

# Oxidantantioxidant Status in Calves Supplemented with Green Tea Extract

Ibrahim I. Elshahawy

**Abstract**—The objective of the present study was to investigate the effect of green tea extract on serum oxidant and antioxidant profile, liver and kidney function. 40 Friesian calves are included in this study and allocated into two groups: Group I (n=20) clinically healthy calves showing no clinical abnormalities, not receiving any treatment and served as control; group II (n=20) received green tea extract (GTE) for 30 days. Non-significant changes in blood urea nitrogen (BUN) were detected between groups, on contrary, serum creatinine and activities of liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) were significantly different between two groups. There were significant increases in the mean values of serum antioxidative parameters (total antioxidant capacity, catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase) in group II. Whereas, the activity of lipid peroxidase significantly decreased in GTE treated calves when compared to control.

**Keywords**—Green tea extract, antioxidants, oxidants, calves.

## I. INTRODUCTION

*CAMELLIA sinensis* plant; the source of green tea, grown in tropical and subtropical regions, is considered a popular drink in many countries for its many beneficial effects for human health [1]. Tea is mainly produced as four varieties; white (made from very young tea leaves or buds), green (made from mature unfermented leaves), oolong (from partially fermented leaves) and black (from fully fermented leaves) [2]. GTE has many benefits including anti-carcinogenic, anti-inflammatory and anti-oxidative powers. Green tea minimizes the free radicals such as harmful oxygen species by chelating them [1]-[3]. GTE plays an important role in combating different micro-organisms as *E. coli*, *Salmonella* spp., *Staph. aureus*, some fungi and viruses [4].

A large number of free radicals are continuously produced from body cells causing oxidative damage to internal organs. Excessive generation and/or inadequate removal of free radicals results in destructive, degenerative and irreversible damage to exposed cells [5].

GTE acts as feed additive and supplement material for growing calves particularly during nursing periods. GTE plays effective role in controlling, prevention and minimization of respiratory and digestive disorders through inhibition of many pathogenic bacteria growth and balance of microflora of intestinal tract, reducing mortality rates [6]. Green tea (*Camellia sinensis*) contains many vitamins and trace elements. The main effective components of tea are called

green tea polyphenols (GTP). It has been known that GTP play an important role in removing of harmful reactive oxygen species generated from different tissues, that affect function of body cells [7]. Green tea is also characterized by its high flavonoid content (polyphenols), mainly catechins (20-30% of the dry weight). The major catechins are epigallocatechin gallate (EGCG), epigallocatechin (ECG), epicatechin gallate (ECG), gallic acid (GA), and catechin (C). The largest proportion of active ingredients in green tea is EGCG [8] which has effective anti-oxidative properties. These C play an important role in disposal of free radicals produced from metabolism [9]. The objective of this experiment was to study the effect of green tea leaves extract on serum metabolites and oxidant and antioxidant properties in newborn Friesian calves.

## II. MATERIAL AND METHODS

### A. Animals

Animals in the respective study were 40 Friesian calves average age 5-8±1.02 months and about 50-80±2.01 kg body weight, belonging to private farms in Behera Governorate, Egypt. Calves were thoroughly and clinically examined and classified into two groups: Group 1 contained 20 healthy calves free of diseases abnormalities and served as control. Group 2 included 20 calves that were supplied with one liter of GTE twice daily for one month.

### B. GTE

GTE was prepared from China green tea and extracted according to the method described by [10].

### C. Blood Samples

Blood samples were collected from jugular vein in plain test tubes, without anticoagulant for separation of the serum. Clear serums samples were transferred into clean, dry, sterile vials and were kept at -20 °C for later analysis.

### D. Serum Biochemical Analysis

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), creatinine were determined using kits supplied by Bio-labo, France according to method described by [11], [12], [13], respectively. Analysis of BUN was carried out by using commercial test kits supplied by Vitro Scient, Egypt according to method described by [14].

