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# Prospective effect of *Calotropis procera* extract as alternative of antibiotic growth regulator for serum biochemical profile of broiler chickens

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**Key words:** *Calotropis procera*, Leaves extracts, Antibiotic growth promotor, Serum biochemical, Broiler chickens

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## Abstract

An experiment for investigation on effects of dietary supplementation of *Calotropis procera* on some of serum biochemical measures of broiler chickens was conducted in the poultry farm Collage of Veterinary and Animal Sciences at Bagdad-ul-Jaded Campus in 2015. Ross-308 breed broiler chicks were purchased from a local hatchery and divided into 5 treatments and 4 replicates based on completely randomized design. Groups as group A or control (fed basal diet, without medicinal plant supplementation), group B (fed 0.25%/ ton enramycin antibiotic), group C (fed 0.5%/ ton *Calotropis procera*), group D (fed 0.1%/ ton *Calotropis procera*) group E (0.15%/ ton *Calotropis procera*). At day-35 two chicks from each replicate were selected and blood samples (whole blood for serum for biochemical measures) were taken. The chicks fed with diet containing pure plant *Calotropis procera leaf powder* (CPLP) 0.15%/ ton had reduced (P<0.05) serum cholesterol, triglycerides and glucose. Supplementation of *Calotropis procera* leaf powder (CPLP) didn't have significant effect on total protein, albumin and globulin. It was concluded that supplementation of *Calotropis procera* leaf powder (CPLP) to broiler diet at level of 0.5, 1.0 and 0.15%/ ton of feed improved significantly serum ALT, AST (liver and kidney functions), total protein, Glucose, antioxidant status, and had beneficial effect to improve immunity status against infection diseases, serum biochemistry and improve the quality of meat. Treatment broiler chicks with *Calotropis procera* leaf powder (CPLP) enhanced nutrients metabolism and improved the absorption of serum minerals.

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## Introduction

*Calotropis procera* belongs to the family Asclepiadaceae with 180 genera and 2200 species. It is commonly known as Aak. It is grown widely throughout the tropical and subtropical regions of Asia and Africa (Ansari and Ali, 2001). Leaf extracts of this specie contains Glycosides, Protein, Triterpenoids (Gupta *et al.,* 2003), Steroids, and Flavonoids (Spring, 2000). Leaves of *calotrops* posse antibacterial contents like cardenolides and procergenin. Roots upper layer stalk have benzoyline-solone β-sitosterol benzoylisolinelone, αlpha-amyrin, βeta-amyrin (Wang *et al.,* 2008; Ahmad, 2009). Different parts of *Calotropis procera* have potential to cure various diseases and disorders (Khare, 2007; Rajesh *et al.,* 2005). In poultry industry, antibiotics are not only used to treat sick broiler chicks, but also to promote faster growth and prevent disease in healthy chicken. Antibacterial were widely used to stabilize and regulate the microorganisms present in intestine with specific dosage to cure which was proved to enhance the growth efficiency in poultry meats for fifty years ago. Although its usage was performing better in growth and development but there was problem of residue accumulation in chick meat. Its residual activity was harmful for human health as it was transferred to human body followed by illness and death due resistance development in antibiotics (Newman, 2002). In addition, carbdox and olaquinodox are common antibiotics used since 1944 with growth promoting efficiency and curative for swine disease but it banned later after its wider usage and rapid accumulation in chick meat in the globe. Use of these two is very dangerous if contaminated meat consumption by humans. Moreover, microbial organisms are posing infectious threat like *Clostridium Botulinum,* Salmonela and Emeria spp. Which are delimiting the growth and developmental performance of chick. These are subsequent threat of economic loss in poultry traders by reducing the weight of meat. Usage of antibiotics in diet for poultry chicks was banned all over Europe by the European Union; whereas antibiotic was banned completely to be used as diet additive in livestock since 2006 (Windisch *et al.,* 2008). So, it was pre-requisite to find alternatives to antibiotics (Chaudhary, 2012).

