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Original Article

Administration of Fenugreek Seed Extract Produces Better Effects in Glibenclamide-Induced Inhibition in Hepatic Lipid Peroxidation: An *in vitro* Study*

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ABSTRACT **Objective:** To evaluate the comparative effects of fenugreek (*Trigonella foenum graecum*) seed extract (FSE) alone and in combination with an antidiabetic conventional medicine, glibenclamide (GLB), on the inhibition of *in vitro* lipid peroxidation (LPO) in liver, the major target organ of a drug. **Methods:** LPO was induced by ferrous sulphate ($FeSo_4$), hydrogen peroxide (H_2O_2) and carbon tetrachloride (CCl_4) and the effects of test seed extract and/or GLB were evaluated. **Results:** While $FeSo_4$, H_2O_2 and CCl_4 markedly enhanced the hepatic LPO, simultaneous administration of FSE reduced it in a concentration dependent manner. However, when both FSE and GLB were added to the incubation mixture, chemically induced hepatic LPO was further inhibited. The test extract also exhibited high antioxidative activity in 1,1-diphenyl-2-picrylhydrazyl radical and in 2,2'-azino-bis,3-ethylbenzothiazoline-6-sulphonic acid radical scavenging assays. **Conclusion:** FSE therapy in moderate concentration along with a hypoglycemic drug may prove to be advantageous in ameliorating diabetes mellitus and other diseases that are LPO mediated.

KEYWORDS fenugreek, glibenclamide, antioxidant activity, lipid peroxidation

Lipid peroxidation (LPO) refers to the oxidative degradation of lipids. It is a metabolic process in which reactive oxygen species (ROS) results in oxidative deterioration of lipids. Among the many different aldehydes which are formed as a secondary product during LPO, malondialdehyde (MDA) appears to be the most reactive product. It has been widely used as a convenient biomarker for LPO. Enhanced LPO plays an important role in the initiation and prolongation of many diseases including diabetes mellitus (DM). Therefore, the drugs/supplements which can inhibit LPO are often used for the amelioration of DM. Currently, several antihyperglycemic drugs are in use for the control of DM. Glibenclamide (GLB) is one such drug that is prescribed very commonly for type 2 DM. *In vivo* studies have indicated its antioxidative potential.[1,2] *In vitro* studies on some other antidiabetic drugs are known to exhibit antioxidative properties.[3] Similarly, many plant extracts are reported to act as antioxidants.[4,5] Fenugreek seed is one of them, which is known to regulate DM through its antioxidative action[6,7] Yet, till today no systematic study has been made to explore whether fenugreek seed extract (FSE) can enhance the inhibition of tissue LPO when administered along with a conventional antidiabetic drug. Therefore, in the present investigation an attempt was made to evaluate the effect of FSE in the augmentation of antiperoxidative property of glibenclamide, if any, in the liver tissues, using ferrous sulphate ($FeSo_4$), hydrogen peroxide (H_2O_2) and carbon tetrachloride (CCl_4) as LPO inducing agents.[8] For this study, hepatic tissues were used because of the fact that liver is considered as the primary target organ of a drug. The test extract was also evaluated for its antioxidative activity with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and in 2, 2'-azino-bis,3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging tests.

METHODS

Animal

Swiss colony bred *albino* male mice (Grade Ⅱ) weighing 28±2 g were used for the study. They were housed in our departmental animal house in a room maintained at 27±1 ℃ and on 10 h light and 14 h dark photo schedule with the provision of mice feed and water *ad libitum*. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals, Ministry of Environment and Forest, Government of India (Regd. No. 779/2012-13) were followed. The approval of the departmental ethical committee for handling and maintenance for experimental animals was

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also obtained before starting the investigation.

Preparation of Plant Material

Fenugreek seeds (2 kg) of high quality were obtained from local market, Indore M.P, India. These were finely powdered and extracted with 70% ethanol, incubated overnight and filtered by whatman filter paper # 1. The filtrate was then dried at 37 ℃ (yield 17.2%, w/w of the original starting material). The powdered extract was dissolved in double distilled water for experimental use.

