



Aqueous extract of *Trigonella foenum graecum* ameliorates cypermethrin-induced circulatory oxidative stress in male Wistar rats

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ABSTRACT

Cypermethrin, a Type II pyrethroid pesticide is commonly used in agriculture and many other domestic applications for pest control. Studies have shown that cypermethrin induces lipid peroxidation and alters the antioxidant status in non-target organisms. Fenugreek, a source of several polyphenols and flavonoids is a potent antioxidant. Hence in the present study we have investigated the possible effect of germinated fenugreek extract supplementation in counteracting the cypermethrin induced oxidative stress in erythrocytes of rats. Male Wistar rats were treated with 25 mg/kg weight (1/10 LD₅₀) of cypermethrin and aqueous extract of germinated fenugreek (GFAQ) (10%) for 60 days. Our results show that lipid peroxidation, an index of oxidative stress, significantly increased in circulation in cypermethrin-treated rats. The activities of SOD, CAT, GPx, GST in RBCs also decreased concomitantly in the cypermethrin treated rats. Treatment with aqueous extract of germinated fenugreek brought the values to near normal showing that fenugreek ameliorates cypermethrin-induced oxidative stress. HPLC analysis of the aqueous extract of germinated fenugreek showed the presence of flavonoids.

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INTRODUCTION

Pyrethroid pesticides are used preferably over organochlorine, organophosphates and carbamates due to their greater field stability, rapid metabolism and elimination from mammalian system, limited persistence in soil and greater potency (Elliot, 1977). Pyrethroids are divided into two types according to their chemical structure. Type I pyrethroid do not contain an alpha cyano group and cause mainly tremors (T syndrome). Type II pyrethroid contains an alpha cyano group and causes choreoathetosis

and salivation (CS syndrome) (Vershoye and Aldridge, 1980). Cypermethrin (CM) is a potent Type II pyrethroid that is extensively used in agricultural farming, livestock industry and to control house hold ectoparasites. Studies in rats show that CM is rapidly metabolized and over 99% eliminated within hours, but accumulation of cypermethrin and its fatty acid conjugates in adipose tissue, brain and liver of rats was reported (Marie et al., 1982). Several studies have indicated that pyrethroids induce oxidative stress (Banerjee et al., 1999).

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Antioxidants can ameliorate the oxidative stress induced by pyrethroids (Yousef et al., 2006 ; El- Demerdash, 2007; Prashanthi et al., 2004) ;

Fenugreek (*Trigonella foenum graecum*) is a leguminous plant traditionally used as a medical herb and spice. Its seeds are a rich source of trigonelline, lysine and L-Tryptophan. They contain a large amount of steroidal saponins and fibres. It is a rich source of flavonoids and polyphenols compounds such as quercetin, luteolin, kaempferol, triclin, gallic acid etc. Apart from possessing several pharmacological properties like antihyperglycaemic activity (Madar, 1984), immunomodulatory activity (Bin Hafeez et al., 2003) and hypocholesterolaemic (Stark and Mazar, 1993) activity, fenugreek is also a potent antioxidant (Anuradha and Ravikumar, 1998; Devasena et al., 2002; Kaviarasan et al., 2007; Thirunavukkuarasu et al., 2003). The present study was undertaken to investigate the antioxidant effect of germinated fenugreek seeds on cypermethrin induced oxidative stress in male Wistar rats.

MATERIALS AND METHODS

Chemicals

Cypermethrin [(RS)-3-phenoxybenzyl (RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] of greater than 95% purity was obtained as a gift from IIBAT , Padappai, Chennai. All fine chemicals were obtained from Sigma chemical Co, U.S.A. All other reagents used were of analytical grade and was obtained either from Merck India Ltd or from Hi Media India Ltd.

Germinated Fenugreek seeds powder (aqueous extract)

Fenugreek seeds were purchased from a local grocery shop, cleared of extraneous matter. Aqueous extract of fenugreek (GFAQ) was prepared by the method of Dixit et al. (2005). Fenugreek seeds were soaked in water and germinated for 24 hours. They were kept at 4 °C for two days, dried in shade and powdered. 10% GFAQ was prepared by

weighing 1g of powder in 10ml of distilled water. The solution was stirred on a magnetic stirrer for one hour. It was then centrifuged and supernatant stored at -20 °C until use.

