

# Evaluation of *Morinda citrifolia (Noni)*, *Azolla pinnata* and *Andrographis panniculata (Kalmegh)* as herbal tonics in indigenous Nicobari fowl

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### **Abstract**

A study was carried out to evaluate the efficacy of Andrographis panniculata (Kalmegh), Morinda citrifolia and Azolla pinnata on serum biochemical parameters in Nicobari fowl. Experiment I comprised of treatments with supplementation of Morinda juice in water @ 5 ml per bird per day, Azolla dry powder in feed @ 3 g per bird per day and Growiplex (Vitamin B complex tonic) in water in comparison with control birds. The SGOT (Serum glutamic oxaloacetic transaminase) level was significantly (P< 0.01) higher with supplementation of *Morinda* juice as water supplement (a) 5 ml per bird per day. Significantly (P<0.01) higher level of total serum bilirubin was observed in Morinda juice, Azolla and Groviplex supplementation. All herbal supplementation groups had significantly (P<0.01) reduced the serum cholesterol level by 13% as compared to control group. Herbal supplementation did not have significant effect on the serum glucose. In the experiment II with Kalmegh, both water and feed supplement had significantly (P<0.01) lowest serum cholesterol (reduction of 23% and 26% respectively) on seventh day of supplementation as compared to control birds. The SGOT was significantly (P<0.01) reduced by 15% on third day supplementation of kalmegh in water. Serum glucose was significantly reduced on fifth day of supplementation of kalmegh as both feed and water supplement and it was sustained till seventh day of supplementation. The bilirubin level was significantly (P<0.01) reduced by 16% on fifth and seventh day of supplementation in both water and feed. Supplementation of Andrographispanniculata as water supplement in Nicobari fowl significantly increased the levels of haematinic mineral iron and immunity enhancing minerals.

Key words: Nicobari fowl, Noni, Azolla, Kalmegh, Serum Biochemical profile, herbal tonics

### Introduction

The poultry industry is one of the most leading agribusiness trades in the world. Feed supplements in poultry production has significant role to improve the productivity of poultry among which antimicrobial compounds are commonly included in poultry diets for promoting growth and to control diseases. The ban for antibiotics as growth promoters by the European Union in 2006 and keeping in view of consumers' food safety and security, the research is now shifted towards the alternative feed supplements and organic additives/plantbased compounds/extracts as herbal growth promoters. It has been well proven that the use of herbal supplements improves the growth, production, immunity and several other beneficial effects in poultry (Narimani-Rad et al., 2011; Sunderet al., 2011) through action of many different bio-active ingredients such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins and tannins

(Wang et al., 1999). Medicinal plants are basically involved in a cascade of physiological reactions, that in turn lead to the alteration of haematological and serum biochemical parameters (Ewuola and Egbunike, 2008). The level of stress poultry undergoes while taking herbal medicines must be evaluated in terms of lowering or elevating the haematological and biochemical values. Growth and production depends on metabolism that is best assessed by biochemical profile. Those values must be maintained within the r1eference ranges for chickens (Mitruka and Rawnsley, 1977).

Morinda citrifolia var. citrifolia, popularly known as Noni is a member in the diversified medicinal plants of A & N Islands. In Andaman & Nicobar Islands, the plant is mainly found in the Nicobar group of islands and is one of the most significant shrubs of traditional medicines among Nicobari tribes of these Islands (Sunder et al., 2007). All the components of this plant are utilised to prepare



alternative and herbal medicines for human ailments (Solomon 1999). Due to its wide range of health benefits and therapeutic value, the studies on the effect of feeding of crude fruit extract to Nicobari fowl and broilers have been carried out(Sunder et al., 2007 and 2011). Nitrogen fixing aquatic fern, Azollahas improved the humoral and cellular immunity of the Nicobari fowl (Kannaiyan and Kumar, 2005, Sujathaet al., 2013). Similarly, Andrographi spanniculata (AP), a shrub found throughout Southeast Asia is a well known medicinal plant as an immune system booster and for treating sore throat, flu and upper respiratory tract infections. However, the biological effect of Morinda citrifolia, Azollapinnata and Andrographis panniculata on biochemical characteristics in chicken is to be cleared even though already few works had been carried out in rats and other species (Mani Saminathan et al., 2014). Hence, experiments were carried out to study the efficacy of Morinda citrifolia, Azollapinnata and Andrographis panniculata on serum biochemical parameters in the Nicobari fowl of A&N islands.