Chemicals

$FeSo_4$, H_2O_2 and carbon tetrachloride (CCl_4), were purchased from Loba Chemie, Mumbai, India. Trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were purchased from E. Merck Ltd., Mumbai, India. GLB (Aventis Pharmaceutical Ltd., Gujarat, India) was purchased from a registered local medical store, Indore, India, while DPPH and ABTS were procured from Sigma-Aldrich Chemicals (St. Louis, MO, USA).

Experimental Design

Determination of Total Polyphenols and Flavonoids

Total polyphenolic contents of the test extract was estimated following the protocol of Leontowicz, et al.[9] In brief, 125 μL of test extract of known concentration (100 mg/mL) was diluted with 0.5 mL of distilled water, and then 125 μL of folin-ciocalteu reagent was added to the mixture. The final volume was made up to 3.0 mL with distilled water and then was incubated at room temperature for another 90 min. Finally the absorbance was measured in Shimadzu UV-Vis spectrophotometer UV-1700 (Japan) against the prepared blank at 765 nm in comparison with standard of known concentrations of gallic acid. The results were expressed in mg gallic acid equivalent/100 g dry weight of the extract. The coefficient of determination was R^2=0.9748. Total flavonoids were determined following the protocol of Leontowicz, et al.[9] The test seed extract of 25, 50 and 100 μg/L concentrations were prepared and each dilution (250 μL) was mixed with 1.25 mL distilled water. It was followed by the addition of 75 μL of 5% sodium nitrite solution and 150 μL of 10% aluminum chloride ($AlCl_3$•$6H_2O$) solution. After incubation of 5 min, 0.5 mL of 1 mol/L NaOH was added. The total volume was made up to 2.5 mL with distilled water. Finally the absorbance was measured against the prepared blank at 510 nm in comparison with standards prepared similarly with known concentrations of quercetin. The results were expressed in mg quercetin equivalent/100 g dry weight of the extract. The coefficient of determination was R^2=0.9748. The results are expressed as mg of quercetin equivalents/100 g dry weight of the extract.

DPPH Radical Scavenging Activity

Different concentrations of extract (25, 50 and 100 μg/L) were taken in separate test tubes. One mL of the test extract was mixed with 0.5 mL of 0.15 mmol/L DPPH and allowed to stand at 20 ℃ for 30 min. Control tubes were prepared by adding all the chemicals, but without the extract. Ethanol was used for baseline correction. Changes in absorbance of the samples were measured at 517 nm. The radical scavenging activity (RSA) was expressed in percent inhibition [RSA = (control OD – sample OD/control OD) × 100%], as described by Leontowicz, et al.[9]

ABTS Radical Scavenging Activity

ABTS radical cation decolorization assay was carried out using an improved $ABTS^+$ decolorization assay.[10] ABTS+ was generated by oxidation of ABTS with potassium persulfate. ABTS was dissolved in deionized water to 7 mmol/L concentration, and potassium persulfate was added to a concentration of 2.45 mmol/L. The reaction mixture was left to stand at room temperature for 24 h in the dark before the use. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 1 mL of diluted ABTS solution to 10 μL of plant extract, absorbance was taken at 734 nm exactly 1 min after initial mixing up to 10 min. All determination was carried out in triplicate and was expressed as: RSA = [($Abs_{t=0}$–Abs_{t-10})/$Abs_{t=0}$ × 100%].

