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EVALUATION OF SAFETY ASPECTS OF ANDROGRAPHIS PANICULATA IN SWISS-ALBINO MICE AT SUB-ACUTE LEVEL

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ABSTRACT

Andrographolide is a diterpenoid lactone present in *Andrographis paniculata.* It is a major active constituent of *A. paniculata*. The present study was focused on the safety aspects of the bioactive fraction of ethanolic extracts of Andrographis paniculata. The safety profile of Andrographis paniculata was evaluated in mice model system at sub-acute levels using different measures such as hematological indices, serum biochemical indices and tissue biochemical indices. In sub-acute toxicity study the ethanolic extracts of Andrographis paniculata upto5mg/kg, 50mg/kg, 300mg/kg and 1000 mg/kg body weight was administered orally and observed animals for 28 days after dosing. No significant variation in the body weight and organ weight, leukocyte count, hemoglobin, SGPT, SGOT, Triglyceride, Cholesterol, Blood Urea Nitrogen, Creatinine Albumin and Globulin were observed between control and treatment groups after administration of Andrographis paniculata upto 5mg/kg, 50mg/kg, 300 mg/kg and 1000mg/kg body while significant increase of erythrocyte count and glutathione were observed in animals treated with 300mg/kg ,significant increase in lymphocyte count with 5mg/kg, 50mg/kg, 300mg/kg and decrease in neutrophils with 5mg/kg, 50mg/kg, 300mg/kg body weight were observed. As far as the safety aspects of Andrographis paniculata is concerned and more studies are required.

Key words: Andrographolide, biochemical indices, erythrocyte, lymphocyte,

INTRODUCTION:

It is a well known fact that the demand for the herbal drug treatment of various ailments is increasing and plant drugs from the Ayurvedic system are being explored more, not only in India but also globally. *Andrographis paniculata* is a plant that has been effectively used in traditional Asian medicines for centuries. *Andrographis paniculata* commonly known as the "king of bitters," is an herbaceous plant belonging to the Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India. Extracts of this plant and andrographolide exhibit pharmacological activities such as those that are immunostimulatory [1, 2], antiviral[3], and antibacterial[4]. As major active constituent, andrographolide exhibits a broad range of biological activities, such as anti-inflammatory, antibacterial, antitumor, antidiabetic, antimalarial, and hepatoprotective[5].

Aims & Objectives of this study:

To evaluate the safety aspect of Andrographis paniculata in Swiss albino mice at sub acute level

Material and methods:

Swiss albino mice were obtained from "Jeevanika" Cimap Lucknow. The animals were maintained at22±3^{O}C with50-70% relative

humidity and 12:12 hr of light and dark cycles. The animals were fed with pellet diet containing 22-24% protein, 4-5% fat, 4-5% crude fibre, nitrogen free extract 45-55% Bengal gram, 15% phosphorus 0.4-0.6%, calcium 1-1.5% and insoluble ash 8%. They were also fed with soaked Bengal gram and water

Experimental animals: virgin inbred strains of Swiss- albino of both sexes weighing 18-30g were used.

Type of the study: case control study

Sample size and study design: In sub-acute experiments a total of 30 adult mice of both sexes mice were used. The animals were divided into 5 groups comprising of 3 males and 3 females in each group. Animals of group 1 were kept as controls and were fed with 0.25 ml distilled water once daily for 28 days and the animals of group 2,3,4 and 5 were considered as experimental group and were treated with the ethanolic extract of @5mg/kg, 50mg/kg, 300mg/kg and 1000 mg/kg body weight once orally for 28 days

Place of the study: The study was performed in CIMAP Lucknow

Duration of the study period:

The experimental groups were treated with the ethanolic extract of @5mg/kg, 50mg/kg, 300mg/kg and 1000 mg/kg body weight and controls and were fed with 0.25 ml distilled water for 28 days.

Animals were carefully observed to check the Mortality, Morbidity**,** respiration rate, convulsion heart beat, salivation, diarrhea, lethargy, coma etc in sub acute toxicity experiments daily for 28days.The whole study was completed in 6 months

All animals' experiments were performed according to the ethical guidelines suggested by the intuitional animal ethics committee (IAEC) and committee for the purpose of control and supervision of experiments on animals, government of India.

Plant Extracts: Bioactive fraction (ethanolic extracts) of A. paniculata was obtained from in- vivo testing lab, CIMAP Lucknow.

Reagents and Diagnostic Kits: SGPT, SGOT, BUN, Creatinine, Triglyceride, total cholesterol and albumin obtained from MERCK Diagnostic. RBC, WBC diluting fluid, Drabkin's reagents were purchased from Randox laboratory ltd, other chemicals like Manoldehyde, Reduced glutathione, Folin's reagent and other routine chemicals of analytical grade were obtained from Merck India limited and Sigma Chemicals Limited.

Collection of Blood samples: Blood samples of all animals of all groups of animals were collected separately from orbital plexus of periods of 28th day in sub-acute experiments and control groups mice to enumerate RBC, WBC and hemoglobin.