#### Materials and Methods

**Preparation of herbs:** Azolla was cultivated, harvested, dried under sun and dried. Morinda juice was prepared as per the protocol (Sunder *et al.*, 2011).

## Experiment I

Forty eight Nicobari fowls of same hatch at 40 weeks of age were randomly subjected to each of four treatmentsviz.,  $T_1$ : Supplementation of Morinda juice in water @ 5 ml per bird per day;  $T_2$ : Supplementation of Azolla powder in feed @ 3 g per bird per day;  $T_3$ :Supplementation of Growiplex in water;  $T_4$  (Control): No supplementation. Each treatment comprised of twelve birds with three replicates of four birds per replicate. Birds were under treatment for 10 days under deep litter system. The common layer feed contained 2600 Kcal of ME kcal / kg of feed with dietary crude protein levels of 18 per cent. The ingredient and nutrient composition of layer feeds are presented in Table 1.







Table 1. Per cent composition of Basal layer ration (Feed)

SI.No	Ingredients	Per cent	
1	Yellow Maize	49.00	
2	Broken rice	4.00	
3	Cumbu/Bajra	5.00	
4	De-oiled rice bran	4.50	
5	Wheat bran	2.00	
6	Sunflower oil cake	3.50	
7	Soybean oil cake	19.50	
10	Dry fish	5.20	
11	Mineral mixture*	2.00	
12	Di-calcium phosphate	0.29	
13	Shell grit	5.20	
	Total	100	
Per cent nutrient composition			
1	Crude protein (%)*	17.85	
2	Calcium (%)*	3.12	
3	Total phophorus (%)*	0.58	



**Biochemical parameters:** Two milliliters of blood was collected from all birds at the 10<sup>th</sup> day of experiment and serum was separated and kept at -20°C for biochemical studies. Serum total cholesterol, glucose, Bilirubin and SGOT was quantified using ERBA Automatic Biochemistry Analyzer by ERBA kit based on CHOD-PAP methodology (Allain*et al.*, 1974) and GPO-PAP method (Bucolo and David, 1973).

# **Experiment 2:**

**Preparation of aqueous Kalmegh extract**: Fifty gram of kalmegh powder was soaked in water (200 ml) whole night and was filtered.

**Experimental design:** Thirty breeders of Nicobari fowls were selected at 35 weeks of age. Fowls were assigned to each of dietary treatments namely, T<sub>1</sub>: **Kalmegh feed supplement** (Kalmegh powder with feed @ 3g per bird per day; T<sub>2</sub>: **Kalmegh water supplement** (Supplementation of Kalmegh ageous extract in water for Oral administration) @ 10 ml per bird per day; T<sub>3</sub>(Control): No Supplementation. All birds were fed ad libitum feed as per Bureau of Indian Standards (BIS, 2007) recommendation under deep litter system with 16 hours light of 3 lux intensity per sqft.

**Biochemical profile:** Blood was collected from all experimental birds on 0, 3<sup>rd</sup>, 5<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> day of experiment. Sera was separated and kept at -20□ C for biochemical studies. Serum total cholesterol, glucose, Bilirubin and SGOT was quantified using Automatic Biochemistry Analyzer by ERBA kit based on CHOD-PAP methodology (Allain*et al.* 1974) and GPO-PAP method (Buccolo and David, 1973).

Micronutrient analysis of serum: The serum collected on third day of kalmegh supplementation in water was mixed with De-ionized water at 1:10 ratio. The samples were assayed for Iron and copper content using Atomic Absorption Spectro-photometry and were expressed as ppm.

**Statistical analysis:** Statistical analysis of data of experimental I and II was carried as per Snedecor and Cochran (1994) by using ANOVA. The significance of the difference among the groups was determined by Duncan's multiple range tests (Petrie and Watson, 1991).

