TOXICOLOGICAL SCREENING OF *Andrographis paniculata* LEAF EXTRACT (APLE) ON SINGLE AND REPEATED DOSE TOXICITY STUDY.

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ABSTRACT

*Andrographis paniculata* (Nees) belongs to the family of acanthacea, has many application in the traditional Indian system of medicine for treating various liver disorders. In addition of *Andrographis paniculata* leaf extract (APLE) was reported to possess an immunostimulatory, anti-viral, anti-cancer, anti-diabetic and anti-helmintic effects. Despite the widespread use of APLE by the traditional Indian system of medicine for treating various diseases, no systematic toxicological studies has been performed on this plant. Therefore in the present study, the safety profile of APLE was assessed by single (2000 mg/kg bw) and repeated dose (500 mg/kg .bw, oral gavages for 30 days and 1000 mg/kg bw, oral gavages for 30 days) toxicity was carried out in male and female albino rats. Biochemical parameters such as haematology, Serum liver-biomarkers (AST, ALT, ALP, GGT, LDH and bilirubin), Serum kidney -biomarkers (urea, uric acid and creatinine), Structural studies (light microscopic and transmission electron micrograph) were assessed in APLE-treated and control Wistar albino rats. No significant changes was observed in all the biochemical parameters on APLE administration indicate the non-toxic effect of APLE.

**Keywords:** *Andrographis paniculata*, Toxicological screening, liver-and kidney biomarkers
INTRODUCTION

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness (Dev, 1997). There are more than 100 drugs of known structure that are extracted from medicinal plants and used in allopathic medicines (Farnsworth, 1990; Cox, 1994). A proper ethno pharmacological search and follow-up studies can lead to many more useful drugs. Even though numerous plant constituents have been proved to have pharmacological activity in experimental animals, the probability of success in the clinical trials is very less (Feres et al., 2006). *Andrographis paniculata* (AP) (Family: Acanthaceae) commonly known as “king of bitters” is used extensively in the Indian traditional system of medicines. It’s a shrub with a considerable reputation as a potent adjunct for various ailments including jaundice, cholestatis and inflammatory conditions. It is found in the Indian Pharmacopoeias and is a prominent component in atleast 26 Ayurvedic formulae (Madav et al., 1995). Despite the widespread use of phytotherapeutic drugs, the licensing regulation and pharmacovigilance regarding herbal products are still incomplete and clear-cut proof of their efficacy against liver diseases are sparse. Apart from therapeutic properties reports are accumulating about toxicity of medicinal plants after their intake. Hence, the natural products used in therapeutics must be submitted to efficacy and safety tests by the same method used for new synthetic drugs (Talalay and Talalay, 2001). Therefore the present study was carriedout to assess safety of aqueous APLE, focusing on its acute and chronic toxicity study and its effect on biochemical parameters.

MATERIALS AND METHODS

Adult male and female albino rats of Wistar strain weighing 170-190g were used for the study. The animals were maintained in the animal house, Pachaiyappa’s College Chennai. They were individually housed under hygienic conditions in polypropylene cages under 12 h light / 12 h dark cycle. The animals used in the present study were cared as per the principles and guidelines of the Institutional Animal Ethical Committee, in accordance with the Indian National Law on Animal Care and Use (Register Number:
CPCSEA/ORG/CH/2006/REG.NO.967/922) and the procedures were approved by the committee.

*Andrographis paniculata* (*Ap*) was collected from rural area near Chidambaram (India). The collected leaves were washed with water, shade-dried and then coarsely powdered. The powder was then subjected to aqueous extraction (20g dry leaf/100 ml of water).

**Toxicity study**

Single and repeated dose toxicity studies were carried out in this study according to OECD guideline No.423 (OECD, 2001). Single dose toxicity study of APLE was conducted in two groups of rats. Group I served as control whereas, Group II received single dose of APLE (2000 mg/kg.bw, oral gavage) and both the groups were sacrificed by cervical dislocation after 0, 2, 4, 6, 8, 12 and 24h of treatment.

In repeated dose toxicity study, animals were divided into three groups: Group I treated as Control, Group II received APLE -500 mg/kg.bw, oral gavage for 30 days and Group III received APLE -1000 mg/kg bw, oral gavage for 30 days.

**Assessment of toxicity by biochemical markers**

Hepatic marker enzymes such as aspartate transaminase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), bilirubin, kidney function markers such as urea, uric acid, creatinine, levels in serum, hematological parameters such as Hb, RBC, WBC and ESR in blood were analysed in control and experimental groups following standard methods (Demma *et al.*, 2007) to assess the toxicity of the plant.

**RESULTS**

Drug toxicity is associated with a variety of pathological conditions varying from a simple intoxication to severe, life-threatening derangement of metabolism. So the attention made in herbal medicine to assess the safety.

**Effect of APLE on the body and organ weight in male and female of control and experimental rats**

The initial and final body weights of the rats and the organ weights at the end of the experimental period were shown in (Fig.1&2). The initial body weights of the rats of the
control and experimental groups were uniform, there being no significant differences. The body weights at the end of the experimental period (30 days) were significantly higher than the respective initial weights, but almost same in all the three groups. Moreover, no lethality was recorded for any dose up to the maximum of 1000mg /kg bw during the 30 days of treatment.

