International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Study of Phytochemical and Antioxidant Activity of Hydro-methanolic Extract of *Trigonella foenum-graecum* Seed and Indigenous Cow Urine Distillate in Broiler Birds

Chandra Prakash Sen[1], Shraddha Nety[1], K. M. Koley[2], Durga Chourasia[3], Meenu Dubey[4]

[1, 2, 3] Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, (CGKV) Anjora, Durg (Chhattisgarh), India

[2]Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Chhatisgarh Kamdhenu Vishwavidyalaya (CGKV) Anjora, Durg (Chhattisgarh), India

[3]Department of Animal Nutrition College of Veterinary Science and Animal Husbandry, Chhatisgarh Kamdhenu Vishwavidyalaya (CGKV) Anjora, Durg (Chhattisgarh), India
Corresponding author: Chandra Prakash Sen; Email: *drcparjunnvet483[at]gmail.com*

Running title: Anti-oxidant activity of *Trigonella foenum-graecum* seed Extract and Indigenous Cow Urine in Broiler Birds

Abstract: ***Oxidative stress in broiler chicken can result in damage to biomolecules, cells and tissues which thereby can decrease immunity, antioxidant system and result in poor growth rate and production of the bird's. Present study was conducted to evaluate the effects of supplementation of hydro-methanolic extract of fenugreek seed extract (1g/L in drinking water) on oxidative stress in broiler birds. In this experiment phytochemical analysis and antioxidant parameters were studied. Dried seed powders of fenugreek seed were extracted with hot methanol by using Soxhlet's apparatus. Hot hydro-methanolic extract of Fenugreek seed was referred hereafter "Fenugreek Seed Extract" (FSE). For study of antioxidant status one hundred fifty-day old Ross AP broiler birds were used and randomly divided into five groups namely control, standard, FSE (T1), CUD (T2) and FSE+CUD (T3). Each group consisted of 3 replicates with 10 birds. The control group was supplemented with basal diet. standard group with 0.25% Enradine and groups FSE, CUD and FSE+CUD were supplemented 1g/L of fenugreek seed extract, Cow Urine Distillate (10 ml/lit) and its combination respectively in drinking water daily for consecutive 42 days. Phytochemical analysis was assessed by different chemical method and results revealed the presence of Phenolic compound, flavonoids, saponin, terpenoid, glycosides etc. Antioxidant status were assessed by MDA level in lipid peroxidation, Reduced Glutathione and Glutathione peroxidase. Increased level of glutathione peroxidase, glutathione and reduced level of lipid peroxidation were observed in this study. Thus, confirming the antioxidant / scavenging action of FSE and this might be due to the presence of many potent phytochemicals flavonoids, polyphenols, terpenoids etc. Therefore, it can be used for ethno veterinary medicine purposes.***

Keywords: Antioxidant, *Fenugreek Seed Extract*, (FSE), *Cow Urine Distillae (*CUD) Glutathione peroxidase, Flavonoids, Phenols

1. Introduction

Oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of over one hundred human diseases. Antioxidants capable of neutralizing ROS and their actions are considered beneficial. In this context natural dietary components with antioxidant activities are very important (Bandyopadhyay *et al.*, 1999; Yoshikawa *et al.*, 2000). Fenugreek (*Trigonella foenum-graecum*-known as '*Methi*' in Hindi) is one such plant used in India and various other Asian, African and European countries. It belongs to the family of fabaceae. Its leaves, tender shoots and germinated seeds are consumed as vegetables. Fenugreek is credited with many medicinal properties and is also one of the oldest medicinal plants being used in many Asian and African countries for its health benefits. Its seeds, leaves and tender shoots show antidiabetic effects and are helpful in digestive disorders such as flatulence, dysentery, diarrhoea, dyspepsia, chronic cough and enlargement of the liver and spleen (Nadkarni and Nadkarni, 1976; Bach, 2003). In type 1 diabetic patients, supplementation of fenugreek in the diet lowers lipid peroxidation (Ravikumar and Anuradha, 1999), induces hypocholesterolemia and hypoglycemia (Sharma 1986; Sharma *et al.*, 1990). The present study investigates the antioxidant activities of different fractions from the extract of dried germinated seeds of fenugreek. Antioxidants protect against ROS induced damage, acting at different levels (Devasagayam *et al.*, 2004), such as radical formation, radical scavenging and prevention of damage to cellular components such as membranes. The recent research states that 1% fenugreek seed extract and its combination with Cow Urine Distillate showed notable reduction the level of MDA lipid in groups supplemented with Fenugreek seed extract showed significantly lower LPO level as compare to all other supplemented groups.