*Calotropis procera* have charismatic properties of killing microbial agents (viral and bacterial), delimiting bioactivity of such organism. Instead all, stem, leaves and root balk has therapeutic properties, increase the digestion of diet, ease in excretion by providing digestive enzymes and enhance the growth of chicks (Ertas *et al.,* 2005; Williams, 2001; Lee *et al.,* 2003). The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition known as secondary metabolites (Karthikeyan *et al.*, 2009). Use of such plant extracts mix with diet are trending to replace antibiotics and other similar agents in chicks. Plant extracted natural diet admixed substances of are usually supposed harmless, recovering and less hazardous for humans and animals. Before no work was done on *Calotropis procera* as alternative antibiotic growth promoter on serum biochemical profile in poultry and we are reporting very first time with various concentration used as treatment. Therefore, present experiment was designed to study the effect of this plant leaves powder on serum biochemical profile of poultry. This is new work on *Calotropis procera* plant and will be helpful in poultry nutrition and poultry industry.

## Materials and methods

The experiment was conducted in a semi environmentally controlled house in controlled broiler farming. Ross 308 breed broiler chicks were used in the experiment and nutrient recommendations for Ross 308 breed broiler were used as control from local industry. The whole experimental period was divided into three phases: starter (3 to 10 d), grower (11 to 22 d) and finisher (22 to 35 d). The experiment was carried out in the poultry farm Collage of Veterinary and Animal Sciences at Bagdad-ul-Jaded Campus during 2015. Experiment was conducted to evaluate the effect of *Calotropis procera* leaves as alternative of antibiotic growth promoter on serum biochemical profile of broiler chickens. *Calotropis procera* fresh leaves for experiment were collected at 5$^{th}$ April 2015 from sandy soil at Biodiversity Park CHOLISTAN desert. Gloves were used during the collection of leaves. The leaves were separated from twigs and branches and

cleaned by washing with water to remove the dirt particles. The leaves used for the preparation of powder dried naturally provided adequate measures are taken to prevent contamination or moisture of the material during the process. Different quantities of powder were mixed in diets. The shed was thoroughly disinfected and white washed before the initiation of experiment. Shed was fumigated using $KMNO_4$ 250g and Formalin 500ml and dividing both formalin and $KMNO_4$ into two parts $KMNO_4$ 125g, 125g and formalin 250ml,250ml used to disinfect the experimental room thoroughly before the initiation of experiment.

On arrival, chicks were weighed and kept in three pens for first three days and local diet was used on third day. Chicks were randomly distributed in 20 pens and then used the experimental diets, and this divided in five treatments with four replicates of 20 chicks in each pen based on a completely randomized design. Chicks were fed by basal diet as control group, the antibiotic group received 3.125mg/ half bag enramycin and no *Calotropis procera* powder was used in this group, *Calotropis procera* powder at levels of 25g/ bag and 12.5g/ half bag and 2g/half bag enramycin used in third group. In fourth group 1g enramycin and 25g/half bag *Calotropis* powder used in fifth group no antibiotic 37.5g/ half bag Calotropis powder is used respectively as natural growth promoter. *Calotropis procera* powder was mixed well with feed every other day to avoid decomposition. Each replicate pan was 5.5×3.2 ft in measurement and the pans were allotted randomly to different experimental units. The experimental room length is 50 feet height 8 feet and width 38 feet. Rice hulls were used as litter material which was stirred regularly to keep it in dry conditions. The wet or moist litter replaced with dry litter to prevent the infection. The temperature was maintained 30°C and humidity was 65%. First vaccination was done at day 5, Vaccine was used Newcastle disease (ND) and second vaccination was done at day 12, vaccine was used Infectious Bronchitis (IB) both these vaccine used by eye drop method and third vaccination was done at day 22, vaccine was used after one hours starvation skimmed milk was added in water after 15 minutes vaccine was added in drinking water.

*Experimental diets*

The basal diet was analyzed in triplicate for their dry matter (DM) 95.87%, either extract, crude fiber (CF) and AA contents. The Nitrogen was recorded in triplicate by the Kjeldahl method and Crude Protein cp was recorded. The fatty substance (fat) was ether extract v.ia protocol of Soxhlat named apparatus. The basal diet contains 2900 kcal/kg metabolizable energy (ME) and 21% protein respectively and crude fiber ether extract were included according to Ross 308 recommendation. The basal diet was distributed in to experimental diets were divided into five groups as:

A = Control, No *Calotropis procera* and no antibiotic

B = No *Calotropis procera* but antibiotic (enramycine 3.125g/ half bag)

C = *Calotropis procera* powder + Antibiotic (Enramycin antibiotic 2g/half bag and *Calotropis procera* powder 12.5g/ half bag

D = *Calotropis procera* powder + Antibiotic (1g/half bag enramycin and 25g/half bag *Calotropis procera* powder

