

Full Length Research Paper

Biochemical and hematological evaluation of *Costus speciosus* as a dietary supplement to Egyptian buffaloes

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Sixty Egyptian buffalo heifers ageing about one year old, with an average weight of about 250 to 260 kg were allotted randomly according to their live body weight and age into three equal groups; Group I which received a basal ration; Group II which received a basal ration with fine ground *Costus speciosus* roots in a concentration of 2.5 kg/ton ration and Group III which received a basal ration with fine ground *C. speciosus* roots in a concentration of 5 kg/ton ration. Blood samples were collected from each group and divided into two blood samples, one for serum separation and the other for hematological study. Separated serum samples were subjected to the biochemical analysis of glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, uric acid and electrophoretic pattern. The obtained data revealed a decrease in serum glucose and cholesterol levels which may be utilized in anabolic pathways during this period. While total protein, albumin, α_1 -globulin, β -globulin, hemoglobin, packed cell volume (PCV) and lymphocytes were significantly increased especially in Group III. In addition, the erythrocytes antioxidant status was significantly improved by *C. speciosus* supplementation. We could conclude that supplementation of *C. speciosus* powder to the Egyptian buffalo heifers improves the health status, total antioxidant capacity and hematology. So, we advise owners to add *C. speciosus* ground powder to the ration of heifers.

Key words: Heifers, *Costus speciosus*, total antioxidant capacity, hematology.

INTRODUCTION

Natural product is a source of bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade, there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment (Jawla et al., 2009). Herbal drugs

are derived either from the whole plant or from their different parts like leaves, bark, roots, flowers, seeds, etc., and also from plant excretory products like gums, resins and latex (Rajashree et al., 2012). Although herbs had been prized for their medicinal, flavoring and aromatic qualities for centuries. However, the blind dependence on synthetics is over and people are returning to nature, with hope of safety and security (Singab, 2012).

Zingiberaceae is a family of about fifty two genera and more than 1,300 species distributed throughout tropical Africa, Asia, and the Americas. Many species are very important for example, shell ginger (*Alpinia*), summer tulip (*Curcuma alismatifolia*), ginger lily (*Hedychium*), torch-ginger (*Etilingera elatior*), ginger (*Zingiber*), turmeric (*Curcuma*) and cardamom (*Amomum Elettaria*) (Jiang et al., 2000). *Costus speciosus* is commonly called Crepe ginger. In Sanskrit, it is known as Keyu and in Hindi as Kust (Khare, 2007). *C. speciosus* is a *Zingiberaceae* erect plant, up to 2.7 m high; root stock tuberous stem, sub-woody at the base, occurring in the moist and wet evergreen areas of the Indo-Malayan region and Sri Lanka. Within India, it occurs from Central and Eastern Himalayas to Southern India (Dutta and Dutta, 1998).

C. speciosus contain diosgenin, 5 α -stigmast-9(11)-en-3 β -ol, sitosterol- β -D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinones. In addition, it contains α -tocopherol (Husain et al., 1992). Traditionally, *C. speciosus* is used in the treatment of fevers, cough, worm infestations, skin diseases and snake bites. The effects of *C. speciosus* with regard to the following compounds diosgenin, prosapogenin B of dioscin, diosgenone, cycloartanol, 25-en-cycloartenol and octacosanoic acid which extracted from it (Qiao et al., 2002). *C. speciosus* has an anti-inflammatory, anthelmintic, astringent, bitter, depurative, purgative, and stimulant effect while its roots were used as a remedy in fevers, coughs, anti-diabetic, hepatoprotective (Biman and Kamaruz, 2008), the antihyperglycemic, antihyperlipidemic and antioxidant properties of *C. speciosus* has been reported by Bavarva and Narasimhacharya (2008). In addition, its rhizome juice is applied on the head for relief of headache (Gupta, 2010).

An alkaloid extracted from *C. speciosus* rhizomes is a smooth muscle relaxant and enhances antispasmodic activities (Srivastava et al., 2011). Extract of *C. speciosus* rhizomes stimulate the uterine contraction due to non-estrogenic effects (Wanwisa et al., 2011). Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthenes, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage, and DNA strand breaking (Jha et al., 2010). *C. speciosus* has an antioxidant activity measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total antioxidant capacity, nitric oxide scavenging activity, ion chelating activity, hydroxyl radical scavenging activity and its correlation with total phenolic content (Nehete et al., 2010).