### E. Determination of Serum Oxidant and Antioxidant Profiles

The following markers were analyzed using test kits supplied commercially by Bio-Diagnostic, Cairo, Egypt:

Total antioxidant capacity (TAC) was determined according

Ibrahim I. Elshahawy is Lecturer of internal medicine, Faculty of Veterinary Medicine, Alexandria University, Egypt (phone: +201009040356; fax: +20452960450; e-mail: hemas\_82@yahoo.com).

to [15]; catalase according to [16]; MDA was measured by method of [17]; level of reduced glutathione was measured according to [18]; glutathione peroxidase (GSH-P<sub>x</sub>) was estimated according to [19] and SOD was estimated according to [20].

#### F. Statistical Analysis

Results were statistically analyzed by SPSS [21]. The obtained results analyzed using ANOVA and significance was declared at  $p < 0.01$ .

### III. RESULTS

Results of biochemical observation of calves could be summarized as follows:

The results of activity of AST and ALT in GTE treated calves decreased significantly ( $P \leq 0.01$ ) compared with control (Table I).

TABLE I

CONCENTRATION OF SERUM ALT, AST IN FRIESIAN CALVES SUPPLEMENTED WITH GTE (GROUP 2) AND CONTROL (GROUP 1) (MEAN±SE)

Parameter	Control	GTE
	Mean± SE	Mean± SE
ALT (Iu/L)	32.14±2.03	27.76±2.01*
AST (Iu/L)	89.1±1.44	72.37±7.75*

\*Significant at  $P \leq 0.01$

As shown in Table II, there were no significant changes in the values of BUN between the two groups while significant  $P \leq 0.01$  decreases in serum creatinine were recorded in GTE group compared with control.

TABLE II

CONCENTRATION OF BUN AND CREATININE IN FRIESIAN CALVES SUPPLEMENTED WITH GTE (GROUP 2) AND CONTROL (GROUP 1) (MEAN± SE)

Parameter	CONTROL	GTE
	Mean± SE	Mean± SE
BUN (mg/dl)	23.04±1.84	23.5±1.43
Creatinine (mg/dl)	1.27±0.15	1.07±0.09*

\*Significant at  $P \leq 0.01$

There were significant  $P \leq 0.01$  increases in levels of TAC, catalase, GSH, GSH-P<sub>x</sub> and SOD activities in GTE group compared with control calves. On the other hand, there was a significant ( $P \leq 0.01$ ) decrease in the activity of lipid peroxidase in GTE calves compared with control.

TABLE III

OXIDANT AND ANTIOXIDANT PROFILE IN FRIESIAN CALVES SUPPLEMENTED WITH GTE AND CONTROL (MEAN± SEM)

Parameter	CONTROL	GTE
	Mean± SE	Mean± SE
TAC (mmol/ml)	1.75±0.15	2.45±0.20*
Catalase (U/L)	2355.7±75.1	3121.8±131.2*
MDA (mmol/ml)	1.89±0.045	1.48±0.07*
GSH (mmol/ml)	8.18±0.31	9.06±0.091*
GSH-P <sub>x</sub> (U/L)	214.4±2.01	247.8±3.12*
SOD (U/ml)	329.4±9.24	424.5±16.01*

\*Significant at  $P \leq 0.01$

### IV. DISCUSSION

Researches performed on animals models showed that the GTE has antioxidative, antiviral and antibacterial properties [22]. AST and ALT enzymes' levels may be increased due to hepatic cellular damage followed by leakage of these enzymes from liver cytosol into the circulation, so calves administered with GTE showed an increase of internal antioxidative power; this enhanced the liver cells for combating the harmful effects of oxidative impurities that might negatively affect liver cells.

The obtained results showed a significant drop in the AST and ALT activities in GTE group. These results are in agreement with [23] who recorded significant decrease in serum activities of AST and ALT in animals supplied with GTE; this may be due to ability of GTE to prevent oxidation of liver cell membranes, with subsequent prevention of leakage of liver enzymes into circulation. Polyphenols are known to protect different organs of the body from oxidative damage, particularly liver and kidney [24].

In the present study, no significant changes were recorded in the values of BUN in both groups. The significant decrease in serum creatinine in GTE group compared with control is not identical to those values obtained by [25]; who indicated that GTE supplementation maintains the creatinine level in the serum. Green tea Cs protect the brain, liver and kidney from lipid peroxidation injury [24]. Moreover, GTE protects against alcohol induced liver and serum lipid peroxidation [26] and gentamicin induced oxidative stress in kidney [27] in a rat model.