### **Results and Discussion**

### Experiment I

# Effect on serum biochemical profile of Nicobari fowl layers

The effect of herbal supplementation on biochemical profile is presented in table 2. The serum bilirubin, cholesterol and SGOT differed significantly among treatment groups. Supplementation of Noni juice @ 3ml per bird in the present study reduced SGOT ranging by 23% as compared to Azolla supplementation, commercial tonic and control groups. This per cent of reduction is agreed with the findings of Lovita et al. (2014) who found that supplementation of Noni juice @ 3% reduced SGOT by 25.94% from 234.67 U/L to 186.33, U/L. Pro-xeronine compound and the enzyme pro-xeronase present in noni juice might be attributing to protect liver cells so that the low level of SGOT was recorded in the study. Liver acts as the most important organ in the body's metabolism. Abnormalities of liver function can be diagnosed from the increased levels of SGOT. Hepatic cell damage can be caused by excessive processes such as reduction, oxidation, hydroxylation and conjugation in the metabolism of medicinal plants. Low level of SGOT in the present study is the indicators of normal functioning of liver cells. Herbs in general have the hepato protective effects as they decrease serum GOT levels (UmitPolat et al., 2011; Soltanet al., 2008; Abou-Elkhair, 2014). Azolla supplementation has decreased the SGOT at statistically comparable to commercial tonic and control. This significant reduction in serum GOT level with Azolla might be due to presence of antioxidants such as pigments.

The noni and Azolla supplementation reduced the secretion of bilirubin to a level that is statistically on par with the commercial tonic. Hossain et al. (2013) has recorded the ability of water plantain (Alisma canaliculatum) to lower serum bilirubin. Low level of serum GOT enzymes and bilirubin provides the research base for the hepato protective effect of Noni and Azolla. It is inferred that low levels of serum bilirubin and SGOT are attributed by bio active compounds present in many



herbal and medicinal plants which in turn have not exertedtoxic effect on the liver which otherwise might have resulted in necrosis or changes in cell membrane permeability and there by these parameters would have increased(Hossain, et al., 2013).

Supplementation of noni and Azolla reduced serum cholesterol significantly by 17% as compared to control group and this reduction is statistically on par with commercial immune booster. These results are opined by previous reports by Sunder *et al.* (2011) and Lovita *et al.* (2014) who recorded the same per cent of reduction in serum cholesterol by supplementing noni @ 3%. Similarly, supplementation of sun-dried azolla at 4.5% in broiler diet reduced cholesterol in serum and meat (Balaji *et al.*, 2009). The reduction in serum cholesterol level is attributed to presence of beta-carotene and flavonoids

in Noni and Azolla(Nuraini *et al.*, 2008). Beta-carotene can inhibit the action of HMG-CoA (3-hydroxy-3-methyl glutaryl Co-A) reductase, enzyme that plays a role in the formation of mevalonate in cholesterol biosynthesis. Both of these compounds will form cholesterol through a series of reactions. The findings of the present study is opined with number of medicinal plants in poultry feed such as Tulsi, Amla, Turmeric, Rosemary, Aloevera, Black pepper and Coriander (Lovita *et al.*, 2014; AbouElkhair *et al.*, 2014; UmitPolat *et al.*, 2011)

Noni and Azolla did not have hypoglycosemic effect since there was no significant reduction in serum glucose level though numerical decrease was recorded with herbal supplementation. However, the recorded mean serum glucose level (242mg/dl) is within the normal range reported as in previous reports.