**Effect of *Andrographis paniculata* on single and repeated dose toxicity study**

In order to evaluate the safety of the acute and chronic use of APLE, the effects of single dose (2000 mg/kg bw) and of multiple doses (500 mg/kg .bw, oral gavages for 30 days and 1000 mg/kg bw, oral gavages for 30 days), Survival, haematology, Serum liver-biomarkers (AST, ALT, ALP, LDH, GGT and bilirubin), Serum kidney -biomarkers (urea, uric acid and creatinine) were assessed in APLE- treated and control Wistar albino rats.

**Single dose toxicity studies in Wistar rats**

**Liver-biomarkers in the serum**

The biomarkers of the liver such as AST, ALT, ALP, LDH ,GGT and bilirubin were assayed in the serum of control and APLE treated (single oral dose, 2000 mg/kg bw) albino rats, at different time intervals (0, 1, 2, 4, 8, 12, and 24 h) post-feeding, and the results are shown in Figures. 3-6. None of the liver-biomarkers was affected by the single oral administration of APLE either in the female or in the male albino rats except increased ALT in female at 8h.

**Repeated dose toxicity studies in Wistar rats**

In clinical evaluation, no behavioral changes were observed in the group treated with the 500 mg/kg dose. In the group treated with 1000 mg/kg dose, the animals presented piloerection, hypoactivity-asthenia and grooming, but all the changes were reverted in a maximum period of 1 day. No death was observed for both the experimental groups.
Figure 1  Effect of APLE on the body weight in male and female of control and experimental rats

Figure 2  Effect of APLE on the organ weight in male and female of control and experimental rats

Group I – Control,  Group II - Received APLE -500 mg/kg bw, oral gavages for 30 days,  Group III - Received APLE -1000 mg/kg bw, oral gavages for 30 days
Values are mean ± SD of n = 6 rats in each group
No significant difference among the means.
Figure 3  Effect of single dose administration of APLE on serum AST levels in male, female albino rats

CON M - control male; CON F- female groups
APLE M- Andrographis paniculata leaf extract administered male groups
APLE F - Andrographis paniculata leaf extract administered female groups
Values are mean ± SD of n = 6 rats in each group
*-significantly different from all other groups
Figure 5 Effect of single dose administration of APLE on serum ALPase levels in male, female albino rats

![ALPase Levels Graph]

Figure 6 Effect of single dose administration of APLE on serum LDH levels in male, female albino rats

![LDH Levels Graph]

CON M - control male; CON F- female groups  
APLE M - Andrographis paniculata leaf extract administered male groups  
APLE F - Andrographis paniculata leaf extract administered female groups  
Values are mean ± SD of n = 6 rats in each group;  
No significant difference among the means
Figure 7 Effect of single dose administration of APLE on serum GGT levels in male, female albino rats

CON M - control male; CON F - female groups
APLE M - *Andrographis paniculata* leaf extract administered male groups
APLE F - *Andrographis paniculata* leaf extract administered female groups
Values are mean ± SD of n = 6 rats in each group
No significant difference among the means

Figure 8 Effect of single dose administration of APLE on serum bilirubin levels in male, female albino rats

CON M - control male; CON F - female groups
APLE M - *Andrographis paniculata* leaf extract administered male groups
APLE F - *Andrographis paniculata* leaf extract administered female groups
Values are mean ± SD of n = 6 rats in each group
No significant difference among the means
DISCUSSION

*Andrographis paniculata* is an Indian herb, well known as ‘king of bitter’. It has an excellent anti-inflammatory, anti-bacterial and anti-viral effect. In the light of the above, a detailed preliminary toxicity study was carried out in normal rats after single and multiple oral dose of APLE administration in order to assess the safety use of APLE.

Liver and kidney are the important organs for metabolism, detoxification, storage and removal of xenobiotics and their metabolites. Liver has a wide variety of functional capabilities; no one single test can provide an accurate assessment of its function. In this study, we have assessed the hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, \( \gamma \)-glutamyl transferase) and bilirubin, kidney function markers such as urea, uric acid and creatinine were analysed to determine the effect of APLE on the functional status of liver and kidney in normal rats.

The obvious sign of toxicity is the leakage of cellular enzymes into plasma (Ramaiah, 2007). AST and ALT are reliable markers of liver function. It is established that AST can be found in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes (in decreasing order of concentration), whereas ALT is present in highest concentration in the liver (Rej, 1978). No significant changes in the activities of these enzymes on APLE administration indicate the non-toxic effect of APLE. Many researchers have assessed the toxicological evaluation of various plants by estimating the levels of AST and ALT in experimental animals (Feres *et al*., 2006, Keplinger *et al*., 1999).

ALP and GGT are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. ALP is excreted by liver via bile and hence when liver is affected, the serum enzyme level increases due to defective excretion. During hepatotoxicity GGT is more frequently elevated than either of the transaminases and ALP. Serum GGT is generally considered to be a useful laboratory marker for liver function (Sillanaukee, 1996) and its estimation is a valuable ‘screening test’ with a high negative predictive value for liver disease (Nemesanszky, 1996). In the present study, acute and chronic APLE administration caused no significant increase in ALP and GGT levels in serum.
Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Harpers, 1961). Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. The normal level of serum bilirubin after acute and chronic APLE administration in rats indicates the non-toxic effect of the APLE in maintaining the normal functional status of the liver.

The present investigation amply demonstrates the safety profile of *Andrographis paniculata*. APLE do not have any adverse effect on single and repeated dose administration and paves the way for further clinical research to identify a therapeutic drug of natural origin to prevent liver and kidney toxicity and to delineate a mechanism of APLE on drug-induced toxicity.

**REFERENCES**