Induction of LPO

In $FeSo_4$ System

Two adult male rats were sacrificed after anaesthetizing with mild chloroform. Liver from each animal was taken out immediately, blood clots were removed, washed in phosphate buffered saline (PBS), cut into small pieces and then homogenized in 10% ice cold PBS. Different experiments were performed with this liver homogenate and at the end LPO was studied using the standardized protocol followed in our laboratory.[8] In brief, to 1 mL of liver homogenate, 100 μL $FeSo_4$ and/or FSE either alone or with GLB (10 μL) were added; while in control set, the same amount of distilled water was mixed. Then the reaction mixture was incubated at 37 ℃ for 1 h, following which 2 mL TCA (10%) was added to the mixture and the samples were centrifuged at 3,000 r/min for 5 min. Two mL supernatant was taken out and to it 1 mL TBA was added followed by boiling in water bath for 45 min. After cooling in

running water OD was taken at 532 nm as routinely done in our laboratory.[8]

Experiment 1: Induction of LPO by $FeSo_4$

In three sets of test tubes (each in triplicate) each containing 1 mL of liver homogenate, three different concentrations (0.5, 5 and 10 mmol/L) of $FeSo_4$ were taken. These concentrations of $FeSo_4$ were retrieved from earlier reports.[11,12] A control set was also run in which all materials other than $FeSo_4$ were added. All the tubes were processed for the estimation of LPO by TBA reaction method[13] as described above.

Experiment 2: Inhibition of $FeSo_4$-Induced LPO by Different Concentrations of FSE

Considering the effective concentration of $FeSo_4$ that showed maximum increase in hepatic LPO, antiperoxidative effect of the test extract was evaluated. As only reference available for *in vitro* study on FSE was 20 μg/mL,[14] we used a lower and a higher concentration other than the referred one. Thus the three concentrations of FSE considered in this experiment were 10, 20 and 40 μg/mL. All three concentrations were taken in triplicate; a set of drug control tubes was processed that contained all the materials except FSE. The protocol for the estimation of LPO was similar to that of experiment 1. Although out of the above mentioned three concentrations of FSE, maximum inhibition in LPO was seen at 40 μg/mL, the percent inhibition was only 16%. Therefore, three still higher concentrations (80, 160 and 240 μg/mL) were also considered to explore the best LPO inhibiting concentration. Here also similar experimental protocol was followed.

Experiment 3: Pilot Experiment on GLB-Induced Inhibition of LPO

From the result of experiment 2, the concentration of FSE that exhibited maximum percent inhibition of LPO was found to be 160 μg/mL. Therefore, 160 μg/mL of FSE was added with different concentrations of GLB to study their combined effects in the possible inhibition of $FeSo_4$-induced hepatic LPO. The concentration of GLB was taken from an earlier report,[15] where 1 μg/mL was taken. So we used three concentrations of GLB. Thus, the concentrations of GLB taken in this study were 1, 2 and 4 μg/mL. For all three concentrations of GLB, FSE amount was kept constant (160 μg/mL, one that maximally inhibited LPO). A set of drug control tubes were also processed that contained all materials except FSE and GLB. Experimental protocol for *in vitro* study of LPO was the same as followed earlier. As here also the concentration of GLB that came out to be the most effective was the lowest one (1 μg/mL), we again tried with further 2 lower concentrations i.e. 0.25 and 0.5 other than 1 μg/mL, using a similar protocol (as described above) to find out the best effective concentration of GLB to lower the hepatic LPO.

Experiment 4: Confirmatory Experiment on GLB-induced Inhibition of LPO

To confirm the best effective concentration, a final experiment was performed where the most effective concentration of $FeSo_4$ and drugs (160 μg/mL of FSE and 0.5 μg/mL of GLB) were used. Similar protocol was adopted for LPO estimation as mentioned above.

In H_2O_2 System

Experiment 1: Induction of LPO by H_2O_2

To five sets of tubes, different concentrations of H_2O_2 (5, 10, 40, 80 and 100 mmol/L) were transferred in triplicate. These concentrations were taken from earlier reports,[16,17] where only 5 and 10 mmol/L of H_2O_2 were used. A control set was run in which all materials other than H_2O_2 were added. Liver homogenates were incubated for 1 h with 100 μL of each concentration of H_2O_2, which were further processed to measure LPO by TBA reaction method as described earlier.