E = *Calotropis procera* powder (*Calotropis procera* powder level 37.5g/ half bag

*Blood parameters*

At the last day of experiment, two birds per pan were selected for taking blood samples. The blood samples were taken from wing vein and were collected into iced tubes. Heparin was used as an anti-coagulant. After centrifugation, plasma was stored refrigerated until analysis for hormones and metabolites. Plasma triiodothyronine ($T_3$) and thyroxin ($T_4$) (triglycerides, uric acid, plasma glucose) content was recorded with recommended apparatus. The serum triglycerides, cholesterol, ALT and AST concentrations were recorded using spectrophotometer and colorimetric method using diazonium salt following standard protocols.

*Statistical analysis*

All the numeric data was collected and entered in excel sheet on which linear regression with probability value of ($P<0.05$) and Tukey's honesty's significant test was applied using Statistix V9.1., (SAS institute). In addition, quadratic regression followed by Linear analyses was also carried out to find the several CP levels (Steel *et al.*, 1997).

**Results**

The higher level of uric acid was observed in groups B, C and in control group as compared to group D and E. The highest level of uric acid 5.200 was observed in group D fed with diet *Calotropis procera* leaf powder 0.1%/ton and antibiotic enramycine 0.08%/ton. The lowest level of uric acid 2.675 was observed in control group fed with basal diet no supplementation of *Calotropis procera* leaf powder (CPLP) and antibiotic (Table 1, Fig. 1).

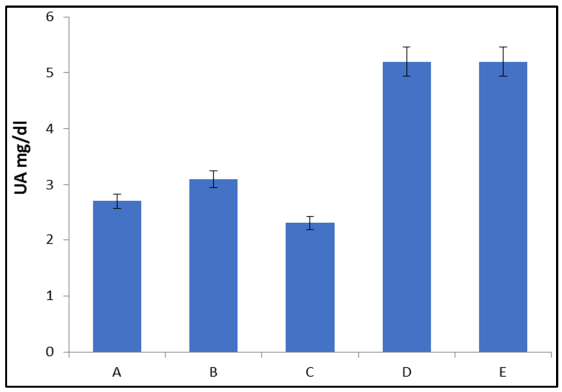


**Fig. 1.** Uric acid concentration in broilers after various treatments of *Calotropis procera*.

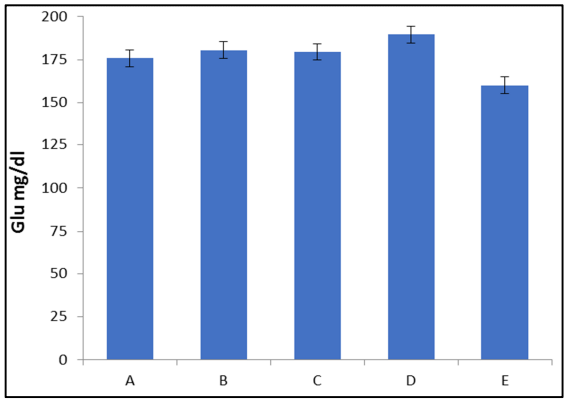


**Fig. 2.** Glucose level in broilers after various treatments of *Calotropis procera*.

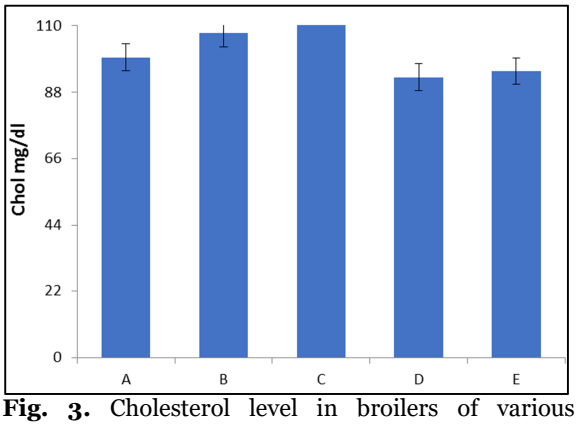


**Fig. 3.** Cholesterol level in broilers of various treatments of *Calotropis procera*.

There was miner increase in glucose value in group B and D as compared to control group and significant decrease in glucose value in group E as compared to groups A,B,C and D. Serum cholesterol values of group B and C on the day 35 of experiment were higher than groups A,D and E and decreasing trend in Cholesterol value was observed in group D as compared to other groups A,B,C and E. Serum triglycerides values of group B at the end of experiment was significantly higher than groups A,C,D and E. A significant decrease in serum triglycerides values were observed in group C and E as compared to other groups A increasing trend in concentration of glucose recorded in group D 189.5mg/dl at 35 day of experiment fed with diet containing plant *Calotropis procera* leaf powder at 0.1%/ton and antibiotic enramycine 0.08%/ton and having slightly difference to group B having 180.5mg/dl glucose value.