Serum Separation: Blood was kept for clotting for 30 min at RT and then at 4 ^{O}C for 1hour for the clot to get shrunk and easy separation of serum. The clotted blood was centrifuged at 4000 rpm for 10 min. serum collected was stored at -20 ^{0}C and used subsequently for enzymatic and biochemical assay.

Tissue Samples and Homogenate: After the treatment period is over at 28th day in acute experiments, the animals of all the groups were sacrificed by cervical dislocation, abdominal, thoracic, cervical and cranial cavities were opened and organs like liver, spleen, kidney, heart and brain were quickly removed and placed in ice-cold PBS buffer (Ph=7.4) minced into small pieces and homogenized immediately by homogenizer. The homogenate was processed for estimation of GSH.

Parameters studied:

Body weight: The body weight of all the experimental animals were noted at the starting and end of the experiment to observe the effect of A. paniculata bioactive fraction of alcoholic extract on the body weight of the animals.

Total erythrocyte count (RBC): The blood was taken directly from the tail of mice (the procedure was done quickly to avoid the coagulation) 1 ml of RBC diluting fluid was added to 5µl of blood. The cover slip was put into the counting chamber and then a small quantity of the diluted blood was put between the cover slip and the plate form of the counting chamber (carefully to avoid the overflow and formation of air bubble).The solution was allowed to settle for a couple of minute and then the counting was done under the high power of microscope.

Total Leukocyte count (WBC): The blood was taken directly from the tail of mice 5 µl of blood was diluted in 100 µl of the diluting solution in an eppendorf. The cover slip was put on the counting chamber and a small quantity of the diluted blood was put between the cover slip and the plateform of the counting chamber .The solution was allowed to settle for a couple of minutes and the counting was done under high power of microscope.

Hemoglobin: 1 ml of drabkin's solution (diluted with distilled water at 1:20) and 4 µl of blood was pipette out to a clean dry test tube Labelled (T).Mixed well and kept at RT for 5 min and read the absorbance of test samples and CMG standard (s) against distilled water at 540 nm.

Liver Function Test: SGOT (AST), SGOT by UV-kinetic IFCC method, cholesterol (by CHOD-POD enzymatic method), Triglycerides (by GPO- POD UV-kinetic IFCC method), Creatinine (By Jaffe calorimetric- kinetic method) while BUN, Albumin and Globulin were measured by kit methods (MERCK Diagnostic).

Reduced Glutathione (GSH):

Reagents: phosphate buffer saline (PBS) of ph7.7.precipating solution: (1 g Meta-phosphoric acid, 0.12g EDTA and 18gm NaCl was added in 500 ml distilled water), 1 % sodium citrate and 10mg DTNB was dissolved in 25 ml of 1 % sodium citrate.

Procedure: 100 µl of liver tissue homogenate was added in equal vol. of PBS and 1800 µl of distilled water. Added 1.5 ml of precipitating solution and centrifuge at 5000rpm for 5 min and discarded the precipitate. Taken 50 ml of supernatant with 200 µl of PBS and 25 µl of DTNB in 96 well plates and taken OD at 412nm in spectrometer.

Protein estimation by lowery methods:

Reagents: 4% Na2co3 in distilled water (reagent A). 0.5%CuSO4.5H_2O in Na-K Tartrate (reagent B).Alkaline cupper solution: Mix 50ml reagent A with 2 ml of reagent B (reagent C). Diluted Folins reagent**:** Ciocalteu Reagent with equal vol. of 0.1 N NaOH.

Standard protein solution: BSA Solution at100mg/ml as a stock standard protein solution.

Procedure: 80µL of tissue samples was taken and 80µL of distilled water, 80µL of reagent C and 8 µL of folins (Ciocalteu) reagents was added. Absorbance was taken by spectrophotometer against standards (BSA) and blank.

Statistical Analysis: Results were expressed as mean±SEM with the help of the software INSTAT. Statistically significant differences between the cases and control were calculated using students t test and $p<0.05$ was considered statistically significant.

Result: Swiss albino mice treated with alcoholic extract of A. paniculata at @ 5mg/kg, 50mg/kg, 300mg/kg and 1000mg/kg body weight once orally for 28 days in sub-acute study showed no mortality and morbidity during the entire experimental period. Paradoxically the treated mice were alert, sound and active like control animals. There were no changes were observed during the cage site observations for lacrimtion, salivation, changes in colour of skin/fur and respiration rate.

Effects on body Weight: The body weight were taken on 0^{th} and 28^{th} day (post dosing) of the control and treated group taken individually and presented in Table1. There is no significant changes were observed.

Effects on Hematological Parameters: The blood of all the groups of animals were collected on 28^{th} day of treatment and used for analysis of total leucocytes count (thousand/ml), total erythrocyte count (million/ml), hemoglobin level (gm/dl) and DLC count. Data were presented in **Table 1**. There was no significant changes were found in WBC and hemoglobin but significant increased in erythrocyte counts in animals treated with 300mg/kg and significant increase in lymphocyte count and decrease in neutrophils in animals treated with 5mg/kg, 50mg/kg, 300mg/kg body weight, however no changes were observed in monocytes basophil and eosinophills.