Animals and treatment

Male albino Wistar rats weighing 120-160 g were taken for the present study. They were maintained in polypropylene cages on a 12 h light – 12 h dark cycle. All animals were kept under controlled conditions of temperature (30 ± 2 °C) and humidity. They were fed with pellet food and water *ad libitum*. The procedures employed in the study was accepted by the animal ethical committee (CPCSEA). Animals were randomly divided into four groups containing 6 animals each. They were treated with 1/10 LD₅₀ (LD₅₀ of CM is 250 mg) of CM, 25 mg/kg body weight/per day in corn oil (Cantalamesa et al.,1993) and 10% aqueous extract of germinated fenugreek for 60 days as shown in Table 1.

Blood sampling

A day or two before the sacrifice blood was collected by sinocular puncture into heparinised tubes and plasma was separated by centrifugation at 2000 x g for 10 minutes.

Preparation of haemolysate

After the separation of plasma, the buffy coat was removed and the packed cells (RBCs) were washed thrice with cold physiological saline. To determine the activity of RBC antioxidant enzymes, RBC lysate was prepared by lysing a known volume of RBCs with cold hypotonic phosphate buffer, pH 7.4. The haemolysate was separated by centrifuging at 3000 x g for 10 minutes at 2 °C

Biochemical investigations

Determination of LPO

Measurement of thiobarbituric acid was used to study lipid peroxidation due to its sensitivity and simplicity. Plasma and RBC LPO were measured according to the method of Okhawa (1979).

Table 1: Experimental design.

Group	Treatment	Dose / Duration
Normal control	-	No treatment for 60 Days
CM	Cypermethrin	25 mg/Kg body weight for 60 days by i.g (intragastric) tubing
CM + GFAQ	Cypermethrin+10% GFAQ	(25 mg/Kg body weight of CM + 5 ml of 10% GFAQ)/day for 60 days by i.g tubing
GFAQ	10% GFAQ	5 ml/day for 60 days

Determination of enzymatic and non enzymatic antioxidants

Superoxide dismutase (SOD) (EC 1.15.1.1) was determined by the method of Kakkar (1984). Catalase (CAT) (EC 1.11.1.6) was assayed colorimetrically by the method of Sinha (1972). Glutathione peroxidase (GPx) (EC 1.11.1.9) was measured by the method of Rotruck (1973). Glutathione S-Transferase (GST) (EC 2.5.1.18) activity was measured by the method of Habig (1974).

HPLC analysis of 10 % aqueous extract of fenugreek

Sample preparation

2.5 g of sample was taken in 50 ml of 60% v/v solution of acetone and refluxed under a reflux condenser for 30 min. The filtrate was collected and the drug residue was extracted a second time in the same manner, using 40 ml of the 60% v/v solution of acetone. 50 ml of the solution was evaporated to eliminate the acetone and transferred to a 50 ml vial rinsing with 30 ml of acetone, 4.4 ml of hydrochloric acid was added, diluted to 50 ml with water and centrifuged. 10 ml of the supernatant liquid was placed in a 10 ml brown glass vial, closed with a rubber seal and aluminium cap and heated on a water bath for 25 min. Allowed to cool to room temperature. 10 mg of quercetin, vitexin, orientin, naringenin was dissolved in 20 ml of methanol and 15 ml of dilute hydrochloric acid was added. Finally 5 ml of

water was added and the mixture diluted to 50 ml with methanol. This was taken as the reference solution. A column of 5 µm was made with Octadecylsilyl silica gel. Sample volume injected was 10 µl. Mobile phase A was 0.3 g/l solution of phosphoric acid adjusted to pH 2.0, Mobile phase B was methanol, flow rate was adjusted to 1.0 ml/min and the wavelength used to detect the eluent was 370 nm.

Statistical analysis

Data from biochemical investigations were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if "p" value was 0.05 or less.

RESULTS

Effect on lipid peroxidation in plasma and erythrocytes

Levels of TBARS in circulation and in tissues in different groups of our study are shown in Figure 1. CM treatment enhanced lipid peroxidation in circulation. Significantly higher TBARS (p < 0.05) was noticed in the CM treated rats when compared with the normal control. The LPO significantly reduced (p < 0.05) in the CM and GFAQ treated rats when compared with the CM treated group.

