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PHYTOCHEMICAL AND TOXICOLOGICAL INVESTIGATION ON AQUEOUS EXTRACT OF POLYHERBAL PREPARATION

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Keywords:

Polyherbal preparation , Acute-toxicity, Sub chronic toxicity study

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ABSTRACT: Polyherbal preparations (PHP) are known to express high effectiveness in a vast number of diseases. In view of this, present investigation was carried out to identify phytochemical constituents of PHP, prepared from the aqueous extract of test plant powders of *Mimusops elengi* Linn., *Strobilanthes barbatus* Nees, *Indigofera zollingeriana* Miquel and *Dillenia indica* Linn. Phytochemical screening of APHP revealed the presence of carbohydrates, Proteins and amino acids, alkaloids, glycosides, phenols, flavonoids, and fixed oils/fats. Acute toxicity studies were conducted on female wister rats. Study design is according to OECD Guidelines no. 423. APHP was administered at dose of 5 mg/kg, 50 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight as suspension (1% Na CMC) along with blank. No-Observed-Adverse-Effect-Level (NOAEL) of APHP was found to be 2000 mg/kg. In connection to that, sub chronic toxicity studies have been carried out to predict the dose to be selected for *in-vitro* and *in-vivo* experiments. APHP at the doses of 250 mg/kg, 500mg/kg and 1000mg/kg were administered and examined for variations in serum biochemical parameters, haematological parameters, body weight and organ weight. Data obtained in this study indicated no significant signs of any toxicity due to administration of APHP at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg to rats.

INTRODUCTION: A number of investigations showed that the plants are the prosperous source of medicines and make a wide array of bioactive molecules, the majority of which most likely evolved as chemical resistance against to predation or disease. Traditional uses of crude extracts of some well known medicinal plants in India against various diseases in recognized systems of medicines such as Ayurveda, Unani, Siddha [1, 2].

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Medicinal value of the extracts lies in bioactive phytochemical constituents (secondary metabolites), which are formed due to the presence of chemical substances that produce a definite physiological action on the human body [3]. The most important of which include: alkaloids, glycoside, phenols steroids, flavonoids, fatty oils, resins, phosphorus and calcium. They are essential for cell growth, replacement and body building [4].

Drug formulation in Ayurveda is based on two principles: Use as a single drug and use of more than one drug, in which the latter is known as PHP. This key traditional therapeutic herbal strategy exploits the combining of several medicinal herbs to achieve extra therapeutic effectiveness, usually known as polypharmacy or polyherbalism.

Historically, the Ayurvedic literature "Sarangdhar Samhita" dated centuries ago in 1300 A. D. has highlighted the concept of polyherbalism in this ancient medicinal system [5]. In the traditional system of Indian medicine, plant preparation and combined extracts of plants are chosen rather than individual ones. It is known that Ayurvedic herbals are prepared in a number of dosage forms, in which mostly all of them are PHP. It has started to gain its popularity recently worldwide, owing to the fact that it possesses some advantages which is not available in allopathic drugs. Effectiveness, safety, cheap, ubiquity and better acceptance, made APHP an ideal treatment of choice, hence higher compliance by the patients and excellent therapeutic effect is ensured.

Hence, the present study aim to investigate the phytochemical constituents and toxicity profile of APHP prepared from the aqueous extract of test plant powders of *Mimusops elengi* L., *Strobilanthes barbatus* Nees, *Indigofera zollingeriana* Miquel and *Dillenia indica* Linn. which will establish a baseline data for further research work.

MATERIALS AND METHOD:

Collection of Plant Material: The test plants selected for this study, *Mimusops elengi* Linn., *Strobilanthes barbatus* Nees, *Indigofera zollingeriana* Miquel, *Dillenia indica* Linn. were collected from ABS botanical gardens, Karipatti and authenticated by Dr. D. Arulbalachandran, Assistant Professor, Depertment of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu.

Standardization of Polyherbal Preparation: The selected four plant materials were evaluated physically for organoleptic properties (colour, odour and taste, density) moisture content or loss on drying, foreign organic matter, ash values, extractive values, microbial load and preliminary phytochemical analysis (WHO, Guidelines, 2005).

Phytochemical Screening: APHP was subjected to phytochemical screening. Various tests for identification of carbohydrates, protein and amino acids, alkaloids, tannins, glycosides, phenols, fixed oils, steroids were conducted to investigate their presence [6]. Results were given in **Table 1**.

Acute - toxicity Study: Study Design and Controls were according to OECD Guidelines No. 423. (516/01/A/CPCSEA). Female wister rats in controlled age and body weights were selected. Animals were handled according to standard guidelines for the use and care of laboratory animals [9]. Animals were divided into six groups (3 per group). The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses. 5 mg/kg, 50 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight. The test item was administered as single dose. After single dose administration period, all animals were observed for 14 days.

TABLE 1: PHYTOCHEMICAL SCREENING OF APHP

Name of the Test	
Carbohydrates	
Molisch's Test	+
Bial's Test	+
Proteins and Amino acids	
Ninhydrin Test	+
Xanthoprotein Test	+
Millon's Test	+
Alkaloids	
Mayer's Test	+
Dragendroff's Test	+
Glycosides	
Borntrager's Test	+
Phenolics -	
Ferric chloride Test	+
Flavonoids	
Alkaline reagent Test	+
NH_4OH Test	+
Fixed oils/Fats	
Spot Test	+
Steroids	+
Libermann-Burchard Test	+

Observation Period: All animals were observed for abnormal clinical signs and behavioural changes. Animals in pain or showing severe signs of distress were humanely killed. The cage side observation includes changes in appearance of skin, fur, eyes and mucous membranes, presence of secretions and excretions. Autonomic activities like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behaviour like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types

(*e.g*. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats.

Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups. Results were given in **Table 2**, **3**, **4**, **5**, **6** and **7**.

