Journal of Complementary and Integrative Medicine

Volume 8, *Issue* 1 2011 *Article* 4

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Sushma Navayath, *Vels University*
Devasena Thiyagarajan, *Anna University*

Recommended Citation:
Navayath, Sushma and Thiyagarajan, Devasena (2011) "Fenugreek Supplementation Imparts Erythrocyte Resistance to Cypermethrin Induced Oxidative Changes *In Vivo*," *Journal of Complementary and Integrative Medicine*: Vol. 8: Iss. 1, Article 4.
DOI: 10.2202/1553-3840.1436

Fenugreek Supplementation Imparts Erythrocyte Resistance to Cypermethrin Induced Oxidative Changes *In Vivo*

Sushma Navayath and Devasena Thiyagarajan

Abstract

Erythrocytes are excellent model to study the xenobiotic induced oxidative changes. Pyrethroid pesticides are increasingly being used in insecticidal preparations from the simple mosquito coils to house hold aerosols to sophisticated ultra low volume foggers and sprays. Cypermethrin a Type II pyrethroid pesticide is used widely in pest control. Fenugreek is a potent antioxidant. We have evaluated the potential of aqueous extract of germinated fenugreek seeds in counteracting cypermethrin induced oxidative changes in erythrocytes of male Wistar rats. Male Wistar rats were treated with 1/10 LD50 (25mg/kg body weight) of cypermethrin and 10 percent aqueous extract of germinated fenugreek for 60 days. Cypermethrin treatment caused significant decrease in non enzymatic antioxidants, glutathione (GSH), vitamin E, vitamin C, increased methemoglobin formation in erythrocytes and increased their mechanical fragility. Treatment with fenugreek reversed the cypermethrin induced oxidative changes in erythrocytes and restored all the parameters to near normal levels. The overall results reveal the ameliorating effect of aqueous extract of germinated fenugreek on cypermethrin induced toxicity in erythrocytes.

KEYWORDS: antioxidants, cypermethrin, fenugreek, methemoglobin, osmotic fragility, ROS

Introduction

Oxygen radicals and reactive oxygen species (ROS) are produced normally by most cells (Fridovich, 1975; Kelvin et al., 1975; Pryor, 1976). ROS are also induced by many xenobiotics and also under many pathological conditions (Mc Cay et al., 1974). ROS can severely damage the macromolecules, lipids, proteins, DNA and produces lipid peroxidation (LPO) and oxidative stress (Kelvin et al., 1975; Stadtman and Berlett, 1997; Imlay and Linn, 1988). Even though membrane lipids are the major targets for cellular damage by ROS, they also enhance the degradation of specific proteins in erythrocytes and reticulocytes (Goldberg, 2003). The ROS may directly alter the protein structure or the coupled LPO may generate species that attacks the cellular proteins (Kelvin et al., 1975). ROS induced modification of the membrane impairs the stability and increases fragility of erythrocytes (Stern, 1986; Wagner, 1988).

Pyrethroid pesticides are used preferably over organochlorine, organophosphates and carbamates due to their greater stability in field, rapid metabolism and elimination from mammalian system, limited persistence in soil and greater potency (Elliot, 1976). ROS have been implicated in the molecular mechanism of toxicity of pesticides and as a result they alter the biochemical and physiological functions of erythrocytes (Verma, 2001; Akhgari et al., 2003; Mansoor and Moosa, 2007). Studies with different types of pyrethroids have shown that they induce LPO and oxidative stress (Kale et al., 1999a). Cypermethrin forms cyanohydrins which decompose to cyanides and aldehydes and other lipophilic conjugates that may mediate oxidative stress (Kale et al., 1999a). A number of studies have reported the structural alterations in erythrocytes and the associated biochemical perturbations as a result of exposure to pyrethroid pesticides, cypermethrin, fenvalerate, permethrin and λ-cyhalothrin (Kale et al., 1999a; Giray et al., 2001; Prasanthi et al., 2005; El-Demerdash, 2007)