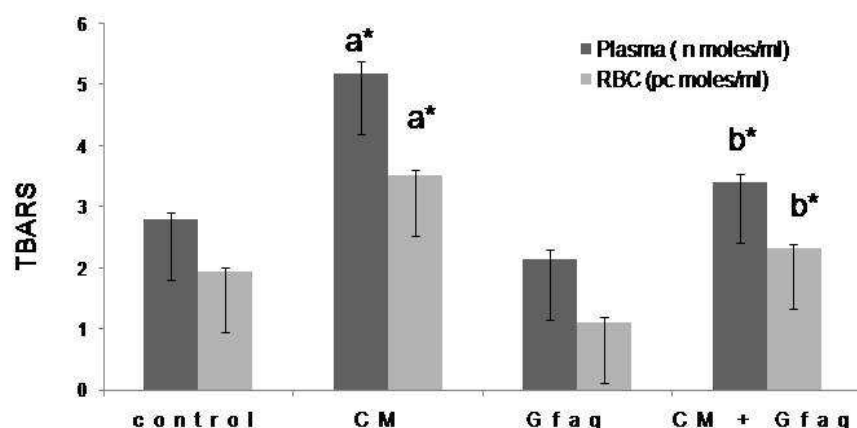


Figure 1. Levels of TBARS in plasma (n moles/ml) and RBC (p moles/ml) in rats treated with CM (25 mg/kg body weight) and 10% GFAQ for 60 days. Values are mean \pm S.D, n = 6. a as compared with Group I (Normal control); b as compared with Group II (CM Treated); (*p < 0.05).

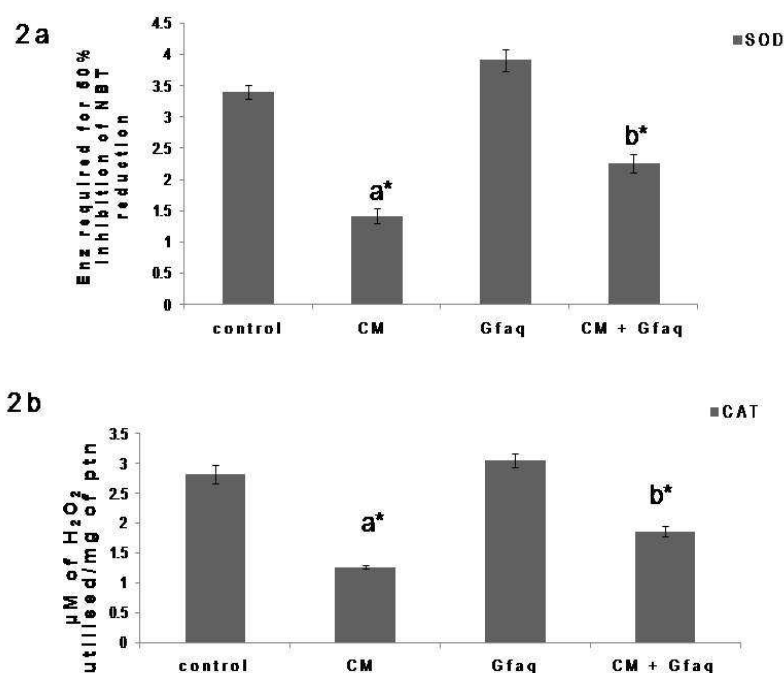


Figure 2. Enzymatic activity of SOD [enzyme required for 50% inhibition of NBT (Nitro blue tetrazolium formazan) reduction], CAT (μ M of H₂O₂ utilised/mg of protein) in RBC in rats treated with CM (25 mg/kg body weight) and 10% GFAQ for 60 days. Values are mean \pm S.D. Values are mean \pm S.D, n = 6. a as compared with Group I (Normal control); b as compared with Group II (CM Treated); (*p < 0.05).

Effect on antioxidant enzymes

The activities of antioxidant enzymes (SOD, CAT, GPx, GST) in control and experimental animals are shown in Figure 2 and Figure 3. The activities of all the enzymatic antioxidants, SOD, CAT, GPx and GST in erythrocytes was significantly lower in the ($p < 0.05$) CM treated rats when compared with the normal control. The activities of these enzymes were found to be significantly higher ($p < 0.05$) in the CM and GFaq treated rats when compared with the CM treated rats.

HPLC analysis of aqueous extract of germinated fenugreek

The HPLC analysis of aqueous extract of germinated fenugreek showed the presence of quercetin (0.1055%), naringenin (0.0776%), vitexin (0.0905%), orientin (0.0105%) and in traces triclin and gallic acid (0.1056%).