TABLE 2: PHYSICAL AND BEHAVIOURAL EXAMINATIONS AFTER ACUTE TOXIC STUDY

Group no.	Dose (mg/kg)	Observation sign	No. of animal affected.
Group-I	5 mg/kg	Normal	0
Group- II	50 mg/kg	Normal	0
Group-III	250 mg/kg	Normal	0
Group-IV	500 mg/kg	Normal	0
Group-V	1000 mg/kg	Normal	0
Group-VI	2000 mg/kg	Normal	0

TABLE 3: HOME CAGE ACTIVITY OBSERVATIONS AFTER ACUTE TOXIC STUDY OF APHP

Functional and Behavioural assessment	Observation	Control n=3	G-I n=3	G-II n=3	G-III n=3	G-IV n=3	G-V n=3	G-VI n=3
Body position	Normal	3	3	3	3	3	3	3
Respiration	Normal	3	3	3	3	3	3	3
Clonic involuntary movement	Normal	3	3	3	3	3	3	3
Tonic involuntary movement	Normal	3	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3	3
Approach response	Normal	3	3	3	3	3	3	3
Touch response	Normal	3	3	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3	3	3

TABLE 4: HAND HELD OBSERVATION AFTER ACUTE TOXIC STUDY (14-DAY) OF APHP

Functional and Behavioural observation	Observation	Control n=3	G-I n=3	G-II n=3	G-III n=3	G-IV n=3	G-V n=3	G-VI n=3
Reactivity	Normal	3	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3	3
Pilo erection	Normal	3	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3	3

TABLE 5: EFFECT OF APHP ON MORTALITY AFTER ACUTE TOXICITY STUDY

Group no.	Dose (mg/kg)	Mortality
Control	Vehicle	0
Group-I	5 (mg/kg)	0
Group-II	50 (mg/kg)	0
Group-III	250 (mg/kg)	0
Group-IV	500 (mg/kg)	0
Group-V	1000 (mg/kg)	0
Group-VI	2000mg/kg	0

TABLE 6: HOME CAGE ACTIVITY OBSERVATIONS AFTER 14-DAY DOSE RANGE STUDY OF APHP

Functional and Behavioural assessment	Observation	Control n=6	APHP 250 mg/kg n=6	APHP 500 mg/kg n=6	APHP 1000 mg/kg n=6
Body position	Normal	6	6	6	6
Respiration	Normal	6	6	6	6
Clonic involuntary movement	Normal	6	6	6	6

Tonic involuntary movement	Normal	6	6	6	6
Palpebral closure	Normal	6	6	6	6
Approach response	Normal	6	6	6	6
Touch response	Normal	6	6	6	6
Pinna reflex	Normal	6	6	6	6
Tail pinch response	Normal	6	6	6	6

TABLE 7: HAND HELD OBSERVATION AFTER 14-DAY DOSE RANGE STUDY OF APHP

Functional and Behavioural observation	Observation	Control n=6	APHP 250mg/kg n=6	APHP 500mg/kg n=6	APHP 1000mg/kg n=6
Reactivity	Normal	6	6	6	6
Handling	Normal	6	6	6	6
Palpebral closure	Normal	6	6	6	6
Lacrimation	Normal	6	6	6	6
Salivation	Normal	6	6	6	6
Pilo erection	Normal	6	6	6	6
Pupillary reflex	Normal	6	6	6	6
Abdominal tone	Normal	6	6	6	6
Limb tone	Normal	6	6	6	6

Mortality and Morbidity: All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days. Results were given in **Table 8**.

TABLE 8: EFFECT OF APHP ON MORTALITY AFTER 14 DAY DOSE RANGE STUDY

Treated group	Mortality
Vehicle(control)	0
APHP250 (mg/kg)	0
APHP500 (mg/kg)	0
APHP1000 (mg/kg)	0

Sub-chronic Toxicity Study: Acute toxicity results showed that the APHP doesn't produce any toxic manifestations even at 2000 mg/kg. So APHP was categorised under Category 5 of Globally harmonised system. In connection to that, sub chronic toxicity studies have been carried out to predict the dose to be selected for *in-vitro* and *in-vivo* experiments.

Experimental Design: Experimental animals were randomly divided into four groups, each com-promising six animals.

Group 1: Normal control group and received only vehicle

Group 2: Animals treated with APHP by an oral gavage at a dose of 250mg/kg

Group 3: Animals treated with APHP by an oral gavage at a dose of 500mg/kg

Group 4: Animals treated with APHP by an oral gavage at a dose of 1000mg/kg

Doses were selected based on the pilot study and literature review.

Assessment of Body Weight: Body weight was measured using a Weighing balance of 5kg capacity and the results were recorded on 15^{th}, 30^{th}, 45^{th} day and 90^{th} day. Results were shown in **Fig. 1** and **2**.

Estimation of Haematological Parameters: Haematological parameters include haemoglobin estimation, total erythrocyte, total leukocyte and platelet count along with packed cell volume using Automated Haematology Analyzer XT-1800i (Sysmex, USA). Results were shown in **Fig. 3 - 14**.

Assessment of Liver Function Tests: [10]

Estimation of SGOT/AST: The SGOT was estimated according to the method described by Gella *et al*., Aspartate amine transferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm; by means of malatedehydrogenase (MDH) coupled reaction. Results were shown in **Fig. 15**.

