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Cumulative toxic effect and identification of non-toxic dose of *Allium sativum* (Garlic) and *Withania somnifera* (Ashwagantha) in Native chicken

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Abstract
The present study was conducted to explore the cumulative toxic effects and the non-toxic doses of *Allium sativum* and *Withania somnifera* individually and in combination in native chicken. Eighty native chicks divided into ten groups and they were supplemented with *A.sativum* bulb powder @ 0.5%, 1.5% and 3% and *W.somnifera* root powder @ 1%, 3% and 5% and their selected three combination doses in feed from 15th day to four months of age. The cumulative dose toxic effects of herbs on vital organs were assessed by estimation of liver function enzymes (SGOT and SGPT) and kidney function parameters (uric acid and creatinine) in UV double beam Spectrophotometer. The data were analysed in one way ANOVA using SPSS software. The significant ($P< 0.05$) differences with was not observed in liver function enzymes value of all groups whereas higher serum uric acid (5.20 ± 0.10 mg/dL) than control group (3.24± 0.05 mg/dL) recorded in *A.sativum* - 3% group. On conclusion, except 3% *A. sativum* dose, other dose of both herbs can be used safely without affecting native chicken performance.

Keywords: *Allium sativum*, *Withania somnifera*, liver and kidney functions, native chicken

Introduction
To assess the safety of herbal products for clinical use, toxicological studies should be carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose. The toxicological evaluation of repeated dose (Subacute/ acute and chronic) studies in experimental animals is relevant in determining the cumulative toxicity of plant material preparations on the target organ and on physiologic metabolic tolerance. To select the ideal dose for a repeated dose study, an acute toxicity study with a range of doses has to be conducted (Rhiouani *et al*., 2008) [7]. A wide variety of toxic effects can be assessed in toxicity studies by monitoring different parameters, including haematological, clinical chemistry, behavioural and histological evaluation (WHO, 1993) [11].
The liver is an important organ involved in biotransformation of xenobiotics, so it is vulnerable to xenobiotics (Sturgill and Lambert, 1997) [10]. The kidneys are principal organs involved in excretion, especially of xenobiotics, so they are easily affected by potentially toxic agents. Some herbal medicines have hepato and nephrotoxic effects. Therefore the liver and kidney functions should be monitored in herbal toxicity studies. The liver contains enzymes such as Alainine transaminase (ALT), Aspartate transaminase (AST), Alkaline Phosphatase (ALP) and Lactate dehydrogenase(LDH). The activities of these enzymes are used to assess the functional status of the liver and used as a biochemical markers of liver injury (Moss and Handerson, 1999) [5]. Hepatotoxic agents cause damage to the liver cell membrane, and these enzymes are leaked into serum and show increased activities (Sturgill and Lambert, 1997) [10]. Drug-induced nephrotoxicity are often associated with marked elevation in blood urea and Serum creatinine (Ferguson *et al*., 2008) [3].
Allium sativum (Garlic) and *Withania somnifera* (Ashwagantha) are the two herbs which have been used traditionally for their growth promoting and immunomodulating properties. The farmers are regularly using the herbs in different doses to augment the performance of native chicken without knowing the exact dose to be used. Hence to findout the safety of these herbs alone or in combination, the present study was conducted in native chicken with the objective of assessment of cumulative toxic effects of different doses of *A. sativum*, *Withania somnifera* and their combination and identification of non-toxic dose of herbs for supplementation in feed.

Materials and Methods
The biological experiment was designed and conducted in the research farm of Department

of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu. Eighty day old native chicks (Aseel cross) were procured and they were randomly divided into ten treatment groups of eight numbers each with one replicate. All the chicks were reared under standard and uniform managemental conditions throughout the experimental period of four months. The native chicken feed, free of toxins, antibiotics and pesticide residues were procured and used as basal diet for formulating the experimental diet.

A. sativum was purchased from local market and the bulb powder was prepared as per the method described by Jafari *et al.* (2008) [4]. *W. somnifera* crude root powder was procured from Natural Remedies Private Limited, Bangalore and used for this experiment. *A.sativum* and *W.somnifera* inclusion levels in the feed for the experiment were fixed as per the existing literatures. Experimental diets containing *A.sativum* bulb powder and *W.somnifera* root powder were prepared and fed to the respective treatment groups, accordingly. The birds were subjected to different treatments from fifteenth day to four months at various inclusion levels of the plant extracts as mentioned below:

Table 1: Experimental design

S.NO	Treatment	Experimental Groups
1	T_1	Control
2	T_2	*A. sativum* bulb powder (0.5%)
3	T_3	*A. sativum* bulb powder (1.5%)
4	T_4	*A. sativum* bulb powder (3%)
5	T_5	*W. somnifera* root powder (1%)
6	T_6	*W. somnifera* root powder (3%)
7	T_7	*W. somnifera* root powder (5%)
8	T_8	*A.sativum* bulb powder (0.5%) + *W. somnifera* root powder (1%)
9	T_9	*A. sativum* bulb powder (1.5%) + *W. somnifera* root powder (3%)
10	T_{10}	*A. sativum* bulb powder (3%) + *W. somnifera* root powder (5%)

Blood samples were collected from all groups at the end of experiment and the serum was separated and liver function enzymes *viz*., Aspartate aminotransferase (AST) / SGOT and Alanine aminotransferase (ALT) / SGPT were estimated by, 2, 4-DNPH Spectrophotometric method (Reitman and Frankel, 1957) in UV double beam Spectrophotometer. Also kidney function parameters *viz*., serum uric acid was estimated as per the method described by Schultz *et al*. (1984) and serum creatinine was estimated by alkaline picrate method in UV double beam Spectrophotometer. The mean values of different treatment groups were compared with control group by one way Analysis of Variance. Critical values are estimated by using Duncan multiple range test using SPSS statistical package, version 17.0.