Table 2: Effect of various herbal supplementations on serum biochemical profile of native Nicobari fowl

Treatment groups	SGOT*(U/dl)	Bilirubin(mg/dl)*	Cholesterol* (mg/dl)	Glucose (mg/ dl)NS
T1:Morinda juice @ 5ml per bird per day	$150.21 \pm 8.46$	$0.045 \pm 0.00$	$98.25 \pm 4.17$	$233.8 \pm 9.62$
T2: <b>Azolla @ 5 gm per bird</b> per day	$165.7 \pm 10.5$	$0.044 \pm 0.00$	$106.1 \pm 3.48$	$246.27 \pm 9.5$
T3:Growiplex (commercial tonic)	$195.1 \pm 17.4$	$0.051 \pm 0.01$	$101.9 \pm 7.83$	$260.1 \pm 16.5$
T4:Control (without supplementation)	$194.3 \pm 9.10$	$0.073 \pm 0.01$	$123.2 \pm 4.23$	$252.1 \pm 9.12$

<sup>\*</sup>Mean values in column having different superscripts vary significantly (P<0.05); NS-Non Significant

### Experiment II

# Biochemical profiles at various days of supplementation of Kalmegh (Andrographis panniculata)

### Serum cholesterol

Serum cholesterol at various days as influenced by kalmegh supplementation is presented in Table 3. The cholesterol was significantly (P<0.01) reduced by 15% on third day supplementation of kalmegh in feed; while the similar trend of significantly (P<0.01) lowest serum cholesterol by 20% was observed with birds fed kalmegh feed supplement as compared to kalmegh water

supplement and control groups. Kalmegh as both water and feed supplement had significantly (P<0.01) lowest serum cholesterol (reduction of 23% and 26% respectively) on seventh day of supplementation as compared to control birds. The early onset of hypo-cholesterolemic effect with kalmegh as feed supplement is due to presence of high fibre content in feed. This finding is in agreement with Mathialagan and Kalaiarasi (2007) who reported the decrease in serum cholesterol with supplementation of *A. panniculata*in broilers. However, the overall mean serum cholesterol of 125, 110 in kalmegh supplemented and control groups of present study was below the level reported in the previous reports which might be due to strain difference. The present study is confirming that



other medicinal plants viz., ginger, cinnamon, coriander,

kalmegh is having potent hypocholesterolemic effect like chicory and aniseed (Bashir et al., 2014; Issa and Omar, 2011; Rahimi et al., 2011; Soltanet al., 2008;).

Table 3: Effect of Kalmegh supplementation on serum cholesterol (mg/dl) at various days of supplementation

Day of supplementation	Kalmegh water supplement	Kalmegh feed supplement	Control
1st NS	$128.28 \pm 6.02$	$127.42 \pm 7.33$	$126.92 \pm 4.79$
3 <sup>rd</sup> **	$126.67 \pm 7.32^{b}$	$105.42 \pm 4.03^{a}$	$125.50 \pm 8.59^{\rm b}$
5 <sup>th</sup> **	$128.58 \pm 3.52^{b}$	$99.50 \pm 7.94^{a}$	$133.58 \pm 6.53^{\mathrm{b}}$
7 <sup>th</sup> **	$116.66 \pm 10.08^{a}$	$110.92 \pm 8.86^{\rm a}$	$151.83 \pm 8.74^{\rm b}$

<sup>\*\*-</sup>Highly Significant, NS- Not Significant

### Serum GOT

The effect of kalmegh supplementation on SGOT level at various days of feeding is presented in Table 4. Serum biochemistry can reflect the condition of an organism and the changes happening to it under the influence of internal and external factors. Liver enzymes GOT can be measured in serum as markers of hepatic damage. The SGOT was significantly (P<0.01) reduced by 15% on third day supplementation of kalmegh in water; while kalmegh supplementation through feed had statistically reduced to moderate level that was comparable with control group. Significantly (P<0.01) lowest SGOT was observed in birds fed with kalmegh through either feed or water on fifth (18% reduction) and seventh (25% reduction) day of feeding as compared to control groups. The serum GOT level was significantly lower in kalmegh fed groups compared to control groups which proved that the hepatoprotective activity of kalmegh due to presence of and rographolide (Handa and Sharma, 1990). The mean level of 180 U/dl in treatment groups is on par with the level reported in previous reports and this finding is in agreement with earlier works of Mathivanan and Kalaiarasi (2007) who reported the significant reduction of SGOT in broilers fed with A. panniculata.