Aerobic organisms are endowed with enzymatic and non enzymatic antioxidants to combat the prooxidant challenges (Halliwell and Gutteridge, 1999). The most important non enzymatic antioxidants are vitamin E, vitamin C and reduced glutathione, GSH. The preventive effect of antioxidants like vitamin E, zinc, ascorbate and selenium in ameliorating pyrethroid induced toxicity has been reported earlier (Kale et al.,1999b; Atehassin et al., 2005). Although there are a number of reports on the ameliorative effect of antioxidant vitamins and micronutrients in pesticide induced toxicity, there are very few reports on the protective effect of medicinal herbs in pesticide induced toxicity.

Fenugreek (*Trigonella foenum graecum*) is a leguminous plant, traditionally used as a medicinal herb and spice. Fenugreek seeds are a rich source of flavonoids and polyphenols. One of the prominent properties of many flavonoids is their ability to scavenge free radicals. Several studies show the

antioxidant potential of fenugreek (Anuradha and Ravikumar, 1998; Thirunavukkarasu et al., 2003; Kaviarasan et al., 2003). Even though many studies have reported the antioxidant potential of fenugreek in pathological conditions such as diabetes mellitus (Siddiqui et al., 2007), ethanol toxicity (Thirunavakkarasu et al., 2003), and colon carcinogenesis (Devasena and Venugopal, 2007, Devasena 2009), there are no reports on the antioxidant potential of fenugreek in pesticide induced toxicity. Hence we have evaluated the protective effect of aqueous extract of germinated fenugreek seeds in cypermethin induced oxidative changes in erythrocytes of male Wistar rats.

Materials and Methods

Chemicals

Technical grade cypermethrin [(RS)-3-phenoxybenzyl(IRS)-cis-trans-3-(2,2-Dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] of greater than 95% purity was obtained as a gift from (International Institute of Biotechnology and Toxicology) 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 Hi Media India Ltd.

Germinated Fenugreek seeds extract (GFaq)

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. GFaq was prepared by weighing 1g of powder in 10 ml of distilled water. The solution was stirred on a magnetic stirrer for 1 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 12h light - 12h 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 were accepted by the animal ethical committee (CPCSEA). Animals were randomly divided into four groups containing 6 animals each and were treated as follows for 60 days by intargastric tubing.

LD_{50} of cypermethrin - The LD_{50} value is 250 mg/kg b.w. in corn oil in rats as reported by Cantalamessa (1993).

Group I – Normal control, Group II- Was treated with 1/10 LD_{50} of cypermethrin (25mg/kg body weight in corn oil), Group III – Was treated with 25mg/kg body weight of cypermethrin and 5 ml of 10% GFaq, Group IV – Was treated with 5ml of 10% GFaq

Blood sampling

Preparation of hemolysate

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

Determination of non-enzymatic antioxidants

The concentration of reduced glutathione (GSH) in erythrocytes and plasma was measured by the method of Ellman (1959) based on the development of yellow colour when 5,5'dithio (bis) nitrobenzoic acid (DTNB) was added to the protein free supernatant of the plasma and /or erythrocytes. Plasma and erythrocyte ascorbic acid concentrations were determined by the method of Roe and Keuther (1943). Vitamin E was determined by the Baker and Frank method (1980).

Erythrocyte osmotic fragility

Osmotic fragility of erythrocytes was determined by the method of Dacie (1960). Small aliquots of samples were mixed with a large excess of buffered saline solutions of varying concentrations (1 - 9 g/ l) in separate tubes. Simultaneously, an aliquot of sample was incubated at room temperature for 30 min and centrifuged at 1200 x g for 10 min. The supernatant from each tube was read colorimetrically at 540 nm using the supernatant of the tube with erythrocytes and 9g/l saline as blank. A value of 100% lysis was assigned to the supernatant of the tube with erythrocytes and distilled water. The percentage hemolysis at each saline concentration was calculated using the formula

$$\% \text{ hemolysis} = \frac{\text{O.D of the tube with RBCs + saline}}{\text{O.D of the tube with RBCs + distilled water}} \times 100$$

Determination of Methemoglobin

Methemoglobin or Met Hb was determined by the method of Evelyn and Malloy (1938). When sodium cyanide is added to a solution of Met Hb, it is converted to cyanmethemoglobin (MHbCN). The resulting change in optical density is directly proportional to the concentration of Met Hb which is measured at 635 nm.