Experiment 2: Inhibition of H_2O_2-Induced LPO by Different Concentrations of FSE

Taking the most effective LPO inducing concentration (80 mmol/L) by H_2O_2, FSE was tested for its antiperoxidative activity. Since in $FeSo_4$ system the most effective LPO inhibiting concentration came out to be 160 μg/mL, we took one higher and one lower concentrations of FSE, other than 160 μg/mL. Thus, the three concentrations of FSE considered in this study were 80, 160 and 240 μg /mL. A set of drug control tubes were also processed that contained all materials except FSE. The protocol followed was similar as described earlier.

Experiment 3: Pilot Experiment on the Role of GLB to Inhibit LPO

The concentration of FSE that inhibited LPO maximally was kept constant and the combined effects of 3 different concentrations of GLB (0.25, 0.5 and 1 μg/mL) were evaluated. These concentrations were also taken on the basis of effective concentrations in $FeSo_4$ system, where 0.5 μg/mL was found to be effective. A set of drug control tubes were also processed that contained all materials except FSE and GLB. At the end changes in hepatic LPO was evaluated.

Experiment 4: Confirmatory Experiment on GLB-Induced Inhibition of LPO

To confirm the best effective concentrations of GLB, H_2O_2 and drugs (FSE and GLB), another experiment was performed, in which concentration of H_2O_2, FSE and GLB were 80 mmol/L, 160 μg/mL and 0.5 μg/mL which were found to be the best effective concentrations in the earlier experiments. The procedure followed was same as mentioned in the previous experiments.

In CCl_4 System

Experiment 1: Induction of LPO by CCl_4

For this study a partially modified protocol was followed as used by Wormser, et al.[18] In brief, after washing in PBS, liver was chopped into small pieces and the liver slices (400 mg) were incubated for 1 h at 37 ℃ with 4 mL of PBS and CCl_4, which was then homogenized. To the three sets of tubes, different concentrations of CCl_4 (10, 20 and 50 μL) were added. These concentrations were taken from an earlier report,[19] where only 10 μL of CCl_4 was used. A control set was run in which all materials were added other than CCl_4. After homogenization the homogenate was centrifuged at 3,000 r/min for 5 min. The supernatant was precipitated with TCA and again centrifuged at 3,000 r/min. Two mL supernatant was taken out and to it 1 mL TBA was added followed by boiling in water bath for 45 min. After cooling in running water OD was taken at 532 nm.

Experiment 2: Standarization of Concentration of FSE for Inhibition of LPO

The concentration of CCl_4 that enhanced the tissue LPO (20 μL) to a greater extent was kept constant and was combined with three different concentrations of FSE (80, 160 and 240 μg/mL), as taken in H_2O_2 system. A set of drug control tubes was also processed that contained all materials except FSE.

Experiment 3: Pilot Experiment on the Role of GLB to Inhibit LPO

Considering the effective concentration of FSE that showed maximum inhibition in hepatic LPO, its combined effect with GLB was evaluated. The concentrations of GLB used were 0.25, 0.5 and 1 μg/mL. Since none of the above concentrations of GLB decreased LPO significantly as compared to the value of FSE treated tubes, further lower concentrations i.e. 0.025, 0.05 and 0.10 μg/mL were taken in triplicate. A set of drug control tubes were also processed that contained all materials except FSE and GLB. Alterations in tissue LPO was measured using the same procedure.

Experiment 4: Confirmatory Experiment on Co-addition of FSE and GLB to Inhibit LPO

Taking the best effective concentration from experiments 1, 2 and 3, the final experiment was performed where CCl_4, FSE and GLB were taken at a concentration of 20 μL, 160 and 0.05 μg/mL respectively and following the incubation, hepatic LPO was studied as done earlier.

Statistical Analysis

Data are expressed as mean ± standard error ($\bar{x} \pm SEM$). Statistical evaluation of the data was made using analysis of variance (ANOVA), followed by student's *t*-test. *P* values of 5% and less were considered to be significant.