Table 4: Effect of Kalmegh supplementation on serum SGOT (U/dl) at various days of supplementation

Day of supplementation	Kalmegh water supplement	Kalmegh feed supplement	Control
1st NS	$209.90 \pm 11.21$	$189.72 \pm 17.58$	$203.37 \pm 11.36$
3 <sup>rd</sup> **	$173.24 \pm 3.74^{\rm a}$	$198.84 \pm 13.18^{ab}$	$204.8 \pm 13.84^{\rm b}$
5 <sup>th</sup> **	$182.34 \pm 10.2^{a}$	$178.6 \pm 8.16^{a}$	$221.32 \pm 14.49^{\rm b}$
7 <sup>th</sup> **	$185.85 \pm 7.88^{a}$	$177.42 \pm 6.9^{a}$	$246.9 \pm 27.7^{b}$

<sup>\*\*-</sup>Highly significant; NS-Not significant

### Serum glucose level

The influence of kalmegh supplementation on serum glucose level at various days of feeding is given in Table 5. On third day of supplementation of kalmegh as water supplement, 9 per cent and 16 per cent reduction was observed as compared to kalmegh as feed supplement and control groups respectively and that was significantly (P<0.01) highest. Serum glucose was significantly reduced on fifth day of supplementation of kalmegh as both feed and water supplement and it was sustained till seventh day of supplementation. Mathivanan and Kalaiarasi could not find any significant reduction in serum glucose with feeding of A. panniculatain broilers.



However, herbal supplementations in poultry have proven their anti-hyperglycemic effect (Hosseinzadeh et al., 2014; Panduranget al., 2011; Soltanet al., 2008). The serum glucose level (210 mg/dl) of the present treatment groups have been claimed to be normal in poultry (Panduranget al., 2011). The antioxidant properties of aqueous leaf extract of the plant has been demonstrated in streptozotocin-induced diabetes. Oral administration of kalmegh plant extract led to significant reductions in blood glucose levels (Dandu and Inamdar, 2009). The plant reportedly showed insulin-releasing actions in vitro, when tested on pancreatic b-cells (Wibudiet al., 2008). *In vitro* a-glucodidase and a-amylase inhibitory effects have been shown for extract of the plant and its component, andrographolide, which suggests that the plant can be a potential candidate for the management of type 2 diabetes mellitus (Subramanian et al., 2008). Anti-hyperglycemicaction in streptozotocin-induced diabetic rats has been shown by an herbal preparation 'Ilogen- Excel', containing extract of the plant, among other constituents (Umamaheswari and Mainzen Prince, 2007). Significant reductions in blood glucose level have been observed when hyperglycemic rats were treated with aqueous extract of the plant grown in Malaysia (Husen*et al.*, 2004). Andrographolide, an active principle found in the leaves of the plant, reportedly demonstrated anti-hyperglycemic effects when administered streptozotocin-induced diabetic rats (Yu et al., 2003). Crude ethanol extract of the plant has been shown to possess anti-diabetic activity, which has been attributed to increased glucose metabolism. Ethanolic extract of the aerial parts of the plant reportedly showed antioxidant and anti-hyperglycemic properties in diabetic rats; notably, oxidative stress is considered an important factor in the development of diabetic complications (Zhang and Tan, 2000).

Table 5: Effect of Kalmegh supplementation on serum Glucose (mg/dl) at various days of supplementation

Day of supplementation	Kalmegh extract @ 50 gm per 200 ml water	In Feed @ 3g per bird per day	Control
1 <sup>stNS</sup>	$226.10 \pm 5.45$	$234.48 \pm 4.11$	$239.24 \pm 5.04$
3 <sup>rd</sup> **	$203.47 \pm 7.50^{a}$	$225.32 \pm 10.18^{b}$	$242.54 \pm 6.10^{\rm b}$
5 <sup>thNS</sup>	$228.20 \pm 9.92$	$239.05 \pm 7.18$	$248.77 \pm 6.92$
7 <sup>th NS</sup>	$230.36 \pm 9.41$	$235.88 \pm 6.77$	$240.18 \pm 6.68$

