek seed was air dried under the shade and grounded into a fine powder using mortar and pestle

Diet formulation

Fenugreek supplemented Diet:-

2.5% of fenugreek (*Trigonella foenum-graceum*) powder was mixed with 97.5% standard animal feed making the total feed formulation (100%) and was considered as 2.5% fenugreek supplement.

Ginger Rhizome Supplemented Diet:-

2.5% of Ginger(*Zingiber officinale*) rhizome powder was mixed with 97.5% standard animal feed, making the feed 100% and was considered as 2.5% Ginger supplement.

Induction of Hyperlipidemia

The animals were intraperitoneally injected with 1.5g /kg of poloxamer 407 for a period of 4 days.

Experimental Design

In the study, 30 Wistar rats weighing between 150 and 200 g were used, each group comprised five rats (n = 5). The animals were grouped as follows:

Group 1: Normolipidemic fed with normal diet for a period of four weeks

Group 2: Poloxamer P407 (1.5g/kg) Induced Hyperlipidemic fed on normal diet for a period of four week

Group 3: Poloxamer P407 (1.5g/kg) Induced Hyperlipidemic administered 0.26g/kg cholestyramine and fed on normal diet for a period of four weeks.

Group 4: Poloxamer P407 (1.5g/kg) Induced Hyperlipidemic fed on Fenugreek-2.5% supplement for a period of four weeks.

Group 5: Poloxamer P407 (1.5g/kg) Induced Hyperlipidemic fed on fermented ginger- 2.5% supplement for a period of four weeks.

Group 6: Poloxamer P407 (1.5g/kg) Induced Hyperlipidemic fed on both fenugreek-2.5% and fermented ginger-2.5% for a period of four weeks respectively.

Collection and Preparation of Serum Samples for Biochemical Assays

Four weeks after the treatment, all the animals were subjected to light anesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box, covered with lid. Blood samples of about 5 ml were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. The samples were collected in Eppendrof tubes and allowed to clot. Thereafter, the serum was separated by centrifugation, using Denley BS400 centrifuge (England) at 3000 *g* for 10 minutes. The supernatant collected was used for the biochemical assay:

Biochemical assays:

Lipid peroxidation biomarker (MDA)

Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substances method (Gallou *et al*., 1993). Serum malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hadley (1990) using Malondialdehyde Assay kits from Northwest Life Sciences Specialties (NWLSS^{TM}, product NWK-MDA01). Butylated hydroxytoluene (BHT) in methanol reagent was used as the control. The method is based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. The MDA formed will therefore be quantified using an extinction coefficient of 1.56 x 10^5/mole/cm (Yagi, 1987). The amount of MDA formed in the control samples is subtracted from the amount in the experimental samples to obtain the amount of MDA in each sample. Since absorbance is directly proportional to the concentration, thus; concentration of MDA in each sample = Absorbance in sample – Absorbance in control x 10^5 nmol/ml ÷ 1.56 x 10^5 $M^{-1}CM^1$

Catalase Activity (CAT)

Catalase activity CAT was assessed using NWLSS^{TM} CAT activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL). Catalase enzyme activity was measured based on the principle of catalase consumption of H_2O_2 substrate at 240 nm (Beers and Sizer, 1952).

Superoxide Dismutase Activity

Activity of SOD in the rabbit serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit is based on the principle of superoxide inhibition of autooxidant of hematoxylin as described by Martin *et al.* (1987).

Glutathione Peroxidase Activity (GPx)

GPx activity was assessed using NWLSS^{TM} cGPx (GPx1) ELISA assay kit (Product NWK-GPx02, Specificity: Glutathione peroxidase, Sensitivity: 12.5pg/ml). The NWLSS^{TM} cGPx Assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA), where sample GPx concentration is determined by comparing the 450 nm absorbance of sample wells to the absorbance of known standards (Takebe, 2002).

Statistical Analysis

Data obtained were expressed in kg as mean ± SEM. The data were analyzed using ANOVA followed by Dunett's *post-hoc* test to show multiple comparisons versus control group. Values of $P < 0.05$ were considered as significant (Duncan *et al.*, 1977).