Statistical Analysis

Data from biochemical investigations were analyzed 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

The level of reduced glutathione (GSH), in control and experimental animals are shown in Fig 1

Cypermethrin (CM) treatment showed a significant decrease ($p < 0.05$) in the levels of reduced glutathione, vitamin E and ascorbic acid when compared with the normal controls and their levels were significantly higher ($p < 0.05$) in CM and GFaq treated rats when compared with the CM treated rats.

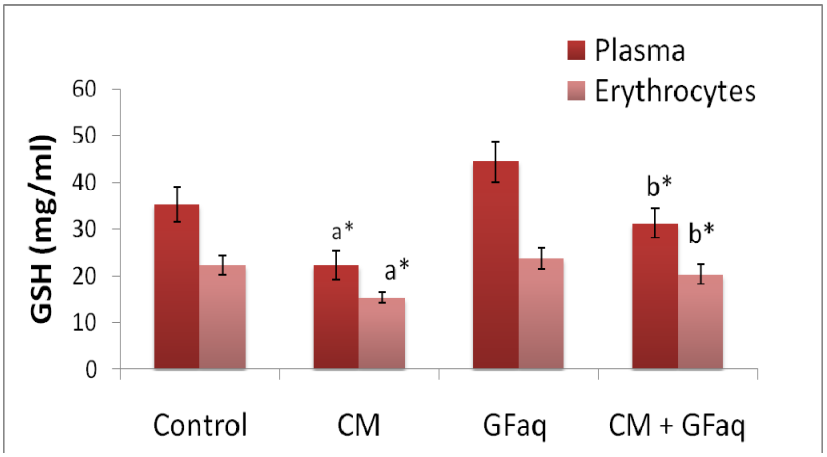

Fig 1 Level of GSH (mg/ml) in plasma and erythrocytes in rats treated with CM (25mg/kg body weight) and 10% GFaq for 60 days. Values are mean ± S.D, n = 6. 'a' as compared with Group I (Normal control); 'b' as compared with Group II (CM Treated); (*$p < 0.05$).

The levels of antioxidants Vitamin E and Vitamin C in control and experimental animals are shown in Fig 2

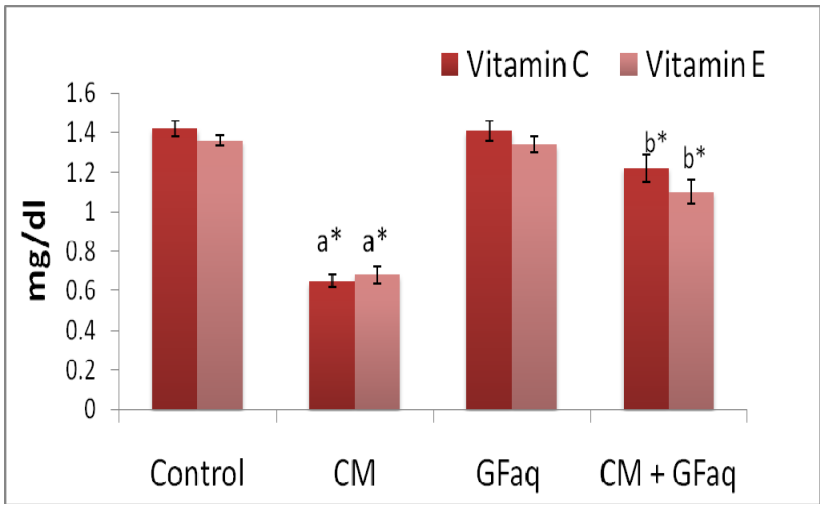

Fig 2 Level of Vitamin E and Vitamin C (mg/dl) in erythrocytes in rats treated with CM (25mg/kg body weight) and 10% GFaq for 60 days. Values are mean ± S.D, n = 6. 'a' as compared with Group I (Normal control); 'b' as compared with Group II (CM Treated); (*$p < 0.05$).