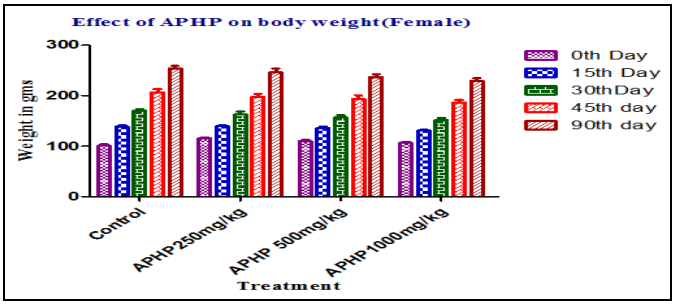

FIG. 1: EFFECT OF APHP ON BODY WEIGHT (FEMALE) AFTER SUB-CHRONIC TOXICITY STUDY

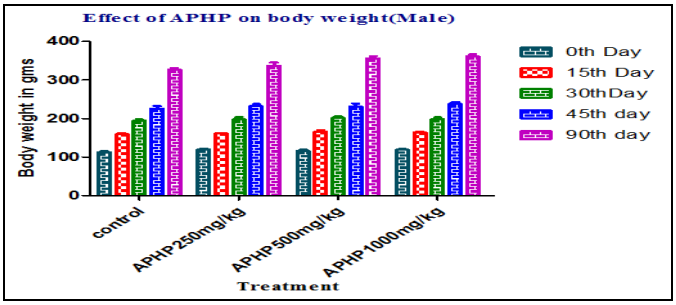

FIG. 2: EFFECT OF APHP ON BODYWEIGHT (MALE) AFTER SUB-CHRONIC TOXICITY STUDY

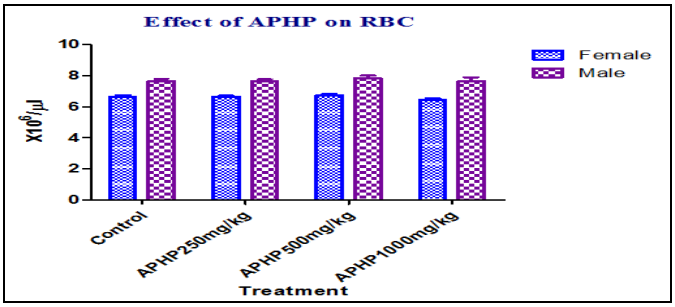

FIG. 3: EFFECT OF APHP ON RBC AFTER SUB-CHRONIC TOXICITY STUDY

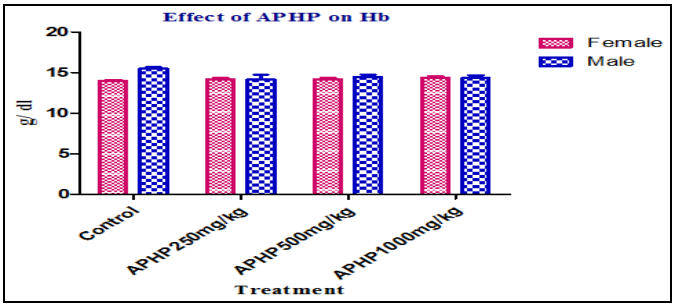

FIG. 4: EFFECT OF APHP ON HB AFTER SUB-CHRONIC TOXICITY STUDY

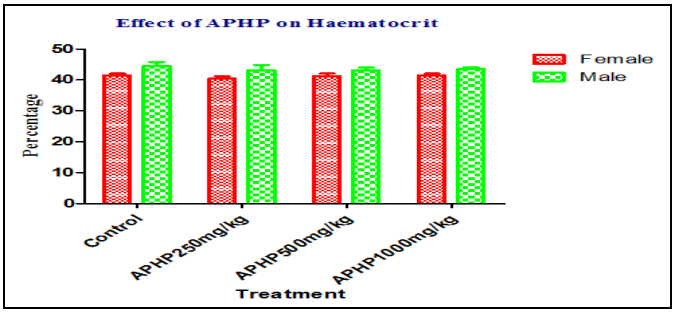

FIG. 5: EFFECT OF APHP ON HEAMATOCRIT AFTER SUB-CHRONIC TOXICITY STUDY

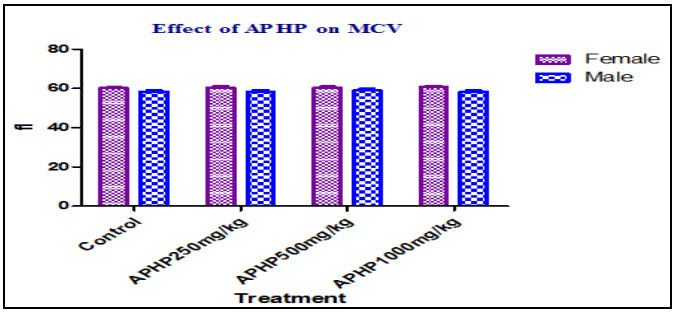

FIG. 6: EFFECT OF APHP ON MCV AFTER SUB-CHRONIC TOXICITY STUDY

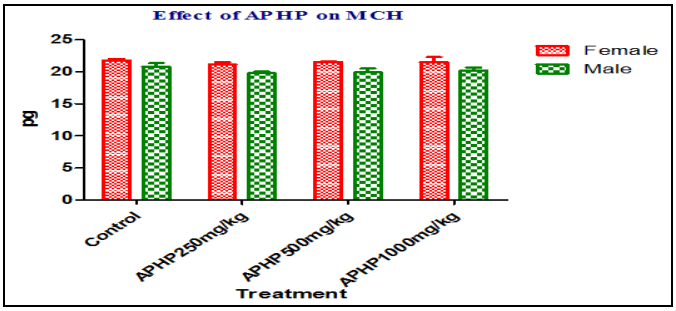

FIG. 7: EFFECT OF APHP ON MCH AFTER SUB-CHRONIC TOXICITY STUDY

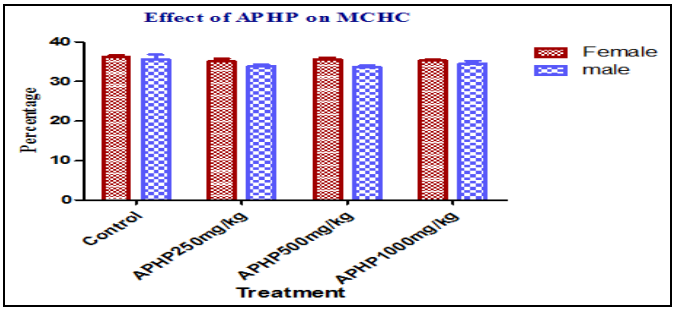

FIG. 8: EFFECT OF APHP ON MCHC AFTER SUB-CHRONIC TOXICITY STUDY

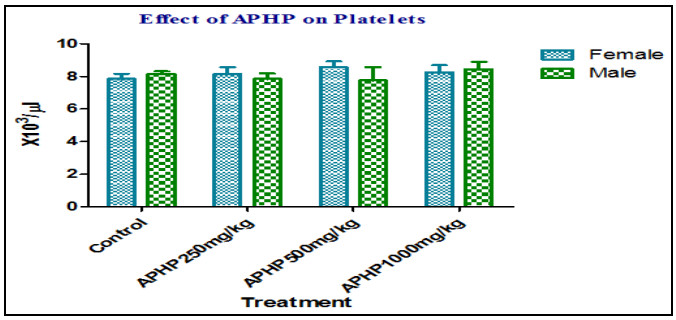

FIG. 9: EFFECT OF APHP ON PLATELETS AFTER SUB-CHRONIC TOXICITY STUDY

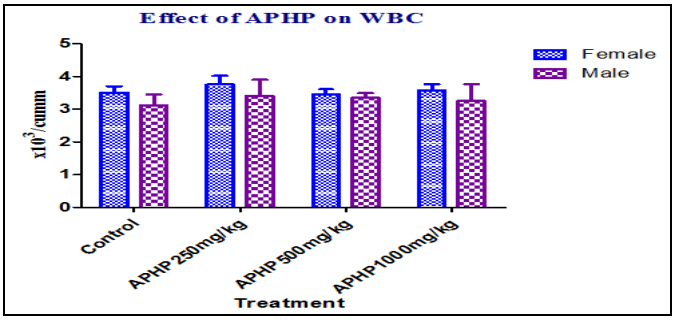

FIG. 10: EFFECT OF APHP ON WBC AFTER SUB-CHRONIC TOXICITY STUDY