The minimum value of glucose 160mg/dl was observed in group E fed with diet containing *Calotropis procera* leaf powder at 0.15%/ton. The serum glucose value in group D at day 35 of experiment was higher in comparison to other groups (Table 1, Fig. 2, 3, 4). A miner higher level of Alanine amino transferase (ALT) in group E was recorded at the end of experiment in comparison to group B, C, D and control group A.

The serum Asparatate amino transferase (AST) levels of groups B and E on day 35 were higher in comparison to groups C, D and control group A. Non-significant difference was observed in serum protein concentration in treated and control group at the end of experiment. There was no significant difference in serum protein Albumin concentration in treated groups as compared to control group.

Similarly, no significant difference was observed in serum protein globulin concentration in treated and control group at the end of experiment. The results of this experiment were successful as no toxic effect of plant extract was observed on serum biochemistry of broilers (Table 1, Fig. 5, 6, 7, 8).

**Table 1.** Serum biodhemistry of broilers fed diets containing various levels of *Calotropis procera* leaf powder (CPLP) and antibiotic growth romoters from 1 to 35 days of age[1].

| Groups | Diets[2] | Glu (mg/dl) | UA (mg/dl) | Chol (mg/dl) | TG (mg/dl) | ALT (µ) | AST (µ) | Pro (g/dl) | Alb (g/dl) | Glo (g/dl) |
|---|---|---|---|---|---|---|---|---|---|---|
| A | Con | 176 | 2.67[b] | 100 | 66 | 11.3 | 139.5 | 2.3 | 1.8 | 0.350 |
| B | CP (0) | 180.5 | 3.1[b] | 108 | 122 | 11 | 156 | 2.4 | 2 | 0.375 |
| C | CP (0.05%/ton) | 179.5 | 2.1[b] | 117 | 26.3 | 15 | 133 | 2.6 | 2.3 | 0.350 |
| D | CP (0.1%/ton) | 189.5 | 5.2[a] | 93 | 48.3 | 16 | 98.3 | 2.7 | 2.4 | 0.300 |
| E | CP (0.15%) | 160.5 | 5.2[a] | 95 | 25 | 25.3 | 155 | 2.8 | 2.3 | 0.475 |
| | SEM | 15.5004 | 0.6368 | 15.4305 | 26.06 | 4.8807 | 15.539 | 0.1942 | 0.1887 | 0.0540 |
| ANOVA | | | | | Probability | | | | | |
| Diets | | 0.747 | 0.022 | 0.799 | 0.105 | 0.280 | 0.111 | 0.315 | 0.155 | 0.271 |

[a-b]Means within a column with different superscripts differ significantly ($P < 0.05$)

[1]Means of 4 replicates with 2 birds in each replicate

[2]Control (no supplementation); *Calotropis procera* leaf powder (2.5kg, 5kg, 7.5kg/ton of feed) and enramycin (125g, 82g, 40g/ton of feed).

Glu; glucose, UA; uric acid, Chol; cholestrol, TG; triglycerids, ALT; alanine amino transferase, AST ; asparatate amino transferase, Pro; protein, Alb; albumin, Glo; globulin.