RESULTS

In $FeSo_4$ System

Experiment 1: Induction of LPO by $FeSo_4$

Following the incubation of liver homogenate with 0.5, 5, and 10 mmol/L $FeSo_4$, there was a significant increase in LPO ($P<0.05$ or $P<0.01$). However, the maximum % increase (301%) was observed with 5 mmol/L of $FeSo_4$.

Experiment 2: Standarization of Concentration of FSE for Inhibition of LPO

The results were shown in Table 1 (Appendix Ⅰ). While $FeSo_4$ (5 mmol/L) markedly enhanced hepatic LPO ($P<0.01$), there was a significant decrease in the same following the incubation with one of the three different concentrations of FSE, i.e 40 μg /mL ($P<0.05$). However, the percent inhibition in LPO was very less (14.8 % as calculated from the average value of $FeSo_4$ added tubes). The other two concentrations were not able to inhibit LPO significantly. With the higher concentration of FSE (80 and 160 μg/mL), there was a significant (both $P<0.01$) decrease in LPO. However, with further increase in concentration of FSE (240 μg/mL) hepatic LPO increased. The concentration of FSE which resulted in greatest inhibition (36%) in $FeSo_4$ induced peroxidation was 160 μg/mL.

Experiment 3: Pilot Experiment on GLB-Induced Inhibition of LPO

With addition of FSE (160 μg/mL) and different concentrations (1, 2 and 4 μg/mL) of GLB to liver homogenates, LPO decreased significantly ($P<0.01$) only at lower concentration of GLB (1 μg/mL), while at higher concentrations (2 and 4 μg/mL), LPO was increased as compared to the value of $FeSo_4$ treated group. Out of the three concentrations of GLB (0.25, 0.5 and 1 μg/mL), the significant inhibition in LPO ($P<0.01$) was seen in 0.5 μg/mL of GLB (67.2%), as compared to the average

value of $FeSo_4$ tubes.

Experiment 4: Confirmatory Experiment on GLB-Induced Inhibition of LPO

Here also the results exhibited a similar pattern as in the above experiment. While the addition of FSE (160 μg/mL) brought a significant decrease (P<0.01, 40.2%) in LPO, simultaneous addition of FSE (160 μg/mL) and GLB (0.5 μg /mL) also significantly decreased LPO with RSA of 67.5% (P<0.01).

In H_2O_2 System

Experiment 1: Standardization of H_2O_2 for the Induction of LPO

After incubating liver homogenate with different concentrations of H_2O_2, there was a significant increase in LPO with 40 and 80 mmol/L of H_2O_2 (P<0.05 or P<0.01) and the percent increases were 61% and 250%, respectively.

Experiment 2: Standardization of Concentration of FSE for Inhibition of LPO

The results were shown in Table 2 (Appendix Ⅱ). Out of the three different concentrations of FSE (80, 160 and 240 μg/mL) only 160 μg/mL showed a significant inhibition (P<0.01) in hepatic LPO. The lower concentration (80 μg/mL) also decreased LPO, but the percent inhibition was only 10% whereas it was 42 % for 160 μg/mL. The highest concentration (240 μg/mL) increased the value of LPO as calculated from the value of H_2O_2 (P<0.01).

Experiment 3: Pilot Experiment on the Role of GLB to Inhibit LPO

When the effective concentrations of FSE (160 μg/mL) was combined with three different concentrations of GLB (0.25, 0.5 and 1 μg/mL), only 0.25 and 0.5 μg/mL showed a synergistic effect, while the higher concentration enhanced LPO. However, out of 0.25 and 0.5 μg/mL, the higher concentration (0.5 μg/mL) exhibited maximum and significant inhibition in LPO (69%, P<0.01) when compared with H_2O_2 treated group, with an additional and significant (P<0.01) inhibition of 50%, while there was 42% decrease in 0.3 μg/mL.