2. Materials and Methods

Freshly dried fenugreek seed was made into powder with help of Pulverizer. Dried seed powders of fenugreek seed were extracted with hot hydro-methanol by using Soxhlet's

apparatus 650c temperature for 24hr. After complete extraction of the plant powder, the extract obtained was transferred to weighed evaporating dishes and kept under a fan for complete evaporation of methanol. The extract remain in the dishes was taken in air tight screw cap vials and stored under refrigerator for use whenever required. This hot hydro-methanolic extract of *Fenugreek* seed was referred hereafter "*Fenugreek Seed Extract*" (FSE)

Preparation of Extract

Fenugreek Seed procured from local market and get powdered by Pulverizer. Extraction of 100 g of dried seed powder with 70% methanol and 30% distilled water by Soxhlet's extraction. The extract thus obtained is hereby referred as hydro methanolic *Fenugreek seed extract* (FSE). Cow Urine Distillate was prepared by collecting in the morning first voided urine from nearby dairy farm in Anjora. The process of distillation was carried out in the laboratory and final distillate was stored in cool place.

Experimental animal

Limit test revealed that the acute oral LD50 in female rats was more than 2000 mg/kg and indicated that the compounds are practically nontoxic. For study of antioxidant parameter 150 day old Ross AP broiler birds procured from registered supplier and randomly divided into five groups Each group consisted of 3 replicates with 10 birds namely control, standard, FSE (T1), CUD (T2) and FSE+CUD (T3). The control group was provided with basal feed, standard group the basal feed was mixed with 0.25% Enradine, group T1 supplemented with FSE (1g/L of *fenugreek seed* extract and T2 group FSE+CUD were, *Cow Urine Distillate* (10 ml/lit) and *its combination* respectively in drinking water daily for consecutive 42 days.

Preliminary Phytochemical Screening of extract

Preliminary phytochemical analysis was carried out to identify the active compounds in hydro-methanolic extract of *Fenugreek seed* such as alkaloids, carbohydrates, flavonoids, terpenes, steroids, saponins and tannins by using test methods of Draggendroff's, Mayer's test, Molisch's, Fehling's test, Lead acetate, Liebermann-Burchard test, foam formation test, ferric chloride test and Alkaline Reagent Test (Raaman, 2006).

Constituents	Name of Test	Inference
Alkaloids	Dragendroff's test/ Mayer reagent test	Positive (+ve)
Saponins	Frothing test	Positive (+ve)
Phytosterols	Liebermann-Burchardt's test	Positive (+ve)
Phenolic compounds	Ferric chloride test	Absent (+ ve)
Tannins	Lead acetate test	Absent (–ve)
Phlobatannin	Hydrochloric acid test	Absent (–ve)
Flavonoids	Alkaline Reagent Test	Positive (+ve)
Detection of Free sugar	Fehling's Reagent test	Positive (+ve)
Detection of Glycosides	Kellar-Kiliani test	Positive (+ve)
Anthraquinone	Borntrager's test	Absent (-ve)
Terpenoid	Salkowski test	Positive (+ve)
Volatile oil	NaOH test	Positive (+ve)