Heifers in this stage of life need special care, so our study aimed to investigate the effect of *C. speciosus* supplementation in Egyptian buffalo on some serum parameters, erythrocytic antioxidant status and its hematological

picture.

MATERIALS AND METHODS

Experimental design

The field experiment was carried out at the farm of Faculty of Veterinary Medicine, Damanshour University, Al-Bostan district to study the effect of different concentrations of *C. speciosus* to the ration of Egyptian buffalo for the duration of one month. Sixty Egyptian buffalo heifers ageing about one year old, with an average weight of about 250 to 260 kg were allotted randomly according to their live body weight and age into three equal groups (twenty Egyptian buffalo heifers in each) and housed in a separate part of a shaded pen. Group I (received a basal ration); Group II (received basal ration with fine ground *C. speciosus* roots in a concentration of 2.5 kg/ton ration) and Group III (received a basal ration with fine ground *C. speciosus* roots in a concentration of 5 kg/ton ration). Concentrate mixtures were given twice daily at 10 a.m. and 2 p.m. while wheat straw was offered *ad lib*. Drinking water was available for animals during the day. The animals in treated groups were noticed for any clinical signs along the experimental period.

Medicinal plant

C. speciosus roots were obtained and identified in the Faculty of Agriculture, Damanshour University. Specimens of *C. speciosus* rhizomes were preserved at -20°C as a standard stock. The rhizomes were washed, cut, grind, and refined. The ground powder was added to the ration at the concentration of (2.5 and 5.0 kg/ton ration).

Blood samples

The blood samples were collected from the jugular vein by using a sterile sharp needle with wide pore. Two samples were collected from each animal; the samples used for hematological analysis and separation of washed red blood cells (RBCs) were collected in clean and dry test tube containing di-sodium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant while serum samples were collected in dry clean tubes and separated by centrifugation at 3,000 RPM for 10 min. Then, clear serum supernatant were aspirated carefully and subjected to biochemical analysis.

Biochemical analysis

Serum samples were subjected to laboratory analysis of blood glucose (Trinder, 1969), cholesterol (Zak et al., 1954) and alanine transaminase (ALT; EC 2.6.1.2) and aspartate transaminase (AST; EC 2.6.1.1) (Reitman and Frankel, 1957), creatinine (Bartles et al., 1972), urea (Kaplan, 1984), and uric acid (Fossati et al., 1980). Electrophoretic patterns of serum proteins were done by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed according to the method recorded by (Laemmli, 1970) while the erythrocytes were washed by using physiological saline, and erythrocyte hemolysates were prepared using digitonin as described by (Kornburg and Korecker, 1955). Hemolysates were used for determination of malondialdehyde (MDA) (Ohkawa et al., 1979), total antioxidant capacity (TAC) (Koracevic et al., 2001) and hemolysates protein (Lowry et al., 1951).

Hematological analysis

Hematological measurements were done by fully automated blood cell count, Exigo, Boule Medical AB, Sweden.

Statistical analysis

The raw data were analyzed according to Statistical Analysis System (SAS) (1996), with one-way analysis of variance (ANOVA), with a value of $P < 0.05$ indicating significance.

RESULTS

All treated animals showed no abnormal clinical signs. Biochemical data due to supplementation of *C. speciosus* were summarized in Tables 1, 2 and 3. After 30 days of treatment with *C. speciosus* by 2.5 kg/ton ration, the serum levels of glucose and cholesterol were significantly decreased. Moreover, there are no obvious changes in the serum ALT, AST, creatinine, uric acid, and urea were stated in comparison to control (Table 1). Furthermore, the concentrations of erythrocytic MDA were significantly decreased. On the contrary, glutathione (GSH) contents in erythrocytes were significantly increased (Table 2).

At the end of the experiment, serum levels of glucose and cholesterol were significantly decreased in the group treated with 5.0 kg of *C. speciosus*/ton ration. Furthermore, the levels of ALT, AST, creatinine, uric acid and urea had no changes in relation to group one. The MDA concentrations in RBCs were statistically highly significantly decreased. On the other hand, GSH was highly significantly increased (Table 2). The electrophoretic patterns of this group were statistically significantly increased in serum total protein, albumin, α_1 -globulin and γ -globulin while serum α_2 -globulin levels were highly significantly increased. Moreover, no significant changes were observed in the level of β -globulin (Table 3).