DISCUSSION

The results of the present study clearly indicate that CM induced oxidative stress in circulation. Previous workers have shown the generation of ROS such as O_2^\bullet , $^\bullet OH$ and H_2O_2 resulting in oxidative stress with altered antioxidant status in pyrethroid treated rats. (Yousef et al., 2006; Prashanthi et al., 2004). Studies on CM have indicated the generation of ROS, LPO and an increased oxidative stress (Giray et al., 2001; Gabianelli et al., 2002). CM, a Type II pyrethroid pesticide containing an α cyano group (Verschoyle and Aldridge, 1980), can decompose to cyanide and aldehydes, and forms lipophilic conjugates and aldehydes, which may also produce oxidative stress in pyrethroid toxicity (Kale et al., 1999a).

CM treated rats showed an increased plasma and RBC TBARS when compared with the normal control. Lipid hydroperoxides produced as a result of LPO, break down in biological systems, producing a great variety of aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (HNE) which may alter the structure, fluidity and permeability of erythrocytes increasing their sensitivity

towards hypotonic saline. The aldehyde products are also reactive and highly cytotoxic (Comporti, 1985). Increased LPO may cause disintegration of biomembranes and subcellular organelles, decreases membrane fluidity leading to gross disturbance in cellular architecture (Howard, 1972; Kagan, 1988). CM and CM metabolites could be responsible for the increased LPO. The decreased TBARS in the plasma and RBC of CM + GFaq treated rats when compared to that of CM treated group suggests that the fenugreek extract counteracted the oxidative stress induced by CM and reduced the LPO.

The endogenous enzymatic and non enzymatic antioxidants scavenge the ROS and counteract the pesticide-induced oxidative stress. Our study has demonstrated a significant decrease in SOD, CAT, GPx and GST in circulation in CM treated rats when compared with the untreated group. SODs scavenge O_2^\bullet by a rapid dismutation reaction (McCord and Fridovich, 1969) and Catalase dismutates H_2O_2 to water and oxygen (Krisinsky, 1992). GPx catalyses the reduction of organic peroxides (ROOH) and transforms lipid hydroperoxides produced at the membrane level into less reactive species, hence it plays a major role in protecting the plasma membrane from LPO (Gabianelli et al., 2002). A significant reduction in these enzymes in CM treated rats show the depletion of enzymatic antioxidants in scavenging CM induced reactive oxygen species. Treatment with deltamethrin at 150 mg/kg body weight (1/10 of LD_{50}) for 30 days in Wistar rats showed altered hepatic antioxidant status with activities of SOD, CAT and reduced GSH content (Manna et al., 2005). Thus, our results show that in CM treated rats the oxidative status of the system is imbalanced as indicated by enhanced LPO and depleted antioxidant status. In CM + GFaq treated rats the LPO and the antioxidant status were reverted to near normal in circulation. The levels of TBARS decreased significantly and the activities of SOD, catalase, GPx and GST were close to the normal control. Earlier studies have shown that antioxidants play a protective role in CM induced toxicity by reducing the LPO and oxidative stress (Atehassin et al., 2005; Kale et al., 1999b). Pretreatment of rats with Vitamin E or allopurinol provided