Collection of Sample

On 35 day of experiment, blood samples collected from six birds of each group (two birds from each replicate) in Eppendroph tubes containing anti-coagulant (acid citrate dextrose)[at]1.5ml/10 ml blood. These tubes were centrifuged at 3000 rpm for 15 min. at 4^0C, followed by removal of plasma and buffy coat. The resulting erythrocyte pellet was washed thrice with phosphate buffer saline (PBS 50 mosmole/L). The erythrocyte pellet obtained was mixed with an equal volume of PBS to form RBC suspension. To prepare haemolysate, 0.5 ml of RBC suspension is taken in a 10-15 ml vial and 4.5 ml of stabilizing solution (EDTA, 2.7 mM and 0.7 mM, beta mercaptoethanol) was added to it.

Analysis of antioxidant enzyme

1) Glutathione Peroxidase (GSH-Px)

Activity of whole blood GSH-Px were determined by spectrometry using RANdox ransel kit (Randox laboratories Ltd., UK) as per the method described by Paglia and valentine, (1967) using heparinized whole blood for the analysis. The decrease in absorbance at 320 nm is measured and GSH-Px activity was expressed as units per litre of blood.

2) Lipid peroxidation

Lipid peroxidation in haemolysate was measured by the method of Placer *et al*. (1966). Determination of malondialdehyde (MDA) by thiobarbituric acid (TBA) is used as an index for the extent of lipid peroxidation. The absorbance was read at 548 nm and the concentration of MDA was calculated based on the formation of pink pigment due to reaction between the malondialdehyde with thiobarbituric acid (TBA).

3) Analysis of non-enzymatic antioxidant: Reduced glutathione

Reduced glutathione concentration in RBC suspension was measured by the method of Prins and Loos (1969). The amount of reduced glutathione (GSH) was expressed as mmol/mg Hb.

Statistical Analysis

Statistical analysis was done using completely randomized design (CRD), one-way classification as per procedure given by Snedecor and Cochran (1994). Duncan's Multiple Range Test was employed for the significant differences amongst the different treatments.

3. Results

The effect supplementation of *Fenugreek Seed Extract*, Cow urine Distillate and its combination of both on malondialdehyde lipid peroxidation (LPO), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) in RBCs of broiler birds were presented in table.

Effect of supplementation of fenugreek seed extract (FSE), Cow Urine Distillate (CUD) and its combination (FSE+CUD) on Antioxidant Stress Parameter on Broiler Birds.

Antioxidant Parameters (Mean ± SE)						
Parameters	Control	Standard	FSE (T1)	CUD (T2)	FSE+CUD (T3)	Level of Significance
LPO (MDA)	8.84 ± 0.97^{a}	8.02 ± 0.48^{ab}	6.46 ± 0.29^{bc}	7.72 ± 0.34^{abc}	6.27 ± 0.34^{c}	*
GSH	0.15 ± 0.01^{c}	0.16 ± 0.01^{c}	0.30 ± 0.02^{a}	0.23 ± 0.01^{b}	0.26 ± 0.01^{ab}	*
GSH-Px	145.57 ± 7.48^{c}	191.57 ± 6.30^{b}	231.45 ± 3.74^{a}	197.24 ± 3.83^{b}	222.30 ± 20.71^{ab}	*

Means having different superscripts in rows differ significantly, * (P< 0.05)
NS-Non-significance, Values show Mean ± SE where n=6,

1) Lipid Peroxidation (nmole/ml)
The levels of malondialdehyde lipid peroxidation (LPO) in groups supplemented Control, Standard, T1, T2 and T3 were 8.84± 0.97, 8.02 ± 0.48, 6.46 ± 0.29, 7.72 ± 0.34 and 6.27 ± 0.34, respectively. Groups T3 showed significantly (P<0.05) lower malondialdehyde level as compare to all other supplemented groups. However, there was no significant difference between Standard and T2.