The oxidative stress and oxidative damage of body cells is controlled by antioxidative power supplied from some enzymes such as superoxide dismutase, catalase, GSH-P<sub>x</sub> and glutathione reductase and non-enzymatic compounds located intracellular and extracellular fluids including some vitamins, trace elements that participate in some enzymes, such as copper, selenium and zinc. High proportion of free radicals as reactive oxygen species with minimal production of antioxidative components create a state of imbalance inside cells, leading to harmful oxidative damages to tissues [28].

Green tea (*Camellia sinensis*) has gained considerable attention as an antioxidant agent [29]. The antioxidative power produced from GTE is enough for combating the oxidative damages produced from reactive oxygen species and other harmful free radicals. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage [30]. The harmful effects of oxidative stress to body cells not only controlled by substances possess antioxidative properties as GTE or its C but also protected by other substances within cells. All cooperate with each other for tissue protection as tocopherols, ascorbic acid, selenium containing enzymes (GSH-P<sub>x</sub> and catalase) and copper containing enzymes (superoxide dismutase [31]. *In vivo* studies showed that green tea C increase total plasma antioxidant activity [32].

Regulation of the synthesis of intracellular glutathione, GSH-P<sub>x</sub> activity and reduction of oxidation inside mitochondria may be achieved by dietary polyphenols which possess potent antioxidative properties. The greatest effect of

body antioxidative defense is due to glutathione [33]. GTE supplementation to animal models during times of oxidative stress has also been shown to increase activities of antioxidant, specifically GSH-P<sub>x</sub>, and increase concentrations of glutathione [34]. Data obtained by [35] showed increased glutathione activity following *ex vivo* treatment of erythrocytes with GTE or dietary supplementation of polyphenol-mixture containing green tea.

Calves treated with GTE showed significant increases in the mean values of TAC, catalase activity, reduced glutathione activity and super oxide dismutase activity and these calves recorded significant decrease in oxidative stress represented by significant lowering in lipid peroxidase (MDA activity) when compared with control calves. GTE participates in increasing the levels of tissues antioxidants; these play an important role in cell protection from ROS [36], particularly in presence of optimum and enough levels of vitamin E and C, and antioxidative enzymes [37]. *In vivo* studies showed that green tea Cs increase total plasma antioxidant activity [32]. GTE flavonoids enhance the level of glutathione and GSH-P<sub>x</sub> [34], these also recorded in rat model treated with GTE [36]. In our study, significant increases in catalase activities and GSH-P<sub>x</sub> were recorded in contrast to that observed by [37] which reported that green tea supplementation showed no effects in activities of GSH-P<sub>x</sub> or catalase. In contrary obtained data coincide with the one recorded by [26], where reactive oxygen species. are neutralized by SOD and catalase, that were further improved and enhanced by addition of GTE [38].

Administration of GTE by friesian calves makes a significant drop in malondialdehyde (MDA) a marker of harmful oxidative stress, these results coincides with that previously observed by [32], [38]. GTE could protect low density lipoprotein against peroxidation [32]. The obtained results are in accordance with those reported by [23], who administered rats with GTE for consecutive 14 days and observed declined kidney and liver peroxidation and decreased MDA within treated rats compared to controls. These explain the beneficial effect of GTE on increasing efficiency of liver and kidney function.

#### V. CONCLUSION AND RECOMMENDATIONS

GTE has effective antioxidant properties that are useful in combating the oxidative stress and damage produced from cellular metabolism, so GTE may be given to animals as feed additive to improve their health, performance and production. Further research is needed to evaluate the beneficial role of GTE in systemic diseases affecting animals.