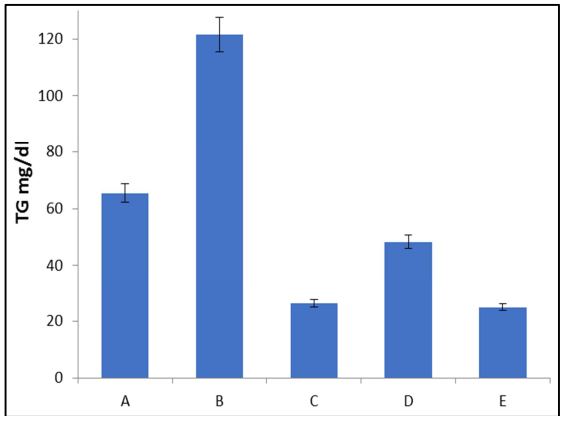


**Fig. 4.** Triglycerides concentration in broilers of various treatments of *Calotropis procera*.

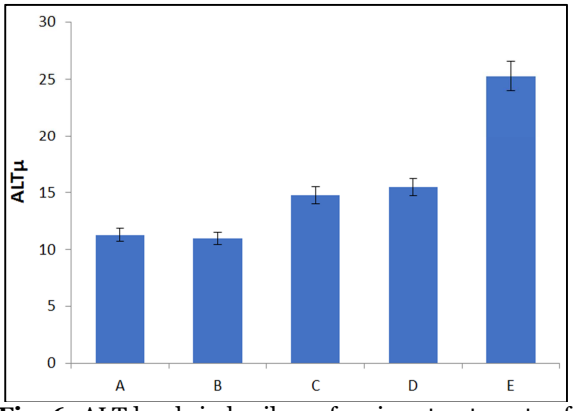


**Fig. 6.** ALT levels in broilers of various treatments of *Calotropis procera*.

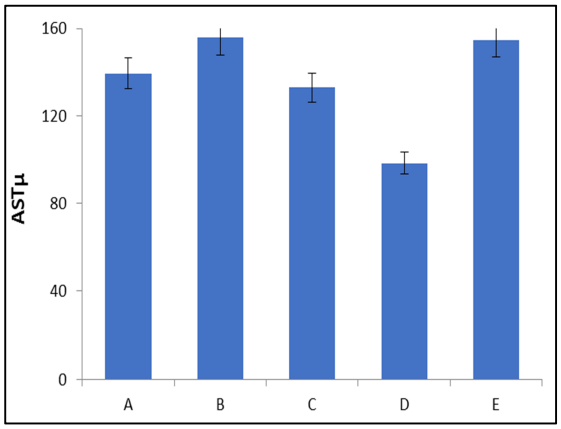


**Fig. 5.** AST levels in broilers after various treatments of *Calotropis procera*.

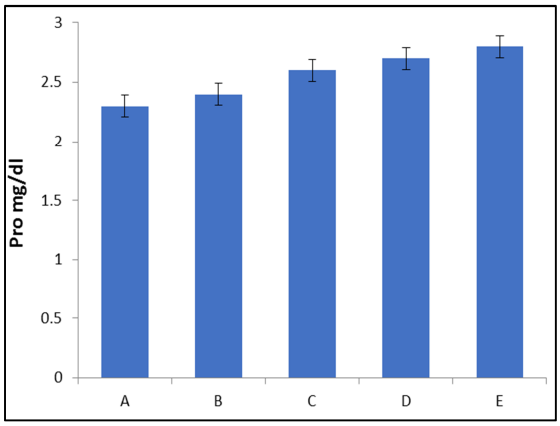


**Fig. 7.** Protein concentration in broilers after various treatments of *Calotropis procera*.

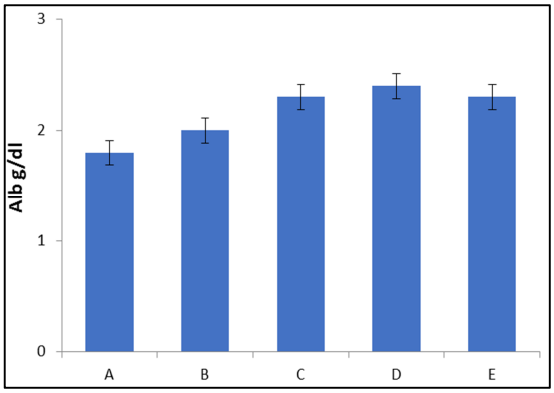


**Fig. 8.** protein albumin levels in broilers after various treatments of *Calotropis procera*.

An increasing trend in level of Alanine amino transferase (ALT) recorded in group E at 35 day of experiment was 25.3µ/L fed with plant *Calotropis procera* 0.15%/ton and having slightly difference to group D Alanine amino transferase (ALT) level is 15µ/L. The minimum level of Alanine amino transferase (ALT) 11µ/L was observed in group B fed with diet containing enramycine 0.164%/ton having slightly difference to control group A. Alanine amino transferase (ALT) level is 11.3µ/L. The serum Alanine amino transferase (ALT) level in group E, at day 35 of experiment was higher in comparison to other groups. An increasing trend in level of Asparatate amino transferase (AST) recorded in group B at 35-day of experiment was 156µ/L fed with diet containing enramycine 0.25%/ton and having silghtly difference to group E Asparatate amino transferase (AST) level was 154.8µ/L. The minimum level of Asparatate amino transferase (AST) 98.3µ/L was observed in group D fed with diet containing *Calotropis procera* leaf powder at 0.1%/ton and enramycine 0.08%/ton having significant difference to all other groups. The serum Asparatate amino transferase (AST) level in group B at day 35 of experiment was higher in comparison to other groups. Higher value of protein recorded in group E at 35-day of experiment was 3g/dl fed with diet containing *Calotropis procera* leaf powder at 0.15%/ton and having slightly difference to other groups. The minimum value of protein 2.3g/dl was observed in control group A fed with basal diet only no supplementation of plant. There was no significantly difference in serum protein values in treated group as compared to control group. The minimum value of protein 1.8g/dl was observed in control group A fed with basal diet only no supplementation of plant. Maximum value of protein globulin recorded in group E at 35-day of experiment was 0.5g/dl fed with diet containing *Calotropis procera* leaf powder at 0.15%/ton and having slightly difference to other treated and control group. The minimum value of protein globulin recorded in treated group D was 0.3g/dl is lower than all other groups. (Table 1, Fig. 8, 9).

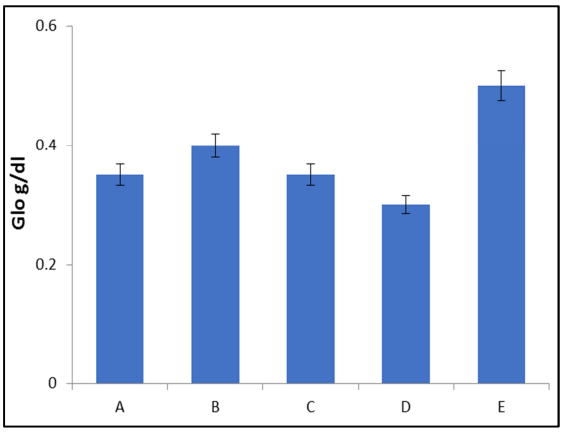