Effects on Serum biochemical parameters:

SGPT AND SGOT: Serum samples were collected from experimental groups of animals on 28^{th} day of treatment and used for the activity of SGPT and SGOT and data presented in Table 1 showed no significant changes in acute toxicity as compared to control.

BUN and Creatinine: Bun and Creatinine levels were estimated from the serum samples of treated and control groups and data presented in Table 1 showed no significant changes in treatments groups as compared to control.

Triglyceride and cholesterol: The level of triglyceride and cholesterol were estimated and presented in Table 1. No significant changes in both triglyceride and cholesterol levels were found.

Total serum protein, albumin, globulin and ratio of albumin and globulin: The levels of Total serum protein, albumin, globulin and albumin/ globulin ratio were estimated from serum samples of treated groups and control and presented in Table 1. No significant changes were observed in levels of total serum protein, albumin, globulin and albumin/globulin ratio in treated group as compared with control.

Effects on Tissues biochemical parameters:

Total liver protein: Hepatic tissue protein contents is estimated from 10% liver tissue homogenate samples of control and treatment groups of animals and data presented in Table 1. There was no significant change observed in animals of treatment group as compared with control.

Tissue antioxidant status: Reduced glutathione contents were estimated from 10% liver tissue homogenate samples of treatment and control group animals. data presented in Table 1 showed that glutathione contents is significantly increased in animals of treatment groups upto 300mg/kg body weight however no changes were observed for higher dose of 1000mg/kg body weight as compared with control

Table 1:effects of ethanolic extracts of Andrographis paniculata on various parameters in Swiss albino mice after oral subacute treatments.

Parameters	Control	5 mg / kg body weight	50 mg/kg body weight	300mg/kg Body weight	1000mg/kg body weight
WBC(thousands/ml)	9.03±1.51	14.19±2.49	15.28±1.24	13.04±0.81	14.51±1.32
RBC(Million/ml)	3.87±0.11	4.48±0.28	3.94±0.2	5.44±0.39*	3.24±0.18
Hemoglobin (g/dl)	26.30±0.40	24.06±1.65	26.79±2.80	25.1±5.17	31.05±71
SGOT(U/l)	14.06±2.18	20.34±5.85	18.99±4.46	11.52±2.1	16.5±1.48
SGPT(U/L)	9.9±0.18	15.65±6.63	14.76±3.09	10.96±2.12	11.23±3.09
BUN(mg/dl)	17.11±0.53	18.56±0.81	15.22±1.64	17.75±1.55	15.71±1.59
Creatinine(mg/dl)	0.52±0.06	0.49±0.04	0.49±0.01	0.55±0.1	0.58±0.12
Triglycerides(mg/dl)	116.91±5.42	132.36±7.12	125.67±18.23	119.27±5.92	111.07±8.13
Cholesterol(mg/dl)	93.13±5.84	75.06±4.88	71.89±2.98	71.69±6.54	73.6±7.58
Total Serum Protein(mg/ml)	14.58±0.71	14.46±0.15	14.56±0.25	14.71±0.25	14.39±0.47
Albumin(g/dl)	2.7±1.18	1.92±0.53	1.91±0.03	1.83±0.3	5.77±4.08
Globulin(g/dl)	11.89±0.47	12.54±0.38	12.65±0.22	12.88±0.06	8.62±3.61
Albumin/ Globulin	0.23	0.15	0.15	0.14	1.05
Total Liver tissue protein(µg/ml)	1214.17±88.41	954.08±205.08	1143.42±206.54	998.91±69.03	1551.59±246.32
GSH(µM/mg Protein)	0.017±0.005	0.034±0.012	0.027±0.007	0.074±0.007*	0.017±0.005

(n=6;*p<0.01? compared to control)

CONCLUSION

The present study is design to evaluate the safety profiling of *A. paniculata* at subacute levels the parameters such as mortality, morbidity, lacrination, salivation, change in skin colour, fur colour, change in body weight, biochemical parameters such as RBC, WBC, DLC Hemoglobin, serum biochemical parameters (SGOT, SGPT, BUN, Creatinine, cholesterol, triglyceride, albumin, globulin, albumin-globulin ratio) and tissue biochemical parameters (GSH, total liver tissue protein) were studied. Subacute level of treatments of ethanolic extracts of Andrographis paniculata resulted in significant increase of erythrocyte count and glutathione contents both these factors showed highest value in the group of animals treated with ethanolic extracts of upto 300mg/kg body weight. Glutathione is most prevalent in RBC and is used for inactivation of free radicals formed inside the RBC .it is involved in erythrocyte membrane integrity and is important in keeping enzymes in the active state. Reduced Glutathione is detoxifies the peroxides and free radicals formed within the RBC. Glutathione helps to detoxify several compounds by transferring to cysteinyl group of organo- phosphorous compounds, halogenated or nitrogenous compounds and heavy metals. It is also involved in detoxifying drugs. Swiss albino mice were monitored as the model system for evaluating changes in biochemical indic-

es and other laboratory measure of toxicity and no problems were found at sub-acute levels of treatment. The use of ethanolic extracts of Andrographis paniculata upto 300mg/kg body weight at subacute is safe.

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