<sup>\*\*-</sup>Highly significant; NS-Not significant

### Serum bilirubin

Serum bilirubin at various days as influenced by kalmegh supplementation is presented in Table 6. The bilirubin level was significantly (P<0.01) reduced by 16% on fifth and seventh day of supplementation in both water and feed as compared to control group. Though, relevant

literatures could be untraceable, the ability of lowering serum bilirubin has been recorded by Kim et al. (2011) and Elias Hossain et al. (2012) with water plantain (*Alisma canaliculatum*). Previous researchers also reported hepatoprotective effects in response to feeding different medicinal plants to broilers (Al-Jaff, 2011; Chand et al., 2011).

Table 6: Effect of Kalmegh supplementation on serum Bilirubin (mg/dl) at various days of supplementation

Day of supplementation	Kalmegh water supplement	Kalmegh feed supplement	Control
1stNS	$0.066 \pm 0.01$	$0.067 \pm 0.00$	$0.062 \pm 0.01$
3 <sup>rdNS</sup>	$0.052 \pm 0.01$	$0.051 \pm 0.01$	$0.062 \pm 0.01$
5 <sup>th</sup> **	$0.054 \pm 0.01^{a}$	$0.052 \pm 0.00^{a}$	$0.069 \pm 0.01^{b}$
7 <sup>th **</sup>	$0.056 \pm 0.01^{a}$	$0.054 \pm 0.01^{a}$	$0.068 \pm 0.01^{b}$

<sup>\*\*-</sup>Highly significant, NS- Not significant



## Micronutrient profile of serum

The effect of kalmegh supplementation on serum micronutrient profile is presented in table 7. Supplementation of Andrographis panniculata had significantly influenced total Serum iron, copper and zinc. The level of iron, copper and zinc was significantly (P< 0.01) higher of 0.27, 0.46 and 0.12 ppm with supplementation of *Andrographis panniculata* extract

as compared to control. Supplementation of medicinal plant *Andrographis panniculata* as water supplement in Nicobari fowl significantly increased the levels of haematinic mineral iron and immunity enhancing minerals viz., copper and zinc in serum by 3.5, 1.2 and 2 times respectively. This improvement in the serum copper and zinc might be the contributing factors to facilitate the immune enhancing properties of andrographolide of *A .panniclata*.

Table 7: Effect of Kalmegh supplementation on micronutrient profile of serum

Micronutrients (ppm)	Kalmegh extract @ 50 gm per 200 ml water	Control
Iron	$0.270 \pm 0.03^{a}$	$0.076 \pm 0.02^{b}$
Copper	$0.456 \pm 0.01^{a}$	$0.367 \pm 0.01^{\rm b}$
Zinc	$0.12 \pm 0.01^{a}$	$0.053 \pm 0.00^{b}$

### **Conclusion and Recommendations**

Supplementation of noni as water supplement, Azolla as feed supplement, Kalmegh as both water and feed supplement reduced significantly serum GOT, cholesterol Kalmegh supplments significantly and bilirubin. reduced serum glucose levels. It is concluded that noni, Azolla and Kalmegh feed and water supplements have hepatoprotective, hypo-cholesterolemic and glycemic effect proving that these supplements do not exert metabolic toxicity in the function of liver cells. Further, serum iron, copper and zinc increased significantly with inclusion of kalmegh supplements facilitating the immunity of Nicobari fowls. Based on this study, noni as water supplement, Azolla as feed supplement and Kalmegh as water and feed supplements are recommended as herbal tonics in indigenous poultry. Andrographis panniculata, Morindacitrifolia Azollacould be recommended as herbal water and feed supplements @ 3g per bird per day, @ 5 ml per bird per day and 3 g per bird per day respectively for the replacement of commercial tonics to enhance the liver function.

### **Authors' Contributions**

All the authors contributed in various phases of this experiment viz., conduction of experiment, guidance to

conduct the research, scientific discussion and preparation of manuscript.

# Acknowledgements

The authors are grateful to the Director, Central Island Agricultural Research Institute, Port Blair for providing the facilities and financial assistance to carry out this work.

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