Each point represents Mean ± S.D (n=5)

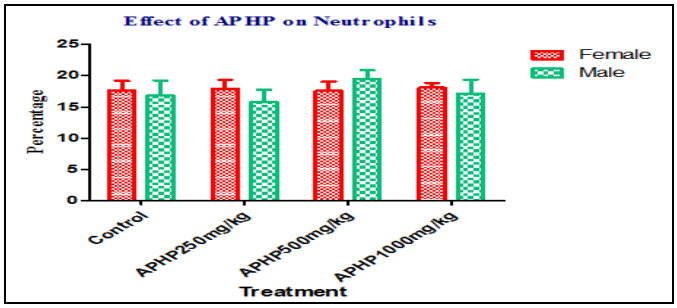

FIG. 11: EFFECT OF APHP ON NEUTROPHILS AFTER SUB-CHRONIC TOXICITY STUDY

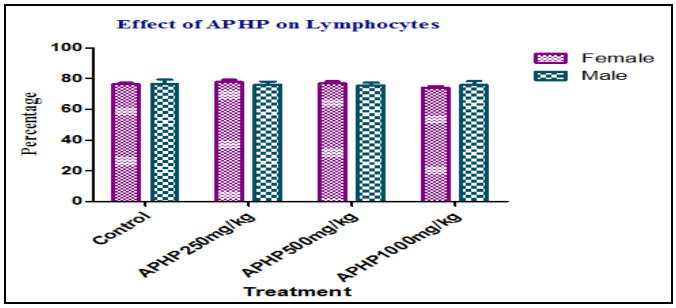

FIG. 12: EFFECT OF APHP ON LYMPHOCYTES AFTER SUB-CHRONIC TOXICITY STUDY

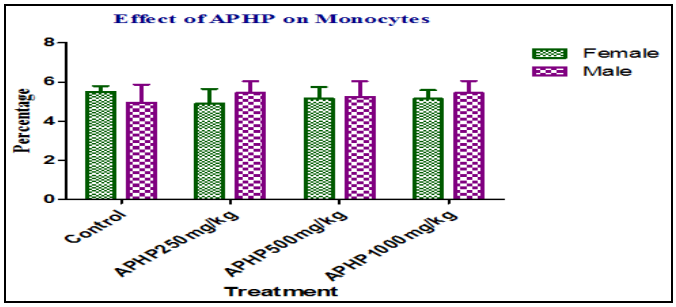

FIG. 13: EFFECT OF APHP ON MONOCYTES AFTER SUB-CHRONIC TOXICITY STUDY

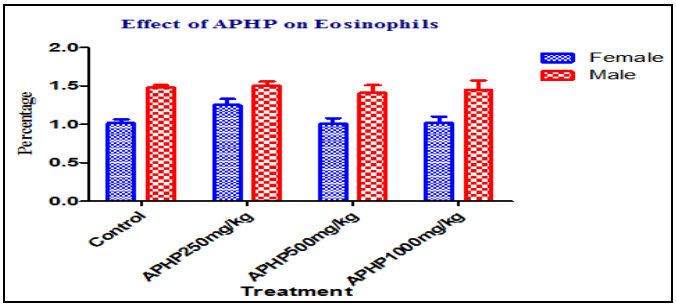

FIG. 14: EFFECT OF APHP ON EOSINOPHILS AFTER SUB-CHRONIC TOXICITY STUDY

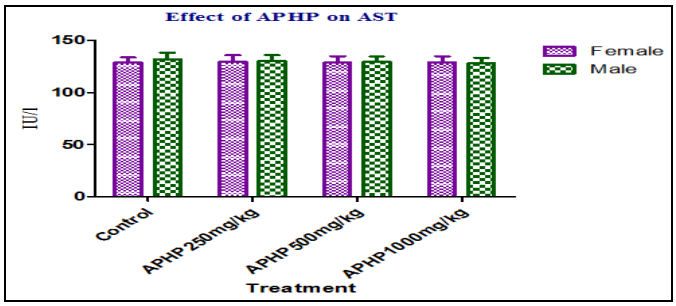

FIG. 15: EFFECT OF APHP ON AST AFTER SUB-CHRONIC TOXICITY STUDY

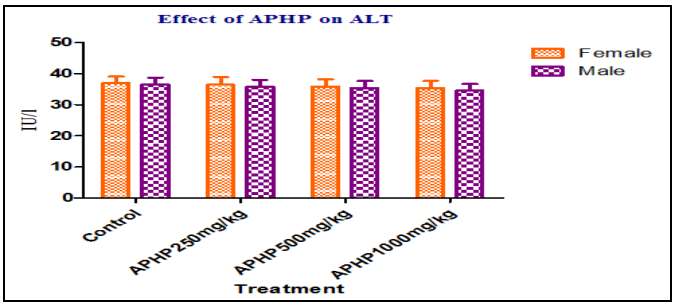

FIG. 16: EFFECT OF APHP ON ALT AFTER SUB-CHRONIC TOXICITY STUDY

Each point represents Mean ± S.D (n=5)

Estimation of SGPT/ALT: Alanine amino transferase catalyses the transfer of amino group from alanine to 2-oxoglutarate, resulting in the formation of pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of lactate dehydrogenase coupled reaction (Gella, *et al*., 1985). Results were shown in **Fig. 16**.