Results and Discussion

The results obtained on biochemical parameters are presented in table 2 and table 3. Biochemical parameters such as SGOT and SGPT levels in serum were assessed in all the groups. The results revealed that, significant ($P< 0.05$) differences in SGOT and SGPT were not observed among the various treated groups, as compared to that of control group.

Table 1: Effect of supplementation of *Allium sativum* and *Withania somnifera* on SGOT and SGPT level (Mean ± S.E) in native chicken

Group/Para Meter	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
SGOT (U/L)	110.38^{ab} ± 0.15	109.63^{a} ± 0.16	111.80^{bc} ± 0.10	111.44^{bc} ± 0.17	111.31^{bc} ± 0.12	110.62^{ab} ± 0.14	111.25^{bc} ± 0.14	111.37^{bc} ± 0.20	111.46^{bc} ± 0.18	110.85^{ab} ± 0.11
SGPT (U/L)	46.78^{ab} ± 0.05	47.04^{bc} ± 0.12	47.07^{bc} ± 0.19	46.74^{ab} ± 0.07	46.84^{ab} ± 0.15	47.17^{bc} ± 0.19	46.84^{ab} ± 0.10	46.53^{a} ± 0.13	46.60^{a} ± 0.13	46.96^{abc} ± 0.10

Overall means bearing different superscripts within the column differ significantly (P<0.05)

Table 2: Effect of supplementation of *Allium sativum* and *Withania somnifera* on Uric acid and Serum Creatinine levels (Mean±S.E) in native chicken.

Group/Para meter	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Uric acid (mg/dL)	3.24^{a} ± 0.05	3.62^{ab} ± 0.12	3.40^{ab} ± 0.10	5.20^{d} ± 0.10	3.64^{ab} ± 0.15	2.94^{a} ± 0.05	3.00^{a} ± 0.07	3.41^{ab} ± 0.14	3.29^{a} ± 0.03	3.43^{ab} ± 0.11
Serum creatinine (mg/dL)	1.42^{a} ± 0.01	1.43^{a} ± 0.00	1.41^{a} ±0.03	1.39^{a} ± 0.04	1.39^{a} ± 0.00	1.40^{a} ± 0.00	1.42^{a} ± 0.00	1.39^{a} ± 0.01	1.41^{a} ± 0.00	1.42^{a} ± 0.00

Overall means bearing different superscripts within the column differ significantly ($P< 0.05$)

The present study was in agreement with Shrivastava, (2012) [9] who reported that the indigenous herbal drug *viz*. Ashwagandha (*W. somnifera*), Satavari (*Asparagus racemosus*) and Kapi-Kachchu (*Mucuna pruriens*) supplementation at 2% in feed of broiler for 42 days had no significant effect on SGOT, SGPT enzyme. SGOT is present in many tissues and is useful in evaluating muscle and liver damage and it is present in both the cytoplasm and mitochondria of hepatocytes (and many other cells) and will elevate in states of altered membrane permeability.

SGPT is considered to be liver specific and it is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. In the present study there was no significant rise in the SGOT and SGPT level. From these results it was inferred that, *A. sativum* and *W. somnifera* can be used individually at the above mentioned doses and in combination without much harmful effect on the normal liver functions. Further elaborate studies are required for more detailed investigation on the effect of *A. sativum* and *W. somnifera* in liver functions.

While, the results of kidney function tests revealed that the serum uric acid value after supplementation of *A.sativum* at the dose rate of 3% (T4) was significantly differ from that of control group. Significant differences were not observed between control and other treatment groups with respect to serum uric acid. also significant differences were not observed between control and other treatment groups with respect to serum creatinine.

The result of present study is in agreement with the Ghalehkandi *et al.* (2012) [2] who reported that, garlic aqueous extract used at 60 mg/kg and 120mg/kg in feed of rats were revealed decrease in serum creatinine and increase in uric acid but these changes were not significant. Also, Amera *et al.* (2013) [1] used garlic oil at 100 mg/kg and 200mg/kg of feed in broilers for 42 days and found non-significant results for uric acid concentration. The reason for elevation of uric acid level in higher dose group of *A. sativum* is not known.

It was found that, the different dose rates of *A. sativum* and *W. somnifera* either individually or in combination had not significantly affected the liver functions, but the kidney function was affected after supplemented with *A. sativum* at 3% level. Hence, it was concluded that, except 3s% *A. sativum* dose, other dose of both herbs can be used individually and in combination without any toxic effects on physiological functions and performance of native chicken.

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