Experiment 4: Confirmatory Experiment on GLB-Induced Inhibition of LPO

Here the results showed the same pattern as in the above experiments. The treatment of FSE (160 μg/mL) brought significant decrease in LPO (P<0.01), which was calculated out to be 42.5%. Co-treatment of FSE (160 μg/mL) and GLB (0.5 μg/mL) also significantly decreased LPO with a inhibition of 62% (P<0.01).

In CCl_4 System

Experiment 1: Standardization of CCl_4 for the Induction of LPO

Incubation of liver slices with all the three concentrations of CCl_4 (10, 20 and 50 μL) enhanced LPO with percent increase of 10%, 119% and 46%, respectively. The significant increase was shown only by 20 and 50 μL (P<0.05 or P<0.01). However, the highest increase in LPO was shown by 20 μL CCl_4. Therefore, this concentration was kept constant for all other experiments.

Experiment 2: Standardization of Concentration of FSE for Inhibition of LPO

Following the three different concentrations of FSE (80, 160 and 240 μg/mL), there was significant percent inhibition only at 80 and 160 μg/mL (P<0.05 or P<0.01; 17% and 44%, respectively). Therefore, the concentration which resulted in maximum percent inhibition (160 μg/mL) was combined with different concentrations of GLB.

Experiment 3: Pilot Experiment on the Role of GLB to Inhibit LPO

The results were shown in Table 3 (Appendix Ⅲ). Addition of FSE (160 μg/mL) with three different concentrations of GLB (0.25, 0.5 and 1 μg/mL) did not show any additive or synergistic effect (Table 3). In fact, the higher concentration (1 μg/mL) significantly increased the value of hepatic LPO (P<0.05). When FSE (160 μg/mL) was added together with three different concentrations of GLB (0.025, 0.05 and 0.10 μg/mL), the percent inhibition was 46%, 61% and 15%, respectively. Only 0.05 μg/mL brought a significant decrease when compared with the value of CCl_4 and FSE treated tubes (P<0.05).

Experiment 4: Confirmatory Experiment on GLB-Induced Inhibition of LPO

Treatment of liver slices with FSE significantly inhibited CCl_4, induced LPO by 45% (P<0.01). As observed in the above experiment, here also simultaneous treatment of FSE (160 μg/mL) and GLB (0.05 μg/mL) significantly inhibited hepatic LPO (P<0.01, 66%). The co-treatment also resulted in a significant inhibition when compared with FSE treated group (P<0.05).

Total Polyphenols and Favonoids

The amount of total polyphenols and flavonoids in FSE was calculated out to be 83.49±3.27 mg garllic acid equivalent/100 g dry weight and 32.39±3.86 mg/100 g

dry weight.

DPPH and ABTS Radical Scavenging Activity

The percent DPPH scavenging activity of FSE was the highest, i.e, 56% at its lowest concentration (25 ppm) as against 90% for the standard antioxidant ascorbic acid at the same concentration. In case of ABTS radical scavenging assay the highest inhibition of free radicals (50%) was shown by 10 mg/mL of FSE.

DISCUSSION

There was an additional decrease in chemically induced hepatic LPO in GLB added samples in which FSE was also added. Of course, the percent inhibition was different depending on the particular LPO inducing agent and its concentration. Following the addition of 5 mmol/L of $FeSo_4$, there was a marked induction in hepatic LPO.[12] However, when FSE was added simultaneously, it could significantly inhibit the tissue LPO. Interestingly, when 160 μg /mL of FSE and different concentrations of GLB were added, there was further inhibition in $FeSo_4$-induced hepatic LPO. However, best inhibition was observed with 0.5 μg/mL of GLB. While other concentrations of GLB proved to be less effective, the highest concentration (4 μg/mL) appeared to be toxic.