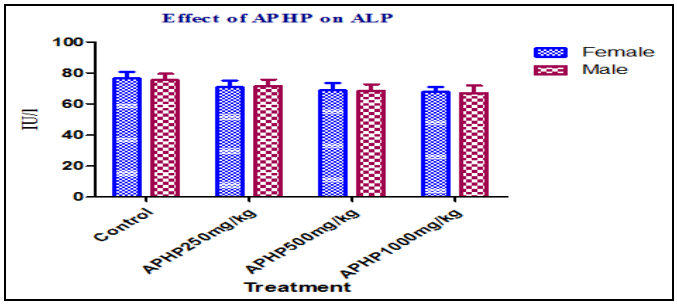

FIG. 17: EFFECT OF APHP ON ALP AFTER SUB-CHRONIC TOXICITY STUDY

Each point represents Mean ± S.D (n=5)

Estimation of Alkaline Phosphatase: Alkaline phosphatase catalyses in alkaline medium the transfer of phosphate group from 4-nitroAPHPnyl phosphate to 2-amino-2-methyl-1-propanol, liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitroAPHPnol formation, measured at 405 nm (Rosalki, *et al*., 1993). Results were shown in **Fig. 17**.

Total Bilirubin: Direct bilirubin in the sample reacts with diazotised sulfanilic acid forming a coloured complex that can be measured by spectro-photometry. Both direct and indirect bilirubin couple diazo in the presence of cetrimide (Pearlman and Lee, 1974). The terms direct and total refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilising reagents. The direct and indirect bilirubin is approximately equivalent to the conjugated and unconjugated fractions. Results were shown in **Fig.18**.

Albumin Estimation: The reaction between albumin in serum or plasma and the dye bromocresolgreen produces a change in colour, which is proportional to albumin concentration (Doumasa, *et al*., 1971) [11]. Results were shown in **Fig. 20**.

Total Protein Estimation: Total proteins were estimated using Total protein reagent from Agappe Diagnostics, Kerala, India (Gomall, *et al*., 1949; Lowry, *et al*., 1951). To 20 µl of serum, 1ml of total protein reagent was added and mixed.

The mixture was incubated at 37 ^{o}C for 15 minutes and the absorbance was measured at 546 nm using a Biochemical Analyzer. Results were shown in **Fig. 21**.

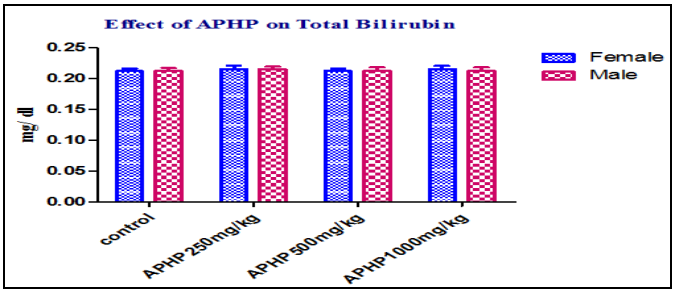

FIG. 18: EFFECT OF APHP ON TOTAL BILIRUBIN AFTER SUB-CHRONIC TOXICITY STUDY

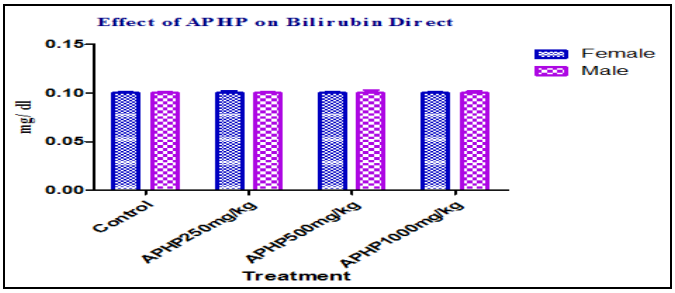

FIG. 19: EFFECT OF APHP ON BILIRUBIN DIRECT AFTER SUB-CHRONIC TOXICITY STUDY

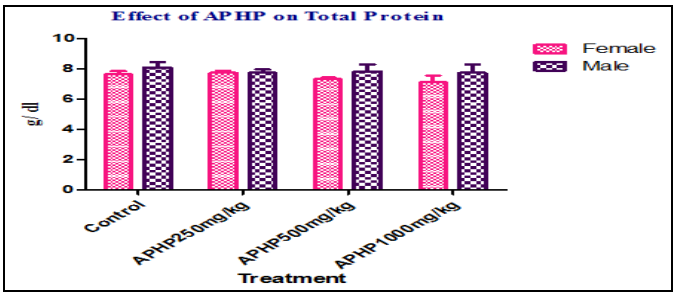

FIG. 20: EFFECT OF APHP ON TOTAL PROTEIN AFTER SUB-CHRONIC TOXICITY STUDY

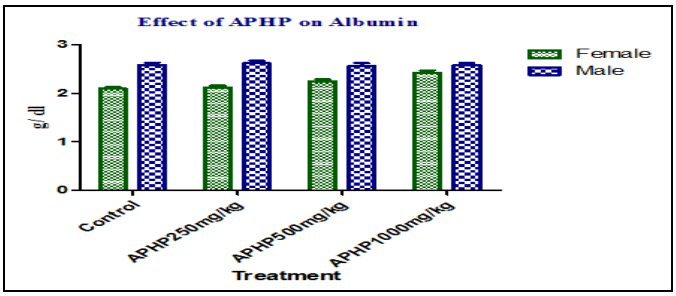

FIG. 21: EFFECT OF APHP ON ALBUMIN AFTER SUB-CHRONIC TOXICITY STUDY

Assessment of Kidney Function Tests:

Estimation of Uric Acid: Uric acid was estimated according to Trivedi and Kabasakalian with a modified Trinder peroxidise method using TBHB. Uric acid is oxidized by uricase to allantoin and hydrogen peroxide. The hydrogen peroxide is reacts with the trinder reagent, catalyzed by peroxidase, to form a quinoneimine dye. The intensity of the colour complex formed is directly proportional to the uric acid concentration of the sample, when measured at 505 nm (500-540 nm) [12]. Results were shown in **Fig. 24**.