Osmotic Fragility curves of different groups of our study are shown in Fig 3

Osmotic fragility curve of CM treated group shows a shift to the right when compared with the normal group. 50% hemolysis of the erythrocytes of rats of CM group took place at a concentration of 6g/l whereas the normal erythrocytes could resist the hypotonicity of 3g/l. The 50% hemolysis of the erythrocytes of CM + GFaq and the GFaq group was same as that of the normal group.

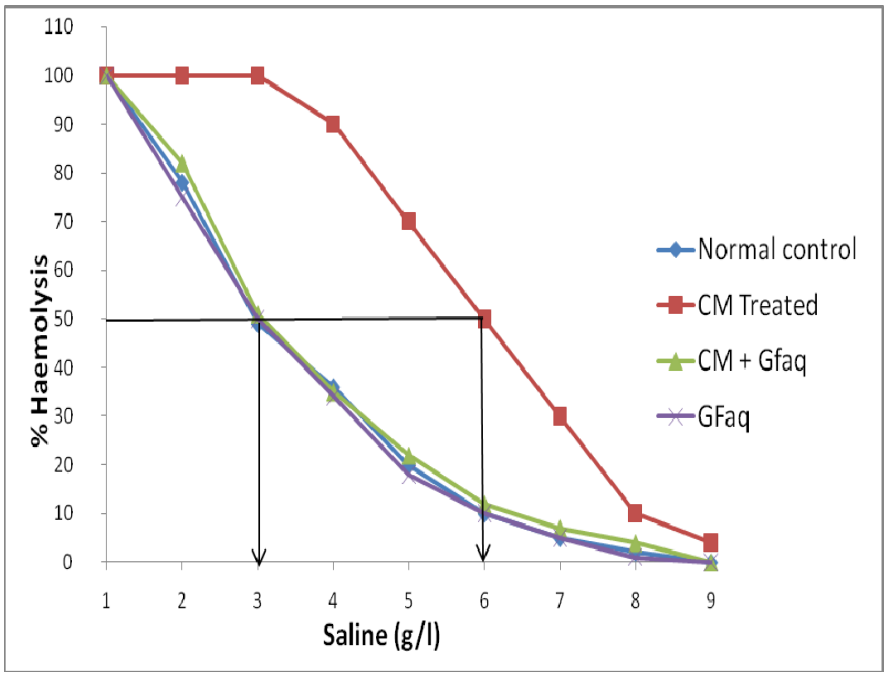

Fig 3 Osmotic fragility curves of rats treated with CM (25mg/kg body weight), CM and GFaq(10%), GFaq and normal rats. n = 6. Mean of % hemolysis was determined and plotted against the corresponding saline concentration. 50% hemolysis of normal control, CM + GFaq and GFaq treated rats occurred at 3g/l while that of CM treated rats occurred at 6g/l.

Methemoglobin levels in erythrocytes in different groups of our study are shown in Fig 4.

The Met Hb levels of CM group was significantly high ($p < 0.05$) when compared with the normal group. The methemoglobin levels of CM + GFaq group was significantly low ($p < 0.05$) when compared with the CM group.