RESULTS

Effect on Serum Malondialdehyde Concentration

The effect of treatments on serum MDA concentration is shown in Table 1. The MDA concentration, an index of lipid peroxidation was higher ($P < 0.05$) in hyperlipidemic control rats (56.26 ± 2.98 µmol/mg Protein), compared to those obtained in the normal

control animals (34.84 ± 1.59 µmol/mg Protein). Fed on 2.5% fenugreek there was a significant change. However, fed on 2.5% fermented ginger significantly ($P < 0.05$) reduced the serum MDA concentration to (42.16 ± 1.27 µmol/mg Protein) when compared with the control group. Furthermore, co-fed on 2.5% both fenugreek and ginger supplements significantly decreased the serum MDA concentration (38.80 ± 1.64 µmol/mg Protein) as compared to control (56.26 ± 2.98 µmol/mg Protein)

Effect on Serum Catalase Activity (CAT)

Table 1 shows a significant ($P < 0.05$) decrease in the activity of serum CAT (68.73 ± 2.71 U/mg Protein) in hyperlipidemic untreated group was obtained when compared to those obtained in rats in normal control group (134.61 ± 2.84 U/mg Protein). There was a significant ($P < 0.05$) increase in CAT activity in the groups fed on 2.5% fenugreek and ginger respectively (98.24 ± 2.02 and 83.61 ± 1.22 U/mg Protein) as compared to control. Co – fed with the two supplements at 2.5% each significantly increased the CAT level (153.74 ± 4.22) when compared to control. Administration of cholestyramine (0.26 ml /kg) significant ($P < 0.05$) increase the serum CAT level as compared to control.

Effect on Serum Superoxide Dismutase Activity (SOD)

The superoxide dismutase activity significantly ($P < 0.05$) decreased (11.56 ± 0.78 U/mL) in hyperlipidemic control. However, rats fed on 2.5 % fenugreek and ginger (34.40 ± 2.51 and 27.75 ± 3.00 U/mL), respectively, significantly increase the SOD level as compared to control. In relation to the group co-fed on 2.5% fenugreek and ginger (18.35 ± 1.28 U/mL) significantly ($P < 0.05$) increased activity of SOD when compared with control as shown in table 1.

Effect on Serum Glutathione Peroxidase Activity(GPx)

The GPx activity significantly ($P < 0.05$) decreased (44.23 ± 2.64 µg/mL) in hyperlipidemic control. However, rats fed on 2.5 % fenugreek and ginger (58.46 ± 2.00 and 52.84± 2.50 µg/mL), respectively, significantly increase the GPx level as compared to control. As regards to the group co-fed on 2.5% fenugreek and ginger (66.29 ± 1.98 µg/mL) significantly ($P < 0.05$) increased activity of GPx as compared to control as shown in table 1.

DISCUSSION

Oxidative stress plays a major role in the causation of diabetes. Free-radicals are generated in disproportionate manner in diabetes mellitus and cause lipid peroxidation (Firdous and Raju, 2014). The free radicals steal electrons from the lipids in the cell membrane, resulting in cell damage. Lipid peroxidation is a late event accompanying rather than causing final cell death. The end products of lipid peroxidation process are aldehydes, hydrocarbon gases and chemical residues including malondialdehyde. One of the most important biomarkers to investigate the oxidative damage on lipid is MDA, a major lipid peroxidation product (Maryam *et al*., 2014). MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation (Karataş *et al.,* 2006). Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and lead to oxidative stress (Mahboob *et al.,* 2005). The degree of tissue damage induced by free-radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism (Sanilkumar and Muthu, 2013). Oxidative deterioration of polyunsaturated fatty acids (PUFA) which are present in abundance in cell membranes, initiates a self-perpetuating chain reaction that yields a wide range of cytotoxic products such as malondialdehyde (MDA), 4-hydroxynonenal. Lipid peroxidation is a free radical-related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components (Mahboob *et al.,* 2005). A rise in MDA indicated that any oxidative stress incurred sufficiently could cause free radical mediated peroxidation of lipid component in cell membrane, thus MDA is a good indicator for evaluating oxidative stress in degenerative disease such as diabetes mellitus (Padalkar *et al.,* 2012).

Table 1. Effects of Fenugreek (*Trigonella foenum-graceum*) and Fermented Ginger (*Zingiber officinale*) Rhizome Supplements on Oxidative Stress and Lipid Peroxidation Biomarkers Poloxamer-407 Induced -Hyperlipidemic Wistar Rats

Group	Treatment	MDA (µmol/mg .Pr)	CAT (U/mg Protein)	SOD (U/mL)	GPx (µg/mL)
1	Normal Control	34.84 ± 1.59*	134.61 ± 2.84*	35.07 ± 1.38*	64.59 ± 1.65*
2	Poloxamer P407 alone	56.26 ± 2.98	68.73 ± 2.71	11.56 ± 0.73	44.29 ± 2.64
3	Poloxamer P407+ cholestyramine	41.84 ± 2.34*	183.99 ± 3.34*	35.40 ± 2.51*	60.98 ± 1.57*
4	Poloxamer P407+Fenugreek	44.36 ± 1.21 ^{ns}	98.46 ± 2.14*	27.75 ± 3.00*	58.46 ± 2.00*
5	Poloxamer P407 + Ginger	42.16 ± 1.23*	83.61 ± 1.22*	18.35 ± 1.28	52.84 ± 2.50
6	Poloxamer P407+Fenugreek+Ginger	38.80 ± 1.62*	153.74 ± 4.23*	22.05 ± 1.36*	66.29 ± 1.98*

Values are expressed as mean ± SEM; n = 5. *: Value considered statistically significant when compared with Poloxamer P407 induced hyperlipidemic control group ($p \leq 0.05$); ^{ns} Value considered statistically significant when compared with control group.