2) Reduced Glutathione (mmol/mg Hb)
The levels of reduced glutathione (GSH) in groups supplemented Control, Standard, T1, T2 and T3 were 0.15 ± 0.01, 0.16 ± 0.01, 0.30 ± 0.02, 0.23 ± 0.01 and 0.26 ± 0.01, respectively. Groups T1 showed significantly (P<0.05) higher levels of reduced glutathione as compared to all other groups. No significant (P<0.05) difference was observed between groups Control and Standard.

3) Glutathione Peroxidase (Units)
The levels of Glutathione Peroxidase (GPX) in groups supplemented with Control, Standard, T1, T2 and T3 were 145.57± 7.48, 191.57 ± 6.30, 231.45 ± 3.74, 197.24 ±3.83 and 222.30 ± 20.71, respectively. Group T1 showed significant (P<0.05) higher as compare to all other groups but no significant difference between Standard and T2.

4. Discussion

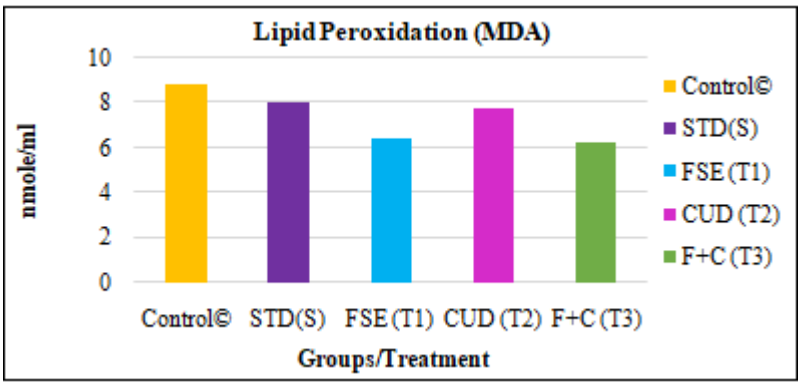
Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical's intermediates, and inhibit other oxidative agents such as thiols, ascorbic acid or polyphenols Stahl and Sies., (2005). Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. The antioxidant potential of medicinal plants may be related to the concentration of phenolic substances (flavonoids, hydrolysable tannins, pro anthocyanin, phenolic acids, phenolic terpenes) and some water soluble vitamins C and fats soluble vitamin A, and vitamin E. The results of present study indicated there was higher level of antioxidant enzymes. This could be due to presence of higher of phenolic compound in *fenugreek seed extract*. Awika *et at.*, *(*2003) who has reported that fenugreek seed extract and its bioactive molecule, polar nature of phenolic compounds, fenugreek yield a relatively high percentage of extract in ethanol and methanol while lower in hexane. The phenolic compounds mainly responsible for antioxidant property. The *fenugreek seed extract* contains phosphorus, iron, β-carotene, sulfur, nicotinic acid, alkaloids, saponins (the origin of its appetite-stimulating properties), flavonoids, carbohydrates, vitamins A, B1, C, magnesium, calcium, lecithin and proteins (30%). In accordance with our study Shimon *et al*, *(*1995) who has also reported that the fenugreek has volatile oil, phenolic acids and flavonoids; therefore, it is a potent source of antioxidants. Similar result was also reported by Yildirim *et al., (*2001) and Siddhuraju *et al*., (2002) that the reducing power of bioactive compounds is associated with antioxidant activity. However, Shan *et al., (*2005) reported that the reducing power of the extracts increases with an increase in the amount of the extract. Lipid peroxidation levels which measured in terms of malondialdehyde formation which showed a statistical difference (p<0.05) among the treatment group T1 and T3 of broiler birds at 42 days of age showed reduction in malondialdehyde level. Glutathione peroxidase is a major selenium containing enzyme found in mammalian cells which catalyzes the degradation of various peroxidase by oxidizing glutathione and protect the cells from oxidative damage. Glutathione is the major free thiol in most living cells and is involved in many biological processes such as removal of toxic compounds and maintenance of the oxidation state of protein sulfhydryl's Parris *et al., (*1997). In the present study the group supplemented with T1 showed significant higher levels of GSH-Px. activity in broiler birds when compared with Control group. In accordance with our study Naidu *et al*., (2010) who has reported that the proximate composition of fenugreek seeds, husk and cotyledons had the highest saponin and protein content. In contrast, husk had higher total polyphenols. At 200 ug concentration, fenugreek seed, extracts of husk and endosperm exhibited 72%, 64%, and 56% antioxidant activities respectively by free-radical scavenging activity.