The data illustrated in (Table 4 and 5) stated the hematological effects of the treated groups at 30th day of experiment and revealed that the total count of erythrocytes (TEC) was significantly ($P < 0.05$) increased in all buffaloes heifers supplemented when compared with control. Consistent with this, the total leucocytic counts (TLC) were ($P < 0.05$) significantly increase, this increase was more pronounced in heifers in Group III. The present finding also revealed that the erythrocytic contents of hemoglobin (Hb) were statistically significantly increased in in Group III in comparison to control one. The same results were observed in packed cell volume (PCV) which was significantly ($P < 0.05$) increased (Table 4). The data summarized in Table 5 indicated that on the 30th day of the experiment, the percentages of lymphocytes were significantly ($P < 0.05$) increased in all buffaloes heifers supplemented with *C. speciosus* when compared with control which more pronounced in Group III. On the contrary, the percentages of

monocytes were significantly decreased ($P < 0.05$) in all buffaloes heifers supplemented with *C. speciosus* after 30th day treatment when compared with one control. Moreover, the percentages of basophils, eosinophils and neutrophils ($P < 0.05$) animals supplemented with *C. speciosus* had non-significant changes.

DISCUSSION

Our study revealed a significant decrease of serum glucose level after supplementation of *C. speciosus* in the buffaloes ration, this finding might be attributed to either the increase in insulin units released by the beta cells of islet of Langerhans and the increase in sensitivity of cell receptors to insulin consequently increased glucose utilization or both. The hypoglycemic action of eremanthin, a component of *C. speciosus* was caused by potentiation of insulin release from the existing beta cells of islets of Langerhans and increased the sensitivity of insulin to uptake glucose (Li et al., 2004). Its hypoglycemic action was accompanied by an increased hepatic hexokinase activity. Hexokinases provided glucose-6-phosphate, the substrate of glycogen synthase which also activated and increased the hepatic glycogen (Bouche et al., 2004).

Generally, blood glucose levels were decreased by *C. speciosus* supplementation due to the increase in glycogenesis and glycolysis and the reduction in gluconeogenesis (Bavarva and Narasimhacharya, 2008). Costunolide isolated from *C. speciosus* was found to possess normo-glycemic and hypolipidemic effect in streptozotocin-induced diabetic rats. In the study of oral administration of costunolide (20 mg/kg bwt) was significantly decreased the plasma glucose level ($P < 0.05$), glycosylated hemoglobin and at the same time markedly increased plasma (Eliza et al., 2009a). In India, diabetics eat one leaf of *C. speciosus* daily to keep their blood glucose low (Benny, 2004). *C. speciosus* leaf water and methanol extracts effectively reduced the insulin resistance in rats by significantly lowering serum glucose at baseline after one month of the onset of experimental medication (Subasinghe et al., 2012).

C. speciosus affects the lipid metabolism by a significant decrease in serum total cholesterol. This finding came in accordance with that stated and the hexane extract of the rhizome possesses a hypolipidemic activity (Daisy et al., 2008). Moreover, costunolide isolated from the plant significantly decreases serum total cholesterol, and triacylglycerol (Eliza et al., 2009a). In addition, the ethanolic extract of *C. speciosus* of administration reduced plasma and hepatic total cholesterol and triacylglycerol concentrations in diabetic rats (Bavarva and Narasimhacharya, 2008).

These results were in agreement with that of ElRokh et al. (2010) who proved that, the hypercholesterolaemic rats treated with aqueous ginger infusion in the three

Table 1. The mean values of serum glucose (g/dl), cholesterol (mg/dl), ALT (U/L), AST (U/L), creatinine (mg/dl), uric acid (mg/dl) and urea (mg/dl) in Group I, Group II and Group III.