**Fig. 9.** protein Globulin levels in broilers after various treatments of *Calotropis procera*.

**Discussion**

Aak Plant extracts showed positive effects on serum biochemistry of broilers and no negative effects were exhibited in broilers. This was resulted in that *Calotropis procera* have a significant positive effect on the serum glucose of Broilers. Present research study was truly consistent with the results obtained by Dehkordi *et al.,* (2010). It was reported by this scientist that glucose content of chick meat was lower down when supplement of ginger extract mixed diet was used for growth. There was no similarity between each treatment within groups. In addition, Jarukamjorn *et al.,* (2006) used *Andrographis paniculata* leaf extract for hyperglycemic rats and observed the glucose level. He reported that resultant level was lower down from the normal rats groups. It was also concluded that significant reduction in glucose content in hyperglycemic rats was due to the presence of diterpenoids and flavonoids compounds in mixed diet.

This was also reported by Jain *et al.,* (2000), and Kin *et al.,* (2006) while similar results were declared by Kim *et al.,* (2006) for anti-hypoglycemic activity in same family of plant. Our result was supported by Al-Jaff, (2011) experiment in which he used coriander as di*et al*ong with its seed @ one and two percent level and demonstrated that content of glucose serum was somehow lowered comparing the control. Similar results were obtained using coriander oil mixed diet by Al-Mashhadani *et al.,* (2011) during hot season. Moreover, anti-hypo and hyper-glycemic activity was resulted using *Veronica amaygdalina due* to the presence of flavonoids in the leaf extract. Reducing level of serum urea was found in our which is in agreement with research findings of Iyayi and Tewe, (1998) who reported that level of protein available in diet was reason of this reduction. Similar results were depicted by Ekpo *et al.*, (2007) in which level of urea was not changed exhibiting function of kidney was disturbed after the use of V*eronica amygdalina*. Serum urea is commonly used to indicate level of renal function and possible damage to kidney architecture (Al-Jaff, 2011). In addition, level of cholesterol was also decrease after the experimentation on rats using coriander herb as diet mixed for feeding the chicks. Mathivanan and Edwin, (2012) resulted in lowering the content of total and LDL cholesterol and triacylglycerol concentrations while cholesterol-HDL level was not altered from the normal. These results true to our findings in which its level was reduced too comparing control. In another research, results of Neto *et al.,* (2013) are truly and significantly agreement with our results in which serum cholesterol was lower down. Moreover, *Andrographis paniculata* was used by Bharathi *et al.,* (2011) and Sivaraj *et al.,* (2011) @ <1% in mix-diet form for twenty-nine to forty-five days duration in broilers-chicks which significantly downgraded serum cholesterol content. It was inferred that one enzyme called catalase was increased from its level which develops esters of serum cholesterol after the usage of plant extract in the plasma. The present results are similar to the result reported by Mchedlishvili *et al.,* (2005) that flavonoids isolated from *Satureja hortensis* had cholesterol-lowering effect in a situation of rising serum cholesterol.