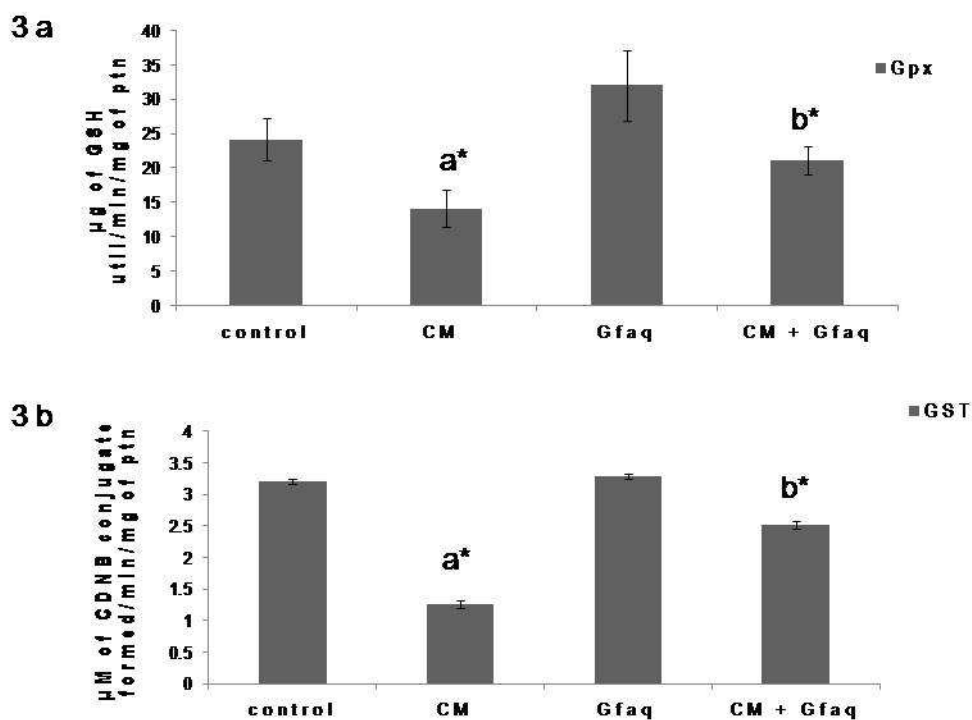
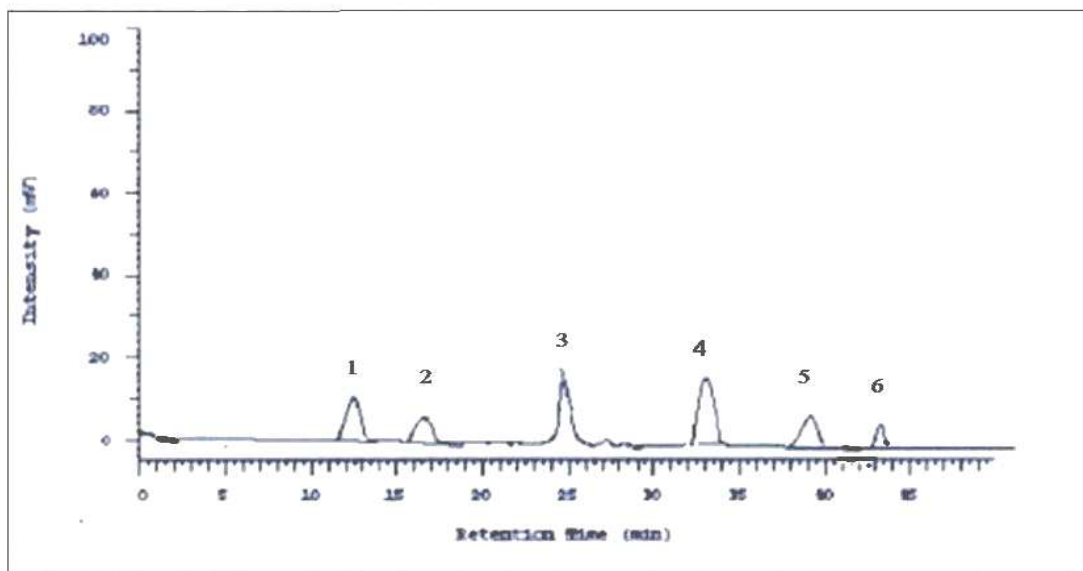


Figure 3. Enzymatic activity of Gpx (μg of GSH utilized/min/mg of protein), GST [μM of CDNB (1-chloro-2,4-dinitrobenzene) conjugate formed/min/mg of protein] in RBC in rats treated with CM (25 mg/kg body weight) and 10% GFAQ for 60 days. Values are mean \pm S.D, n = 6. a as compared with Group I (Normal control); b as compared with Group II (CM Treated); (*p < 0.05).



significant protection against the elevation of TBARS levels in cerebral and hepatic tissues, induced by cypermethrin (Giray et al., 2001). Pretreatment with Vitamin E reduced the LPO in cypermethrin and fenvalerate treated rats (Kale et al., 1999b). Studies show that fenugreek seeds have antioxidant properties. A study of aqueous extract of fenugreek on experimental ethanol toxicity showed that the seeds exhibited appreciable antioxidant property in vitro compared to that of Glutathione and α Tocopherol (Thirunavukkarasu et al., 2003). Treatment with fenugreek seed powder normalized the enhanced lipid peroxidation and increased susceptibility to oxidative stress due to the depletion of antioxidants in diabetic rats, while normal rats showed increased antioxidant status with decreased lipid peroxidation (Madar, 1984).