Nearly similar observations were made with respect to CCl_4 and H_2O_2 to induce hepatic LPO.[8] However, the amount of CCl_4 and H_2O_2 used was different. When three different concentrations of FSE were added with either CCl_4 or with H_2O_2 in both the cases 160 μg/mL of FSE proved to be quite effective in inhibiting hepatic LPO. On administration of 20 μL CCl_4 and 160 μg/mL of FSE along with GLB, further inhibition was observed with 0.05 μg/mL of GLB as compared to FSE alone, suggesting that the addition of FSE with a milder amount of GLB may prove to be more beneficial in reducing the CCl_4-induced hepatotoxicity. When 80 mmol/L of H_2O_2 was added with FSE, here also the maximum inhibition was observed in 160 μg /mL, but the higher concentration of FSE (240 μg/mL) appeared to be toxic a *in vivo* study.[20] On further addition of different concentrations of GLB along with 160 μg /mL of FSE, GLB at 0.5 μg/mL appeared to be most effective. Thus, FSE was also found to augment the beneficial effects of GLB in inhibiting hepatic LPO. While some literature is already available on the induction of hepatic LPO by $FeSo_4$, CCl_4 and H_2O_2,[8] on the use of plant extract in inhibiting the chemically induced LPO in *in vitro* condition, literature is extremely limited.[12,19] The present findings indicate that FSE in a suitable concentration inhibits chemically induced hepatotoxicity as had been reported previously.[21,22] This antioxidative/free radical scavenging property of was further supported through the findings with DPPH and ABTS systems.[23] However, the most interesting finding is that FSE when treated with, GLB, a further inhibition in hepatic LPO was observed clearly. This may hold true for the regulation of other diseases that are LPO mediated. This may be emphasized that despite the popularity of fenugreek for the treatment of diabetes mellitus,[6] its use along with antidiabetic drug was not explored by any other worker, till to date.

Oxidative damage to DNA, proteins and lipids can ultimately lead to outcomes such as disorganisation, dysfunction and destruction of membranes, enzymes and proteins.[24] The TBA reaction is commonly used to measure free-radical damage to amino acids, carbohydrates and nucleic acids. In all of these systems MDA is predominantly formed from intermediate precursor molecules which break down during the acid-heating stage of the TBA test.[25] A significant increase in LPO is always related to the perturbation in membrane structure and cell function and has been implicated in a variety of pathological processes.[26] Therefore, this is considered as an important tool in scientific research. In our study, FSE not only inhibited the induced hepatic LPO by all three toxicants, but also enhanced the LPO inhibition in GLB added samples, clearly suggesting the possible advantage for the diabetic patients receiving conventional antidiabetic drugs. This beneficial effect of FSE is believed to be due to its different active components.[27,28] In fact, appreciable amount of the polyphenols and flavonoids were measured in FSE, which do suggest that the observed free radical scavenging activity of fenugreek might be the result of its phenolic and flavonoid content as also suggested earlier.[28] The radical scavenging capability of phenolic compounds are due to their hydrogen donating ability, which in turn is closely related both to chemical structure and spatial conformation, that can modify the reactivity of the molecules.[29,30]

GLB is also known to play an important role as a potential antioxidant and exerts its direct effect on liver enzymes.[31] Therefore, a greater antioxidative effect following the addition of both FSE and GLB appear to be combined LPO inhibiting effects of both the drugs. High performance liquid chromatography analyses of FSE (data not shown) revealed the presence of trigonellin, quercetin and rutin as indicated by other workers.[28,32,33] However, trigonellin appears to be the major contributor, because of the fact that its concentration in the test sample was more than the other

two. In conclusion, present report showed the beneficial role of FSE on the enhancement of antiperoxidative activity exerted by GLB. This may hold true also for the *in vivo* study.

Conflict of Interests

The authors have no conflict of interests to declare.

Author Contributions

Ms Neha conducted the experiment and studied the different parameters. Dr. Kar designed the experiment and corrected the manuscript. Dr. Panda helped in conducting the experiment, in the biochemical analyses and in interpretation of data.

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