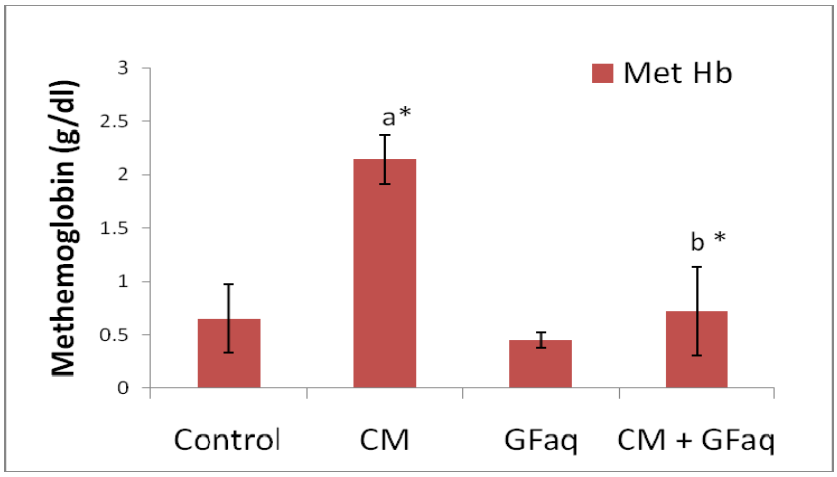

Fig 4 Level of methemoglobin (g/dl) in plasma and erythrocytes in rats treated with CM (25mg/kg body weight) and 10% GFaq for 60 days. Values are mean ± S.D, n = 6. 'a' as compared with Group I (Normal control); 'b' as compared with Group II (CM Treated); (*$p < 0.05$).

Discussion

The results of our study show that treatment with 25mg/kg body weight of cypermethrin (1/10 of $LD_{50,}$ $LD_{50,}$ of cypermethrin is 250 mg) caused perturbations in oxidative stress biomarkers in the erythrocytes as shown by significant reduction in the levels of non enzymatic antioxidants GSH, vitamin E and vitamin C. The erythrocytes showed increased mechanical fragility and elevated levels of methemoglobin on cypermethrin treatment. The above oxidative changes in erythrocytes were effectively counteracted by GFaq bringing all the parameters to near normal levels.

ROS and oxidative stress have been implicated in the pathogenesis of pesticide toxicity. Aerobic cells are equipped with a wide array of antioxidant defenses that scavenge the ROS and prevent LPO. Reduced levels of GSH in CM treated rats validate the oxidative insult caused by CM treatment. GSH potentially scavenges large number of organic peroxides. Lower glutathione (GSH) levels may reflect increased rates of oxidation of GSH, by reduction of H_2O_2 or other oxidants. Giray et al., (2001) have reported a reduction in total GSH levels as a result of repeated dosing of CM at 75mg/kg body weight for 5 days. CM induced ROS could damage the membrane lipids producing lipid peroxy radicals. Vitamin E is an important lipid soluble antioxidant present in cells. It is a major chain terminating antioxidant present in biological membranes (Burton et al., 1983) and scavenges the lipid peroxy radical (LOO·) as they are excellent hydrogen ion

donors. Ascorbic acid is a water soluble antioxidant capable of rapidly scavenging a number of ROS. A significant decrease in vitamin E and ascorbate in CM treated rats when compared with normal control indicates their depletion in neutralizing the CM induced oxidative stress and the resulting changes in membrane fragility.

Our results show that in CM treated rats the oxidative status of the system is imbalanced as indicated by depleted antioxidant status. In CM + GFaq treated rats the levels of GSH, vitamin E and vitamin C were reverted to near normal.

Peroxidative processes taking place in erythrocytes, leads to changes in the permeability, flexibility, fragility and antigenicity of erythrocytes (Stern, 1986; Pfafferott et al., 1982). The osmotic fragility of red cells is often performed to evaluate the sensitivity of erythrocytes towards hypotonic saline. This test reflects the ability of erythrocytes to take up a certain amount of water before lysing (Debouzhy, 1992). Therefore, the measurement of osmotic fragility is a useful indicator of the normalcy of erythrocytes (Devasena et al., 2001). Our study shows that the erythrocytes of rats of CM group have increased osmotic fragility as they could not resist hypotonicity beyond 6g/l while the erythrocytes of CM + GFaq treated rats could resist hypotonic saline of 3g/l. CM induced ROS could be responsible for the increased mechanical fragility of erythrocytes, while in the CM + GFaq treated rats the cells had normal mechanical fragility.