5. Conclusion

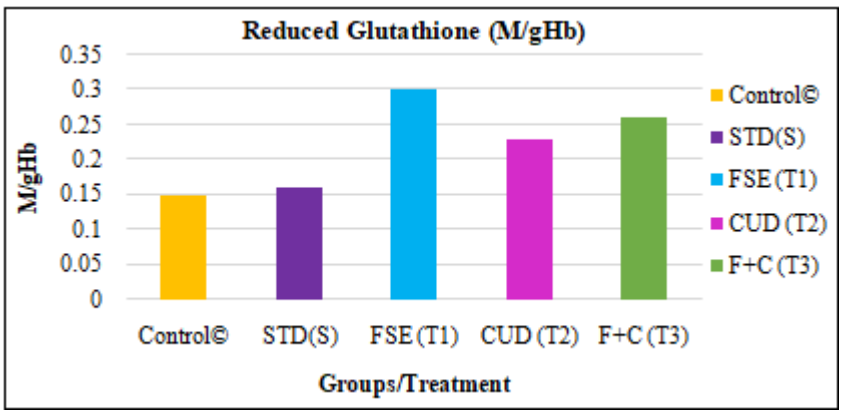
In conclusion, oxidative stress which is the major cause of concern for rapid growth rate in broiler chicken can be minimized by the supplementation of Extract of *Trigonella foenum-graecum* seed and Indigenous Cow Urine Distallate through water thereby ameliorating the stress and can be safely used as seen in the present study.

Acknowledgements
The authors are thankful to the ICAR project on Outreach Programme on Ethno Veterinary Medicine ICAR, New Delhi for providing necessary facility and financial support for conducting research.

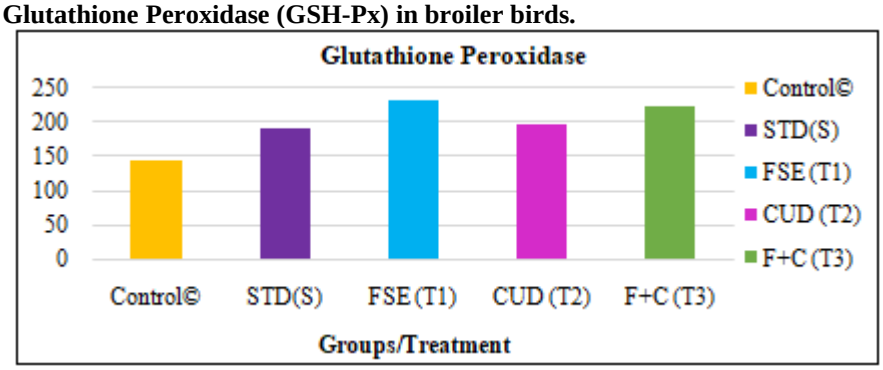
(a) Effect of supplementation of fenugreek seed extract (FSE), Cow Urine Distillate (CUD) and its combination (FSE+CUD) on LPO (MDA) in broiler birds.



(b) Effect of supplementation of fenugreek seed extract (FSE), Cow Urine Distillate (CUD) and its combination (FSE+CUD) on Reduced Glutathione in broiler birds.



(c) Effect of supplementation of fenugreek seed extract (FSE), Cow Urine Distillate (CUD) and its combination (FSE+CUD) on Glutathione Peroxidase (GSH-Px) in broiler birds.