Estimation of Creatinine: Serum creatinine is estimated by using Jaffe's method. The method is based on the Jaffe reaction. Creatinine reacts with picrate ion formed in alkaline medium to develop a red-orange colour. The colour produced from the sample is then compared in a colorimeter at wavelength of 520 nm with that produced by a known amount of creatinine under the same condition. Results were shown in **Fig. 25**.

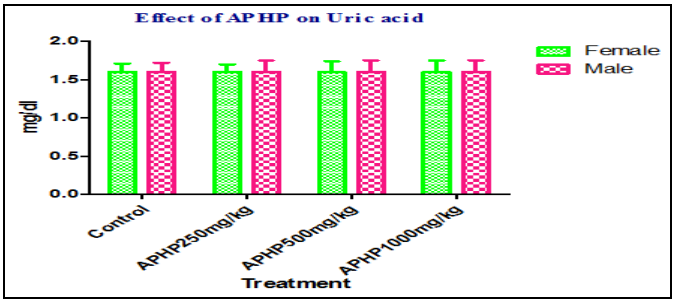

FIG. 24: EFFECT OF APHP ON URIC ACID AFTER SUB-CHRONIC TOXICITY STUDY

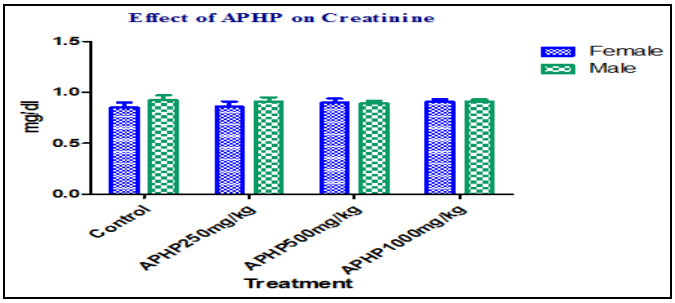

FIG. 25: EFFECT OF APHP ON CREATININE AFTER SUB-CHRONIC TOXICITY STUDY

Estimations of Blood Urea Nitrogen (BUN): BUN was estimated according to GLDH-Urease method, Talke and Schubert, Tiffany *et al*., Urea is the principle waste product of protein catabolism. It is synthesized in the liver from ammonia which is produced as a result of the deamination of amino acids. Normally, urea nitrogen in the blood comprises only about 45 % of the non-protein nitrogen. The importance of urea nitrogen determination is its value as an indicator of liver and kidney functions. Urea is catalytically converted to ammonium carbonate by the use of urease. The reaction rate is dependent upon the concentration of the influence of glutamic dehydrogenase. The rate of this second reaction is dependent upon the first and can be measured by the rate of conversion of NADH to NAD by the change of absorbency at 340 nm [13]. Results were shown in **Fig. 22**.

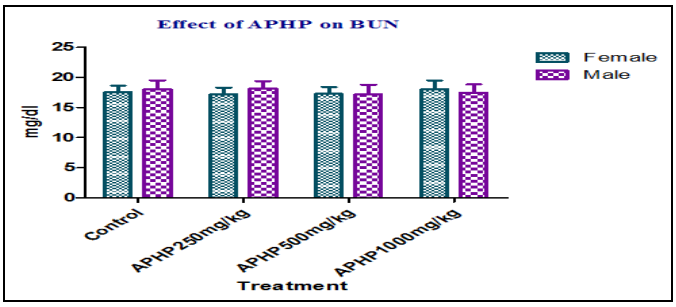

FIG. 22: EFFECT OF APHP ON BUN AFTER SUB-CHRONIC TOXICITY STUDY

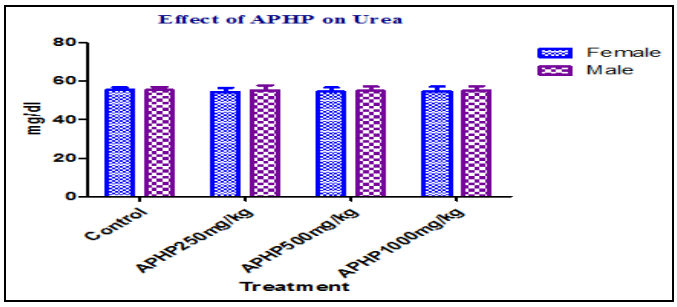

FIG. 23: EFFECT OF APHP ON UREA AFTER SUB-CHRONIC TOXICITY STUDY

Parameters to Assess Metabolic Activity:

Estimation of Glucose: The substrate β-D-glucose is oxidized by glucose oxidase to from gluconic acid and hydrogen peroxide. The hydrogen peroxide so generated oxidizes the chromogen system consisting of 4-aminoantipyrine and phenolic compound to a red quinoeimine dye. The intensity of the colour produced is proportional to the glucose concentration and is measured at 505 nm (490-530 nm) or with green filter (Trinder, 1969). Results were shown in **Fig. 26**.

Estimation of Lipid Profile:

Total Cholesterol: Enzymatic determination of total cholesterol was performed according to the following equation (Allain, *et al*., 1974). Results were shown in **Fig. 27**.

Estimation of NA and K: Potassium-The potassium in the filtrate from the magnesium hydroxyl quinolinate was determined by the method of Shohl and Bennett as modified by Hald.