Gupta and Nair (1999) have shown that the fenugreek seeds are rich in flavonoids (>100 mg/100g). Shang et al. (1998) have isolated five different flavonoids from fenugreek, namely vitexin, tricetin, naringenin, quercetin and tricetin-7-O- β -D-glucopyranoside. The HPLC analysis of the 10% aqueous extract of fenugreek used in our study showed the presence of flavonoids quercetin, vitexin, naringenin, tricetin, rutin, orientin and gallic acid. Plant phenolics are known to be strong antioxidants and exhibit radical scavenging property. In particular phytochemicals naringenin and quercetin in fenugreek could be potent $^{\circ}\text{OH}$ radical scavengers due to the presence of active phenolic groups (Kaviarasan et al., 2007). An aqueous extract of fenugreek seeds inhibited lipid peroxidation in liver samples incubated with Fe^{2+} ascorbate system and by glucose *in vitro* (Anuradha and Ravikumar, 1998). Elicited sprouting improved the phenolic and antioxidant property of fenugreek when compared to the dry seeds (Randhir et al., 2004). GFaq the aqueous extract of fenugreek was reported to be rich in phenolics and flavonoids and showed highest radical scavenging activity when compared with other fractions. (Dixit et al., 2005). This clearly

suggests that GFaq has potent antiradical and antioxidant properties, attributed to the active phenolics and flavonoids present in aqueous fraction of germinated fenugreek, which is responsible for amelioration of CM induced toxicity and oxidative stress in circulation.

Conclusion

We conclude that cypermethrin mediates circulatory toxicity by enhancing the oxidative stress. Germinated aqueous fenugreek extract ameliorates cypermethrin induced toxicity by counteracting LPO and restoring antioxidant status. This may be due to the presence of various flavonoids which could function as antioxidant and antitoxic components. Fenugreek being cheap and easily affordable, on regular consumption can impart significant protection from pesticide induced toxicity. The emphasis of the future studies will be characterization and identification of the active constituents of the aqueous extract of fenugreek for drug formulation.

ABBREVIATIONS

GFaq- Aqueous extract of germinated fenugreek seeds, LPO-lipid peroxidation, SOD- Superoxide dismutase, Catalase- CAT, GPx- Glutathione peroxidase, GST- Glutathione S-Transferase, TBARS- Thiobarbituric acid reactive substances, CM- Cypermethrin, ROS- Reactive oxygen species.

REFERENCES

- Anuradha CV, Ravikumar P. 1998. Anti lipid peroxidative activity of seeds of fenugreek (*Trigonella foenum graecum*). *Med. Sci. Res.*, **26**: 317-321.
- Atessahin A, Yilaraz S, Karahan I, Tasdimir B, Turk J. 2005. The effects of Vitamin E and selenium on cypermethrin induced oxidative stress in rats. *Vet. Anim. Sci.*, **29**: 285- 391.
- Banerjee BD, Seth V, Bhattacharya, A, Pasha ST, Chakraborty AK. 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. *Toxicol. Lett.*, **107**: 33-47.