Group		Glucose	Cholesterol	ALT	AST	Creatinine	Uric	Urea
Group I	0 day	45.67±0.13 ^a	145.32±0.01 ^a	25.34±0.01 ^a	35.23±0.01 ^a	0.92±0.01 ^b	1.60±0.01 ^a	14.08±0.04 ^a
	30th day	45.33±0.49 ^a	143.41±0.49 ^b	25.47±0.39 ^a	37.33±0.35 ^a	1.01±0.01 ^a	1.50±0.12 ^a	14.10±0.04 ^a
Group II	0 day	45.47±0.02 ^a	145.23±0.01 ^a	26.23±0.01 ^a	36.80±0.01 ^a	0.91±0.01 ^b	1.60±0.01 ^a	14.12±0.04 ^a
	30th day	37.30±0.45 ^b	124.80±0.41 ^c	26.07±0.43 ^a	35.50±0.24 ^a	0.90±0.01 ^b	1.40±0.08 ^{ab}	13.70±0.16 ^a
Group III	0 day	45.24±0.01 ^a	145.55±0.01 ^a	25.24±0.01 ^a	36.65±0.01 ^a	0.90±0.01 ^b	1.60±0.01 ^a	14.03±0.03 ^a
	30th day	35.47±0.33 ^c	106.32±0.37 ^d	20.97±2.64 ^a	35.4±0.37 ^a	0.89±0.01 ^b	1.51±0.04 ^a	13.93±0.21 ^a

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means \pm SE.

Table 2. The mean values of erythrocytic MDA (nmol/mg protein) and TAC (mmol/mg protein) levels in Group I, Group II and Group III.

Group		MDA	TAC
Group I	0 day	0.25±0.01 ^a	0.27±0.01 ^d
	30th day	0.27±0.01 ^a	0.31±0.01 ^c
Group II	0 day	0.25±0.01 ^a	0.27±0.01 ^d
	30th day	0.13±0.01 ^b	0.64±0.01 ^b
Group III	0 day	0.25±0.01 ^a	0.27±0.01 ^d
	30th day	0.10±0.02 ^b	0.84±0.01 ^a

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means \pm SE.

different induced significant decreases in all lipid profile parameters. It has been reported that ginger improves dietary (cholesterol, fructose, or high-fat diet) or streptozocin-induced lipid derangements in rodents (Beattie et al., 2011).

C. speciosus supplementation has a healthy effect on liver and kidney functions. Bavarva and Narasimhacharya (2008) reported that the diabetic control group exhibited significantly higher amounts of urea and creatinine while the ethanolic extract of *C. speciosus* administered diabetic rats registered significantly lowered urea and creatinine serum level. Other herbs from *Zingiberaceae* family as *Zingiber officinale* is a useful agent for the prevention of renal ischemia reperfusion-induced injuries (Maghsoudi et al., 2011) and carbon tetrachloride renal induced injuries (Hamed et al., 2012). The ginger extract rendered significant protection against induced nephrotoxicity, which was evident from the lowered serum urea and creatinine levels in the mice (Ajith et al., 2007). It may regard to the fact that the ginger exhibit antioxidant activity and anti-free radicals abilities that stimulate the liver performance and urea synthesis (Polasa and Nirmala,

2003).

Free radicals play an important role in oxidative stress related to the pathogenesis of various important diseases. Many properties of plant products are associated with the presence of phenolic compounds which are essential for plant development and play an important role in their defense mechanisms. The inclusion of these compounds in the regular diet might be beneficial to health by lowering the incidence of diseases (Halliwell, 1997). Oxidative stress of erythrocytes of the 30th day was investigated by determination of the MDA level (as lipid peroxidation product) and total antioxidant capacity. The antioxidant activity of *C. speciosus* extracts might be due to redox properties of the phenolic contents which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Nehete et al., 2010; Baskar et al., 2012). Administration of either costunolide (20 mg/kg daily) or eremanthin (20 mg/kg day), a constituent of *C. speciosus*, for 60 days, caused a significant reduction in thiobarbituric acid reactive substances (TBARS) level and a significant increase in GSH content in the treated rats when compared to untreated diabetic rats (Eliza et al., 2010).

The antioxidant activity of *C. speciosus* rhizome might be due to the presence of phytoconstituents such as flavonoids and phenolic compounds (Jha et al., 2010). In regard to serum protein, the electrophoretic pattern had shown a significant increase in total protein in Group III in comparison to control. This result is in accordance with that of Eliza et al. (2009b). Concentration of total protein in serum of ginger-supplemented broilers tended to be higher at 21 days and was higher at 42nd day of age compared with that of control broilers (Zhang et al., 2009). The blood plasma chemistry analysis revealed that protein, albumin and globulins levels of experimental fish supplemented by ginger at the rate of 5 g/kg of diet were significantly higher than that of control fish (Immanuel et al., 2009).