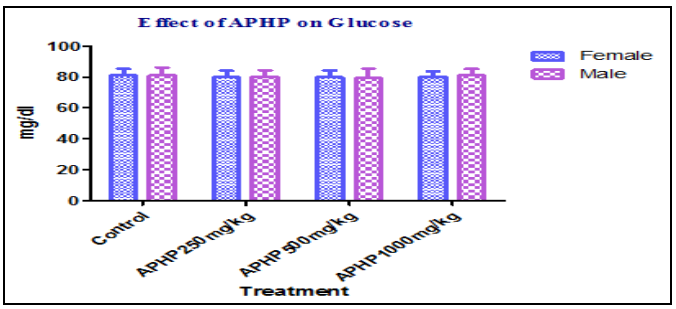

FIG. 26: EFFECT OF APHP ON GLUCOSE AFTER CHRONIC TOXICITY STUDY

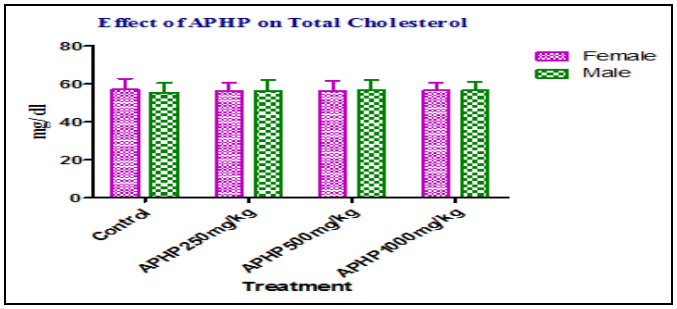

FIG. 27: EFFECT OF APHP ON TOTAL CHOLESTEROL SUB-AFTER SUB-CHRONIC TOXICITY STUDY

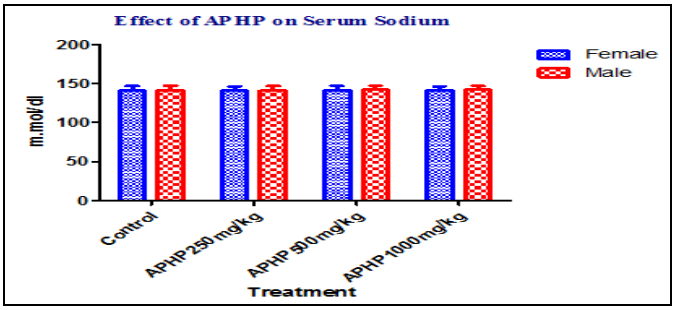

FIG. 28: EFFECT OF APHP ON SERUM SODIUM AFTER SUB-CHRONIC TOXICITY STUDY

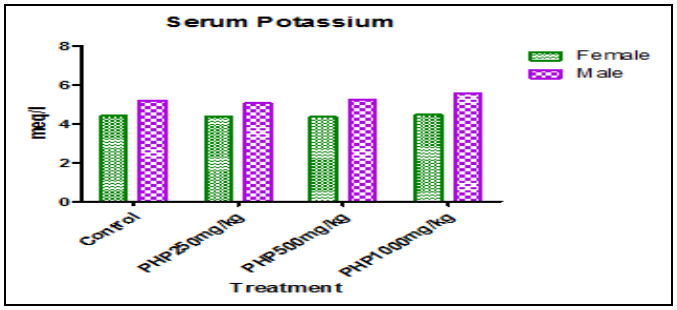

FIG. 29: EFFECT OF APHP ON SERUM POTASSIUM AFTER SUB-CHRONIC TOXICITY STUDY

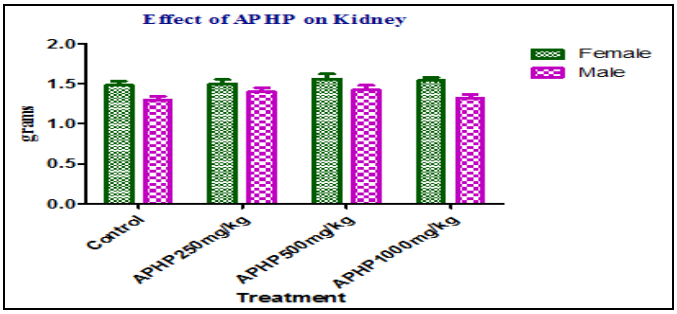

FIG. 30: EFFECT OF APHP ON KIDNEY AFTER SUB-CHRONIC TOXICITY STUDY

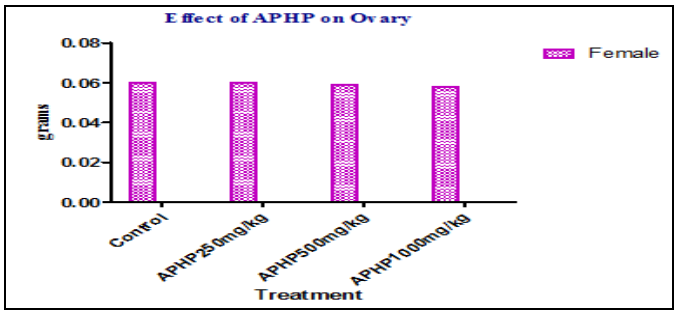

FIG. 31: EFFECT OF APHP ON OVARY AFTER SUB-CHRONIC TOXICITY STUDY

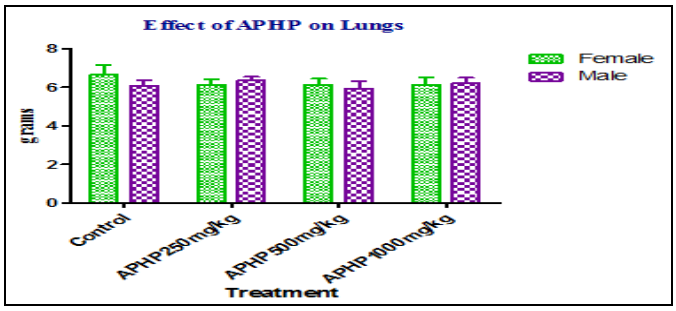

FIG. 32: EFFECT OF APHP ON LUNGS AFTER SUB-CHRONIC TOXICITY STUDY

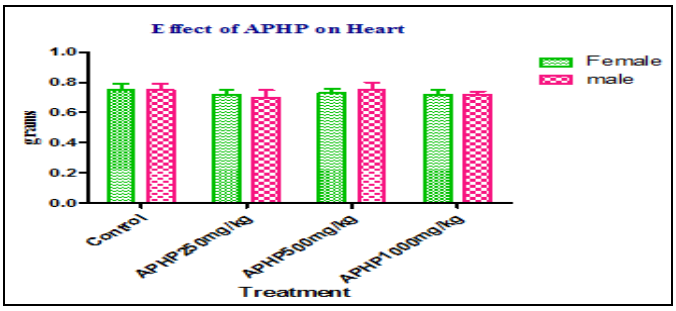

FIG. 33: EFFECT OF APHP ON HEART AFTER SUB-CHRONIC TOXICITY STUDY

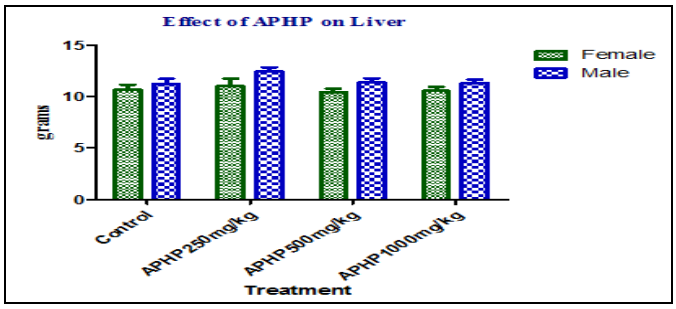

FIG. 34: EFFECT OF APHP ON LIVER AFTER SUB-CHRONIC TOXICITY STUDY

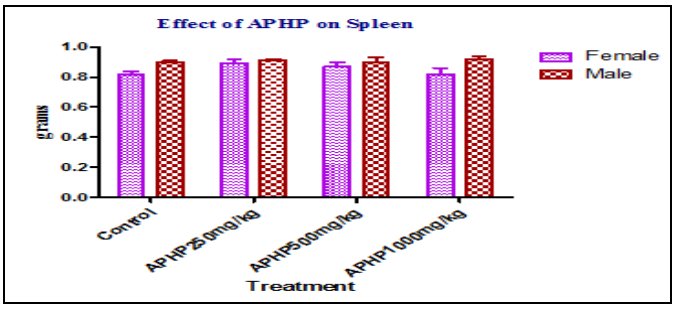

FIG. 35: EFFECT OF APHP ON SPLEEN AFTER SUB-CHRONIC TOXICITY STUDY

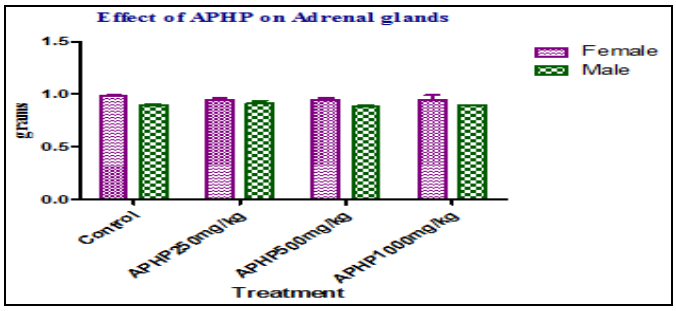

FIG. 36: EFFECT OF APHP ON ADRENAL GLANDS AFTER SUB-CHRONIC TOXICITY STUDY

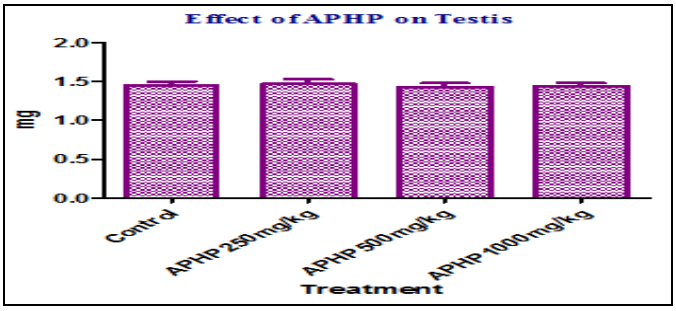

FIG. 37: EFFECT OF APHP ON TESTIS AFTER SUB-CHRONIC TOXICITY STUDY

Each point represents Mean ± S.D (n=5)

Sodium- The sodium in the filtrate from the potassium determination was determined by the gravimetric method of Barber and Kolthoff and Kohhoff as applied by Butler [14]. Results were shown in **Fig. 28** and **29**.