Polyunsaturated fatty acids (PUFA) of membrane, an oxygen rich environment and abundant iron rich haemoglobin (Hb), increases the susceptibility of erythrocytes to peroxidative damage (Fridovich, 1975). The predominant protein in erythrocytes is Hb. Hb is damaged either directly by ROS or induced by lipid radicals or lipid hydroperoxides generated during LPO (Hochstein and Jain, 1981). The degeneration products of Hb, precipitate within the red cells, forming Heinz bodies. Heinz body formation is normally coupled with the formation of methemoglobin or Met Hb (Harley and Mauer, 1960). Hemoglobin may be a useful biomarker to monitor oxidative stress in the biological system (Sadighara, 2009). Our studies show that the concentration of methemoglobin increased significantly on treatment with CM for 60 days when compared to the normal group. This indicates that CM induces Hb oxidation resulting in increased methemoglobin. This supports the findings that methemoglobin production is an essential step in the conversion by drugs of oxyhaemoglobin to late degradation products. This is due to the proteolytic systems in red cells, that play a major role in degrading proteins that were synthesized correctly but have undergone free radical induced oxidative damage. The intracellular accumulation of damaged, non functional proteins (Heinz bodies) represent a threat to normal cell function (Beutler, 1969). Thus it is possible that CM induced increased ROS mediate the protein degradation resulting in oxidation and damage of Hb. Our results show that simultaneous

treatment with CM and GFaq significantly reduced the Met Hb formation when compared with the CM treated group.

Antioxidants scavenge the ROS protecting the cells from the deleterious effects of free radicals. Vitamin E was reported to alleviate the cyfluthrin induced oxidative stress in erythrocytes (Eraslan et al., 2007). Supplementation with vitamin E and vitamin C alleviated λ-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes (El- Demerdash, 2007). Plants are important source of polyphenols that are utilized as important components of both human and animal diets. Polyphenolics were reported to enhance the resistance of erythrocytes to oxidative stress in vitro and in vivo maintaining the membrane integrity (Youdim et al, 2000). Fenugreek is a potent source of antioxidants due to the presence of volatile oil, phenolic acids and flavonoids (Shimon et al, 1995).

Polyphenolic flavonoids of fenugreek were reported to protect various cell types against oxidative stress mediated cell injury under different pathological conditions. Polyphenolic extract of fenugreek seeds are rich in flavonoids and was reported to be effective in reducing oxidative damage in both normal and diabetic erythrocytes in vitro, in a dose dependent manner (Kaviarasan et al., 2003). An aqueous extract of the fenugreek seeds inhibited lipid peroxidation in liver samples incubated with Fe^{2+}-ascorbate system and by glucose (Anuradha and Ravikumar, 1998). Aqueous extract of fenugreek exhibited appreciable Antioxidant property in experimental ethanol toxicity comparable to that of glutathione and α-Tocopherol (Thirunavukkarasu et al., 2003). Fenugreek seeds are a rich source of flavonoids, (> 100mg/100g) (Gupta and Nair, 1999). The phenolic content and related antioxidant properties improved considerably in the fenugreek by elicited sprouting (Randhir et al., 2005). Flavonoids commonly found in herbs inhibit the oxygen radical formation through enhanced oxidation of Fe^{2+} ion as the pro oxidant (Palasuwan et al., 2005). GFaq used in our study was reported to be rich in flavonoids quercetin, vitexin, naringenin, tricin, orientin and gallic acid (Sushma and Devasena, 2010). The active components of GFaq scavenge the free radicals and ROS produced by cypermethrin and metabolites protecting the erythrocytes and its membrane from the oxidants. This is consistent with the antioxidant and membrane stabilising effects of fenugreek observed in our study. Hence, the amelioration of oxidative changes in the erythrocytes could be attributed to the flavonoids and polyphenols present in the aqueous extract of germinated fenugreek seeds that scavenge free radicals and protect the erythrocytes.