Different broiler chicks were given variable concentration up to ten percent level of neem plant extract used as diet mixed seed cake and recorded results demonstrated that crucial enzymes ALP, AST, ALT with nitrogen, and creatine were not significantly affected. This type of supportive research was conducted by Obikaonu *et al.,* (2011) in similar plant extract was utilized and same ezyme profile was tested on broilers with starter diet plan. These investigations were supporting our research work. In addition, it was also concluded by same researcher that level of sugar on blood was enhanced while enzymatic profile tested was not prominently lowered. The increase in ALT level was indicating hepatotoxicity effect due to release of its content in blood stream. This concluding remark was undertaken by Mansour *et al.*, (2002) after its observations on chicks and its finding are strongly in line with our findings. Similarly, AL-Homidan, (2005) reported that chicks fed two percent for 7 weeks *Zinger officinale* diet showed no significant change in ALT activity between *Zinger officinale* group and control chicks.

AST and ALT used to find out liver and kidney damage which might be due to tumor or kidney problem. When either of the enzyme level enhances, it is indication of kidney or liver function problem (Modu *et al.,* 2000) but mean value of Alkaline Phosphatase levels did not increase significantly. In the previous study the neem leaf aqueous extract proved to be toxic for both liver and kidney of chicken as compared to the present study where dry neem improved the function of these two organs. This difference in the findings of the two studies might be due to dissimilarity in forms of neem given and the method of application of the herb. The results of this study were not in-line with the work done by Tollba *et al.,* (2009) who used neem leaves powder in broiler feed and observed that creatinine, ALT and AST levels were not affected. The significant decrease in the levels of biochemical marker enzymes like ALT.

The APPs assay may have potential for monitoring adverse environmental and/or management stressors, thus enabling better control of animal welfare (Murata, 2007).

The reduction in Albumin/ Globulin ratio observed by inclusion of rosemary diets in our study was also accompanied by a significant lower total cholesterol level.

A decrease in Albumin/Globulin ratio related to a decrease in total cholesterol levels was reported by Ghazalah and Ali, (2008). Ratio of A/G was decreased with the addition of experimental additives which may indicates improved immunity for the birds this was implied by the increase in the globulin level compared to albumin which was stated before by Zomrawii *et al.,* (2012) that the increase of serum globulin indicates that birds are immunologically strong and suggested that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects.

The results obtained from *Zingiber officinale* treatment were similar to AL-Homidan, (2002) findings who claimed that, the chicks fed 2% (w/w) for 7 weeks *Zingiber officinale* diet showed that there is a noticeable decrease in the Albumin/Globulin ratio compared to control chicks.

**Conclusion**

Based on the obtained results, supplementation of *Calotropis procera* leaf powder (CPLP) to broiler diet at level of 0.5, 1.0 and 0.15%/ ton of feed improved significantly serum ALT, AST, total protein, Glucose, antioxidant status, and had beneficial effect to improve immunity status against infection diseases, serum biochemistry and improve the quality of meat. Treatment broiler chicks with plant leaf powder enhanced nutrients metabolism and improved the absorption of serum minerals in this. Furthermore, these findings strongly recommend that *Calotropis procera* leaf powder (CPLP) is a promising novel natural health hypolipidemic treatment that may act by multiple mechanisms to reduce serum total cholesterol, triglyceride and oxidation enzymes. The future quest should also include the processing of *Calotropis procera* leaf powder with certain elements and methodologies to eliminate *Calotropis* toxins especially the triterpenoids so that the herb can be incorporated in to the poultry diet in a safer way.

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