- Bin Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I, Raisuddin S. 2003. Immunomodulatory effects of fenugreek in mice. *International Immunopharmacology*, **3**(2) : 257-265.
- Cantalamesa F. 1993. Acute toxicity of two pyrethroids, permethrin and cypermethrin in neonatal and adult rats. *Arch. Toxicol.*, **67**: 510-513.
- Comporti M. 1985. Biology of disease: lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.*, **53**: 599-623.
- Devasena T, Venugopal PM, Rajasekharan KN. 2002. Enhancement of circulatory antioxidants by fenugreek during 1,2-DMH-induced colon carcinogenesis. *J. Biochem Molbiol. Biophys.*, **6**(4): 289-292.
- Dixit P, Ghaskadbi S, Mohan H, Devasagayam T P. 2005. Antioxidant properties of germinated fenugreek seeds. *Phytother. Res.*, **19**(11): 977-983.
- Elliott M. 1976. Properties and applications of Pyrethroids. *Environmental Health Perspectives.*, **14**: 3-13.
- El-Demerdash FM. 2007. Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants. *Toxicol. In Vitro.*, **21**: 392-397.
- Gabbianelli R, Falcioni G, Nasuti C, Cantalamessa F. 2002. Cypermethrin induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. *Toxicology.*, **175**: 91-101.
- Giray B., Gurbay A., Hincal F., 2001. Cypermethrin induced oxidative stress in rat brain and liver is prevented by vitamin E or Allopurinol. *Toxicol. Lett.*, **118**: 139-146.
- Gupta R, Nair S. 1999. Antioxidant flavonoids in common Indian diet. *South. Asian. J. Prev. cardio.*, **13**: 83-94.
- Habig WH, Pabst MJ, Jacoby WB. 1974. Glutathione S- Transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**: 130-139.
- Howard JA. 1972. *Advances in Free Radical Chemistry*. Academic Press: New York.
- Kagan VE. 1988. Molecular mechanisms of biomembrane damage caused by LPO. In *Lipid Peroxidation in Biomembranes*, Kagan VE (ed). CRC Press: Boca Raton FL ; 55-117.
- Kakkar P, Das B, Vishvanathan PN. 1984. A modified spectrophotometric assay of SOD *Indian. J. Biochem. Biophys.*, **21**: 130-132.
- Kale M, Rathore N, John, S, Bhatnagar D, Nayyar SS, Kothari V. 1999a. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: A possible involvement of reactive oxygen species. *Toxicol. Lett.*, **105**: 197-205.
- Kale M, Rathore N, John S, Bhatnagar D, Nayyar SS, Kothari V. 1999b. The protective effect of Vitamin E in Pyrethroid induced oxidative stress in rat tissues. *J. Nutr. Environ. Med.*, **9**: 281-287.
- Kaviarasan S, Naik GH, Ganga Bhagirathi R, Anuradha CV, Priyadarshini KI. 2007. In vitro studies on antiradical and antioxidant activities of fenugreek seeds. *Food. Chem.*, **103**: 31-37.
- Krinsky NI. 1992. Mechanism of action of biological antioxidants. *Proc. Soc. Exp. Med.*, **200**: 248- 254.
- Madar Z. 1984. Fenugreek (*Trigonella foenum graecum*) as a means of reducing postprandial glucose levels in diabetic rats. *Nutr. Rep. Int.*, **29**: 1267-1273.
- Manna S, Bhattacharya D, Mandal TK, Das S. 2005. Repeated dose toxicity of Deltamethrin in rats. *Indian. J. Pharmacol.*, **37** (3):160-164.
- Marei AEM, Ruzo LO, Casida JE. 1982. Analysis and persistence of permethrin, cypermethrin, deltamethrin and fenvalerate in the fat and brain of treated rats. *J.Agric.Foodchem.*, **30**: 558-562.
- Mc Cord JM, Fridovich I. 1969. Superoxide dismutase. An enzymatic function for erythrocyte peroxidase. *J. Biol. Chem.*, **244**: 6049-6055.

- Okhawa H, Oshini N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**: 351-358.
- Prasanthi K, Muralidhara , Rajini PS. 2005. Morphological and biochemical perturbations in rat erythrocytes following in vitro exposure to Fenvalerate and its metabolite. *Toxicol. In Vitro.*, **19**: 449-456.
- Randhir R, Yuan tog Lin, Shetty K. 2004. Phenolics their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors, *Asia. Pacific. J. Clin. Nutr.*, **13**(3): 295-397.
- Rotruck JT, Pope AL, Gauther HE, Swanson AB. 1973. Selenium : biochemical roles as a component of glutathione peroxidase. *Science.*, **179**: 588-590.
- Shang M, Cais HJ, Li J, Zhao Y, Zheng J, Namba T, Kadota S, Tezuka Y, Fan W. 1998. Studies on flavonoids from fenugreek (*Trigonella foenum graecum*). *Zhongguo Zhong Yao Za Zhi.*, **23**(10): 614-639.
- Sinha AK. 1972. Colorimetric assay of Catalase. *Anal. Biochem.*, **47**: 389-394.
- Stark A, Mazar Z. 1993. The effect of an ethanol extract derived from fenugreek on bile acid absorption and cholesterol levels in rats. *Br. J. Nutr.*, **69**(1): 277-287.
- Thinavukkarasu V, Anuradha CV, Viswanathan P. 2003. Protective effect of fenugreek (*Trigonella foenum graecum*) seeds in experimental ethanol toxicity. *Phytother. Res.*, **17**(7): 737-743.
- Verschoye RD, Aldridge WN. 1980. Structure activity relationships of some pyrethroids in rats. *Arch. Toxicol.*, **45**: 325-329.
- Yousef MI, Awad TI , Mohameda EH. 2006. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology.*, **227**: 240-247.