References

[1] Awika J. M., Rooney L. W., X. Wu, Prior R. L. and Cisneros-Zevallos L., (2003) *J. Agric. Food Chem.*, 51 6657.
[2] Bach E. (2003). Therapeutic applications of fenugreek. Altern Med Rev **8**: 20–27.
[3] Bandyopadhyay U, Das D, Banerjee RK.1999. Reactive oxygen species: oxidative damage and pathogenesis. Curr Sci **77**: 658–666.
[4] Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. (2004). Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India **52**: 794–804.
[5] Nadkarni KM, Nadkarni AK. (1976). Indian Materia Medica. Popular Prakashan: Bombay; 1240–1243.
[6] Naidu, M. M., Shyamala, B. N., Naik, P. J., Sulochanamma, G., Srinivas, P., (2010). Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. Food Sci. Technol.44, 451–456
[7] Parris M. kidd. (1997). Glutathione systemic protectant against oxidative and free damage. Alternative medicine review. Volume 2, No 3.
[8] Placer, Z. A., Cushman, L. and Johnon, B. (1996). Estimation of product of lipid peroxidation (malonldialdehyde) in biochemical system. Analytical Biochem.16: 359-364.
[9] Prins and Loos. (1969). Biochemical methods in Red cell Genetics. Academic Press, London, pp.127-129
[10] Raaman, N. (2006). Phytochemical Techniques. New India publishing Agency, Pitampura, New Delhi.19-24
[11] Ravikumar P, Anuradha CV. (1999). Effect of fenugreek seeds on blood lipid peroxidation and antioxidants in diabetic rats. Phytother Res **13**: 197–201.

[12] Ravikumar P, Anuradha CV. (1999). Effect of fenugreek seeds on blood lipid peroxidation and antioxidants in diabetic rats. Phytother Res **13**: 197–201.
[13] Shan B., Cai Y. Z., M. Sun and H. Corke, (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents *J. Agric. Food Chem.*, 53; 7749.
[14] Sharma RD, Raghuram TC, Rao NS. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. Eur J Clin Nutr **44**: 301–306.
[15] Sharma RD. (1986). An evaluation of hypocholesterolemic factor of fenugreek seeds (T. *foenum graecum*) in rats. Nutr Rep Int **33**: 669–677.
[16] Shimon M., Joseph K., Bezalel A. and Sonia P. H., (1995) *J. Agric. Food Chem.*, 43; 1813.
[17] Siddhuraju, P. S. Mohan and K. Becker, (2002) *Food Chem.*, 79 61.
[18] Snedecor, G. W. and Cochran, W. G. (1994). Iowa State University Press. Ames, Iowa.
[19] Stahl, W. and Sies, H., (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*.*1740* (2): 101-107.
[20] Yildirim, M. Oktay and L. Bilalog, (2001). *Turkish. J. Med. Sci.*, 31 23.34. P.
[21] Yoshikawa T, Toyokuni S, Yamamoto Y, Naito Y. (2000). Free Radicals in Chemistry Biology and Medicine. OICA International: London.

Author Profile

Chandra Prakash Sen, PG Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H. Anjora, Durg (C. G.) 491001
E mail – drcparjunnvet483[at]gmail.com
Mobile no.9926383497

Dr Shraddha Nety, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H. Anjora, Durg (C. G.) 491001
E mail-dr_shraddha20[at]yahoo. co. in
Mobile no-09407687345

Dr K. M. Koley, Professor and Head, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H. Anjora, Durg (C. G.)
E mail-drkmkoley[at]yahoo. co. in

Dr Durga Choursia, Associate Professor, Department of Veterinary Anatomy, College of Veterinary Science and A. H. Anjora, Durg (C. G.)
E mail-drdurgavet2010[at]gmail.com

Dr Meenu Dubey, Assistant Professor, Department of Veterinary Animal Nutrition, College of Veterinary Science and A. H. Anjora, Durg (C. G.)
E mail-drmeenu. dubey[at]gmail.com