#### REFERENCES

- [1] Gupta, D. A., Bhaskar, D. J., Gupta, R. K., Karim, B., Jain, A. and Dalai, D. R. *Green tea: a review on its natural anti-oxidant therapy and cariostatic benefits*. Biol. Sci. Pharm., Res. 2014. 2, 8–12.
- [2] Jigisha, A., Nishant, R., Navin, K. and Pankaj, G. *Green tea: a magical herb with miraculous outcomes*. Int. Res. J. Pharm., 2012. 3, 139–148.
- [3] Serafini, M., Del Rio, D., Yao, D. N., Bettuzzi, S. and Peluso, I. "Health benefits of tea", in *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd Edn, Chapter 12, eds I. F. F. Benzie and S. Wachtel-Galor (Boca Rotan, FL: CRC Press), 2011. 239–262.
- [4] Steinmann, J., Buer, J., Pietschmann, T. and Steinmann, E. *Anti-infective properties of epigallocatechin-3-gallate (ECGC), a component of green tea*. Br. J. Pharmacol., 2013. 168, 1059–1073.
- [5] Nazifi, S., M. Saeb, N. Ghafari, I. Razeghian, M. Razavi, F. Vosoughi and H. Orangi: *Reference values of oxidative stress parameters in adult native Iranian goats*. Bulgarian Journal of Veterinary Medicine, 2009. 12: 119-124.
- [6] Ishihara, N.; Chu, D.C.; Akachi, S. and Juneja L.R.: *Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts*. Livestock Production Science, 2001. 68(2):217-229 · March 2001.
- [7] Guo Q., Zhao B., Li M., Shen S. and Xin W.: *Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes*. Biochim. Biophys. Acta, 1996. 1304, 210±222.
- [8] Wang, H., Provan, G. J. and Helliwell, K. *Tea flavonoids: their functions, utilization and analysis*. Trends in Food Science and Technology, 2000. 11, p. 152-160.
- [9] Horzic, D., Komes, D., Belscak, A, Ganic. K. K., Ivekovic, D. and Karlovic, D. *The composition of polyphenols and methylxantine in teas and herbal infusions*. Food Chem., 2009. 115: 441-448.
- [10] Yen, G. C. and Chen, H. Y. *Comparison of antimutagenic effect of various tea extracts (green, oolong, pouchong and black tea)*. J. Food Prot., 1994. 57, 54-58.
- [11] Doumas, B. T. and Biggs, H. G. *Determination of serum albumin, standard methods of clinical chemistry*, Acad. Press. N.Y., 1972. 7: 175-188.
- [12] Curtis, E. R., Silverman, L. M., Christensen, R. H. and Tietz N.W. Text book of clinical chemistry, 3<sup>rd</sup> Ed. C.A.P. 1995. p: 523-524.
- [13] Young, D. S. *Effect of drugs on clinical laboratory tests*, 4<sup>th</sup> Ed. 1995. P: 3-190 to 3-211.
- [14] Rock, R. C., Walker, W. G. and Jennings, C. D. *Nitrogen metabolites and renal function*. In: Tietz NW, ed. Fundamentals of clinical chemistry. 3<sup>rd</sup> ed. Philadelphia: WB Saunders" 1987. 669-704.
- [15] Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., Cosic, V. *Method for the measurement of antioxidant activity in human fluids*. Journal of clinical pathology, 2001. 54 (5), 356-361.
- [16] Aebi, H. *Catalase in vitro*. Methods Enzymol. 1994.105:121–126.
- [17] Ruiz-Larrea, M. B., Leal, A. M., Liza, M., Lacort, M. and De Groot, H. *Antioxidant effects of estradiol and 2-hydroxyestradiol on iron induced lipid peroxidation of rat liver microsomes*. Steroids, 1994.59: 383 – 388.
- [18] Beutler, E., Duron, O. and Kelly, B. M. *Improved method for the determination of blood glutathione*. J. Lab. Clin. Med., 1963.61:882–90.
- [19] Paglia, D. E. and Valentine, W. N. *Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase*. J. Lab. Clin. Med., 1967. 70: 158 – 169.
- [20] Nishikimi, M., Rao, N. A. and Yagi, K. *The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen*. Biochemical and Biophysical Research Communications, 1972. 46 (2):849 – 854.
- [21] SPSS, *Statistical Package for Social Science*, Computer Software, IBM, SPSS Ver. 16.0, SPSS Company, London, UK, 2008.
- [22] MacKay, D. L., Blumberg, J. B. *The Role of Tea in Human Health: An Update*. Journal of the American College of Nutrition, 2000.21, 1-13.
- [23] Shereen, B. Gad and Doaa, M. Zaghoul. *Beneficial Effects of Green Tea Extract on Liver and Kidney Functions, Ultrastructure, Lipid Profile and Hematological Parameters in Aged Male Rats*. Global Veterinaria, 2013. 11 (2): 191-205.
- [24] Lin, A. M., Chyi, B. Y., Wu, L. Y., Hwang, L. S., and Ho, L. T. *The anti-oxidative property of green tea against iron-induced oxidative stress in rat brain*. Chinese Journal of Physiology, 1998. 41: 189-94.
- [25] Elshater, A. A., Salman, M. A. and Moussa, M. A. *Effect of green tea consumption on level of glucose, lipid profile and kidney functions in alloxan induced-diabetic rats Egypt*. Acad. J. biolog. Sci. 2008. 1(2): 125 – 134.
- [26] Skrzydlewska, E., Ostrowska, J., Farbiszewski, R. and Michalak, K. *Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain*. Phytomedicine, 2002. 9:232-238.
- [27] Abdel-Raheem, I.T., El-Sherbiny, G.A. and Taye, A. *Green tea ameliorates renal oxidative damage induced by gentamicin in rats*. Pakistan Journal of Pharmaceutical Sciences, 2010. 23(1): 21-28.
- [28] Papp L.V., Holmgren A., and Khanna K.K: *Selenium and selenoproteins in health and disease. Antioxid Redox Signal*, 2010. 12:793–5.
- [29] Phung O.J., Baker W.L., Matthews L.J., Lanosa M., Thorne A.,