In the same context, hematological investigation, at 30th day, in all treated animals supplemented with *C. speciosus*

Table 3. The mean values of electrophoretic pattern at 30th day in Group I, Group II and Group III.

Group	Total protein (g/dl)	Albumin (g/dl)	α_1 -globulin (g/dl)	α_2 -globulin (g/dl)	β -globulin (g/dl)	γ -globulin (g/dl)
Group I	6.16±0.066 ^b	2.89±0.003 ^b	0.15±0.01 ^b	0.97±0.012 ^c	0.86±0.003 ^a	1.29±0.045 ^b
Group II	6.34±0.026 ^b	2.80±0.003 ^b	0.16±0.01 ^b	1.13±0.007 ^b	0.80±0.009 ^b	1.45±0.006 ^b
Group III	7.09±0.058 ^a	3.12±0.009 ^a	0.19±0.01 ^a	1.17±0.006 ^a	0.85±0.009 ^a	1.76±0.03 ^a

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means \pm SE.

Table 4. The mean values of TEC (10/mm), TLC (10/mm), Hb (g/dl) and PCV (%) in Group I, Group II and Group III.

Group		TEC	TLC	Hb	PCV
Group I	0 day	8.50±0.26 ^c	6.88±0.36 ^c	9.43±0.31 ^b	29.67±0.24 ^c
	30th day	8.43±0.26 ^c	7.03±0.37 ^c	9.72±0.28 ^b	30.33±0.57 ^{bc}
Group II	0 day	8.58±0.20 ^c	7.45±0.52 ^{bc}	9.65±0.27 ^b	29.00±0.37 ^c
	30th day	10.50±0.30 ^b	7.45±0.52 ^{bc}	9.57±0.35 ^b	32.00±0.52 ^b
Group III	0 day	8.68±0.23 ^c	7.50±0.54 ^{bc}	9.68±0.25 ^b	29.83±0.56 ^c
	30th day	12.5±0.54 ^a	10.23±0.29 ^a	11.42±0.23 ^a	37.00±0.55 ^a

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means \pm SE. TEC = total erythrocyte count, TLC = total leukocyte count, Hb = haemoglobin, PCV = packed cell volume.

Table 5. The mean values of differential leucocytes count (%) in Group I, Group II and Group III.

Group		Lymphocyte	Monocyte	Basophil	Eosinophil	Neutrophil
Group I	0 day	58.17±0.97 ^b	4.00±0.41 ^{ab}	0.33±0.15 ^a	6.17±0.46 ^a	31.00±0.75 ^a
	30th day	59.67±0.91 ^{ab}	3.83±0.56 ^{ab}	0.33±0.15 ^a	5.33±0.39 ^a	29.83±0.34 ^a
Group II	0 day	58.17±0.97 ^b	4.00±0.41 ^{ab}	0.33±0.15 ^a	6.17±0.46 ^a	31.00±0.75 ^a
	30th day	61.67±0.6 ^{ab}	2.33±0.24 ^{bc}	0.33±0.15 ^a	5.00±0.41 ^a	30.67±0.35 ^a
Group III	0 day	59.50±0.83 ^{ab}	4.17±0.34 ^a	0.33±0.15 ^a	5.83±0.42 ^a	29.67±0.65 ^a
	30th day	62.33±0.96 ^a	1.33±0.35 ^c	0.33±0.15 ^a	4.83±0.67 ^a	31.67±0.57 ^a

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means \pm SE.

there showed a significant increase in the total count of erythrocytes, the total count of leukocytes, hemoglobin and lymphocytes. These results are in accordance with that induced by ginger (Nya and Astin, 2009) and turmeric (Harikrishnan and Balasundaram, 2008). Moreover, a dietary supplement protects mice against radiation-induced lethality by protecting the bone marrow from radiation, this will be evidenced by a significant increase in RBCs count and hemoglobin (Jagetia et al., 2004).

Conclusion

From the obtained results, we advise to use *C. speciosus* ground roots as a feed additive supplement in Egyptian buffalo to improve the health status of those heifers by enhancement of immunity and antioxidant status.

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