Conclusion

CM induced disequilibrium in pro oxidant and antioxidant status in favour of the former is responsible for the oxidative changes in the erythrocytes. Restoration of

the normal membrane fragility and the levels of antioxidants, GSH, vitamin E, vitamin C, reduction in methemoglobin levels in the erythrocytes of rats treated with CM + GFaq shows that flavonoids and polyphenols present in GFaq could scavenge the ROS, and hence prevent the damage and degradation of haemoglobin and other proteins in erythrocytes. This is also responsible for reducing the CM induced oxidative stress and membrane damage.

References

Abdollahi M, Ranjbar A, Shadnia S, Nikfar, S, Rezaic A 2004. Pesticide and oxidative stress: a review. *Med Sci Monit* 10(6): 141-147.

Akhgari M, Abdollahi M, Kebryaeezadeh A, Hosseini R, Sabzevari O 2003. Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum Exp toxicol* 22: 205.

Anuradha CV, Ravikumar P 1998. Anti lipid peroxidative activity 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.

Baker H, Frank O, De Angelis B, Feingold S 1980. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Res Int* 21: 531-536.

Beutler E 1969. Drug induced hemolytic anaemia. *Pharmacol Rev* 21: 73- 103.

Burton GW, Cheeseman KB, Doba T, Ingold KU 1983. *Ciba Found Symp* 101: 4-18.

Cantalamessa F 1993. Acute toxicity of two pyrethroids, permethrin and cypermethrin in neonatal and adult rats. *Arch Toxicol* 67: 510-513.

Dacie JV 1960. Hemolytic anaemia:Part I. The congenital anaemias 2nd edn. New York: Grune and Stratton 35-42.

Debouzhy JC, Fauvelle F, Vezin H, Brasme B, Chancerelle Y 1992. Interaction of Malondialdehyde molecule with membrane. A differential learning colorimetry NMR and ESR study. *Biochem pharmacol* 44(9): 1787-1793.

Devasena T, Lalitha S, Padma K 2001. Lipid peroxidation, osmotic fragility and antioxidant status in children with acute post streptococcal glomerulonephritis. *Clin Chim Act* 308: 155-161.

Devasena T, Venugopal MP 2007. Fenugreek seeds modulate 1,2-dimethylhydrazine induced hepatic oxidative stress during colon carcinogenesis. *Ital J Biochem* 56(1): 28-34.

Devasena T. Antitumoreigenic action of fenugreek seeds. *Asian journal of Bioscience* 2009; 4(1): 83-87.

Dixit P, Ghaskadbi S, Mohan H, Devasagayam TP 2005. Antioxidant properties of germinated feugreek seeds. *Phytother Res* 19(11): 977-983.

El- Demerdash FM 2007. Lamda cyhalothrin induced change in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants. *Toxicol In Vitro* 21: 392-397.

Elliott M 1976. Properties and applications of Pyrethroids. *Environ Health Perspect* 14: 3-13.

Ellman GL.1959. Tissue sulphydryl groups. *Arch Biochem Biophys* 82: 70-7.

Eraslan G, Saygi S , Essiz D, Aksoy A, Gul H, Macit E 2007. Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocyanidin and *N*-acetylcysteine against exposure to cyfluthrin in mice. *Pest Biochem Physiol* 88: 43–49.

Evelyn KA and Malloy HT 1938. Microdetermination of Oxyhemoglobin, Methemoglobin and Sulfhemoglobin in a single sample of blood. *J Biol* 655-662.

Fridovich I 1975. Superoxide dismutases. *Annu Rev Biochem* 44: 147-159.

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.

Gold berg AL 2003. Protein degradation and protection against misfolded or damaged proteins. *Nature* 426: 895-899.

Gupta R and Nair S 1999. Antioxidant flavonoids in common Indian diet. *South Asian J Precardiol* 13: 83-94.

Halliwell B, Gutteridge J 1999. JMC (Eds.), Free Radicals in Biology and Medicine, Oxford University Press, New York, 105–245.

Harley JD, Mauer AM 1960. Studies on the formation of Heinz bodies-The nature and significance of Heinz Bodies I Methemoglobin production and oxyhaemoglobin destruction. *Blood* 16: 1722-1735.