- Coleman C.I. : *Effect of green tea catechins with or without caffeine on anthropometric measures: a systematic review and metaanalysis*. Am. J. Clin. Nutr., 2010. 91:73–81.
- [30] Halliwell, B. and Gutteridge, J. M. C. *Free Radicals in Biology and Medicine* Oxford: Carendon Press. 1985.
- [31] Abdel-Raheim, M. A. M., Enas, A. H. and Khaled, A.,E. *Effect of green tea extract and vitamin c on oxidant or antioxidant*. Indian J. Clin. Biochem, 2009. 24(3):280-287.
- [32] Yokozawa, T., Nakagawa, T. and Kitani, K. *Antioxidative activity of green tea polyphenol in cholesterol-fed rats*. J Agric. Food Chem., 2002. 50:3549-3552.
- [33] Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., and Telser J. : *Free radicals and antioxidants in normal physiological functions and human disease*. Int. J. Biochem. Cell Biol., 2007. 39:44–84.
- [34] Ramesh, E. Jayakumar, T., Elanchezian, R., Sakthivel, M., Geraldine, P. and Thomas P.A. *Green tea catechins, alleviate hepatic lipidemic-oxidative injury in Westar rats fed an atherogenic diet*. Chem. Biol. Interact. 2009. 180:10–9.
- [35] Kumar N, Kant R, and Maurya P.K. : *Concentration-dependent effect of (-) epicatechin in hypertensive patients*. Phytother Res., 2010. 24:1433–6.
- [36] Babu, P. V., Sabitha, K. E. and Shyamaladevi, C. S. *Green tea impedes dyslipidemia, lipid peroxidation, protein glycation and ameliorates Ca<sup>2+</sup>-ATPase and Na<sup>+</sup>/K<sup>+</sup> -ATPase activity in the heart of streptozotocin diabetic rats*. Chemico Biological Interaction, 2006.162(2): 157-164.
- [37] Basu Arpita, Nancy M. Betts, Afework Mulugeta, Capella Tong, Emily Newman and Timothy J. Lyons. *Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome*. Nutr. Res., 2013. 33(3): 180–187.
- [38] Yokozawa, T., Nakagawa, T., Lee, K. I., Cho, E. J., Terasawa, K. and Takeuchi, S. *Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK1 cells and rats*. J Pharm Pharmacol, 1999. 51:1325-1331.