Poloxamer 407 significant increased the MDA concentration. However, administration of cholestyramine 0.26 ml/kg which served as positive control also significantly decrease the MDA concentration as compared with the control. When compared with normal control there was a significant decreased in the MDA level as compared to control. Furthermore, the group fed on fenugreek 2.5% supplement for a period of 4 week significantly decreases the MDA concentration as compared with control. Also, there was a significant decrease in the group fed on 2.5% ginger supplement as compared to control. The groups that were co-fed with fenugreek and fermented ginger supplements, significantly ($P < 0.05$) decreased the MDA concentration as compared with the control group, indicating that the combination or singly fed may have inhibited lipid peroxidation.

The findings agreed with previous study that reported increased levels of lipid peroxidation in hyperlipidemic rats (Gopalakrishnan and Dhanapal, 2014). In the present study, significant ($P < 0.05$) increase in the levels of lipid peroxidation observed in the poloxamer 407 control rats might be due to reduction in antioxidant defense or due to increased free radical generation Ceretta *et al.* (2012). The intensified free radical production during poloxermer -mediated experimental rats resulted in the elevated levels of lipid peroxides and hydroperoxides by oxidative degradation of polyunsaturated fatty acids. These are unstable, cytotoxic and highly reactive, leading to free-radical damage to proteins and DNA and finally caused various diabetes-mediated complications. These observed effects of the ginger and fenugreek supplements may probably be due to its various antioxidant defense strategies, most especially the scavenging of two of the reactive oxygen species (ROS): singlet molecular oxygen and peroxyl radicals (Atessahin *et al.,* 2005).

Oxidative stress can result from either an overproduction of ROS or from the inactivation of the AOS, thus shifting the oxidative stress / antioxidant system balance in favor of stress with overproduction of ROS (Dröge, 2001; Fang *et al.,* 2002). The antioxidant enzymes play a crucial role in the cellular defence against ROS (Bernabucci *et al.,* 2002). The major antioxidant enzymes, including superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPx) are regarded as the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress and act cooperatively at different sites in the metabolic pathway of free radicals (Cheng and Kong 2011). SOD, CAT and GPx are enzymatic antioxidants that prevent cells from being exposed to oxidative damage by direct scavenging of reactive oxygen species (Bouayed and Bohn, 2010). GPx (Chen and Schopfer, 1999), and CAT (Liedias, 1998) are involved in the elimination of H_2O_2, and SOD acts to dismutate superoxide radicals to H_2O_2, which is then acted upon by GPx. Humans have evolved with antioxidant systems to protect against deleterious productions of free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from diet (exogenous) (Kangralkar *et al.,* 2010; Vijayakumar *et al.,* 2012). An imbalance between oxidation and antioxidant status has been shown to play an important role in mediating oxidative stress (Ramesh and Saralakumari, 2012). Overwhelming free radicals generated due to oxidative stress may develop several adverse effects commonly seen in diabetes such as neuropathy, nephropathy, retinopathy, and vascular disorders (Al-Azzawie and Alhamdani, 2006). Administration of Poloxamer 407 significant decrease in the SOD activity, while there was a significant increase in the normal group. Group fed on 2.5% fenugreek significant increase the SOD activity as compared to control. Also, there was no significant change in the group fed on fermented ginger alone as compared to control. Furthermore, combination of fenugreek and ginger significantly decreased the SOD activity as compared to the control. The SOD offers the first line of defence against ROS by scavenging and catalyzing dismutation of superoxide, produced by cellular metabolism, into hydrogen peroxide (H_2O_2) and oxygen (O_2) (Lin *et al.,* 2005; Das *et al.,* 2011). In relation to GPx group fed on 2.5% fenugreek supplement significantly decrease the GPx activity as compared to control. However, there was no significant change in the group fed on 2.5% fermented supplement. Co –fed with the two supplements significantly decreased the levels of GPx as compared to control. Hence, the observed effects of may be attributed to the secondary metabolites in the supplements. The CAT and GPx are involved in the reduction of H_2O_2 into H_2O and O_2 (Kaute *et al.,* 2010). However, as regards to the catalase activity group fed on 2.5% fenugreek and 25% ginger supplements separately for a period of 4 weeks, significant decreased the catalase activity as compared with negative control. Also, co- fed with both supplement significantly increase the catalase activity as compared to control. The finding is in agreement with the work of Maher (2014) In conclusion supplements of fenugreek and fermented ginger reduced reactive oxygen free radicals, improved the activities of the antioxidant enzymes and decreased the malondialdehyde concentration.

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