Hochstein P, Jain SK 1981. Association of lipid per oxidation and polymerization of membrane proteins with eryth rocyte aging.*Fed. Proc* 40: 183-188.

Imlay JA, Linn S 1988. DNA damage and oxygen radical toxicity. 240: 1302-1309.

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.

Kale M, Rathore N, John S., Bhatnagar, D., Nayyar, S.S., 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.

Kaviarasan S, Vijayalakshmi K, Anuradha CV 2004. Polyphenol-Rich extract of Fenugreek seeds protect erythrocytes from oxidative damage. *Plant Foods Hum Nutr* 59: 143–147.

Kelvin JA, Davies, Goldberg AL 1987. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. *J Biol Chem* 282 (17): 8220-8226.

Mansour SA , Moussa ATH 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pest Biochem Physiol* 93: 34–39.

Mc Cay PB, Lai EK, Poyer JL, Dubose CM, Janzen EG 1984. Oxygen and carbon centered radical formation during carbon tetra chloride metabolism. Observations of lipid radicals *in vitro* and *in vivo*. *J Biol Chem* 259: 2135-2143.

Nasuti C, Cantalamessa F, Falcioni G, Gabianelli R 2003. Different effects of Type I and Type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats. *Toxicology* 191(2-3): 233-244.

Palasuwan A, Swgarun S, Lertlum T, Pradhiwat P, Wiwanitkit V 2005. Inhibition of Heinz body formation in an in vitro model and total antioxidant activity of medical Thai plants. *Asian Pacific Journal cancer Prev* 6: 458-463.

Pfafferott C, Meiselman HJ, Hochstein P 1982. The effect of malonyldialdehyde on erythrocyte deformability. *Blood* 59: 12-15.

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.

Pryor WA 1976. In Free radical Biology (Pryor W A., ed). Academic Press New York 1-49.

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.

Roe JM, Keuther CA 1943. Detection of ascorbic acid in whole blood and urine through the 2,4- dinitro phenyl hydrazine derivative of dehydroascorbic acid. *J Biol Chem* 147: 399-407.

Sadighara P 2009. RBC: Tool for Oxidant Agents Screening Test. *Aust J Basic appl sci* 3(3): 2970-2973.

Shimon K, Joseph A, Bezalel, Sonia PH 1995. *J Agric Food Chem* 43:1813.

Siddiqui MR, Taha A, Moorthy K, Hussain ME, Basir SF, Baquer NZ 2005. Amelioration of altered antioxidant status and membrane linked functions by Vanadium and Trigonella in alloxan and diabetic rat brains. *J Biosci* 30(4): 483-490.

Stadtman ER, Berlett BS 1997. Reactive oxygen-mediated protein oxidation in aging and disease. *Chem Res Toxicol* 10: 485-494.

Stern A 1986. Red cell oxidative damage. In: Oxidative stress (Sies H., ed). Academic Press London U.K. pp 331-349.

Sushma N, Devasena T 2010. Aqueous extract of *Trigonella foenum graecum* ameliorates cypermethrin induced circulatory oxidative stress in male Wistar rats. *Int J Biol Chem Sci* 4(1): 25-33.

Thirunavukkarasu V, Anuradha CV, Viswanathan P 2003. Protective effect of fenugreek (*Trigonella foenum graecum*) seeds in experimental ethanol toxicity. *Phytother Res* 17(7): 737-743.

Verma RS 2001. Chlorpyriphos induced alterations in levels of thiobarbituric acid reactive substances and glutathione in rat brain. *Indian J Exp Biol* 39: 174-177.

Wagner GM, Lubin BH, Chiu DTY 1988. Oxidative damage to red blood cells. In: Cellular Antioxidant Defense Mechanisms (Chow, C. K., ed.), vol. 1, pp. 185-195. CRC Press Inc., Boca Raton, FL.

Youdim KA, Shukitt-Hale B, MacKinnon S, Kalt W, Joseph JA 2000. Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. *Biochem Biophys Acta* 1523:117-122