Organ to Body Weight Ratio: All groups were sacrificed after 90 days for calculating organ to body weight ratio. All vital organs, *viz* spleen, thymus, adrenals, isolated and weighed. Results were shown in **Fig. 30 - 37**.

RESULTS AND DISCUSSION:

Acute Toxicity:

Cage Side Observation: Treated groups recorded normal behavioural, motor, and neuronal functions for all the administered OS extracts with no mortality observed. The monitoring of skin and fur,

eyes, behavioural pattern such as gait and posture, and autonomic and central nervous system activities of treatment rats remained unchanged with the treatment of APHP when compared with those of control group. This showed that the oral LD50 of APHP was greater than 2000 mg/Kg body weight. (**Table 2**, **3**, **4**, **5**, **6** and **7**)

Sub-chronic Toxicity Studies: Our findings have reported that there is no significant variation in body weight of APHP administered groups when compared to normal. No variation was observed in haematological parameters, bio chemical parameters, Individual organ weight, Serum electrolytes, glucose and cholesterol ($P > 0.05$).

DISCUSSION: For several centuries, medicinal plants and herbal remedies continue to enrich the healthcare needs of animals and human. Many medicinal herbs play important role in the management of various disorders [15]. The beneficial effects of various medicinal plants are widely established in scientific literature and the preparations that contain single or multiple herbs have been indicated for restoring health in ethno medical practices and traditional medicinal systems of many countries [16]. However, herbal preparations, in spite of being popularly claimed as naturally safe, need to be authenticated by scientifically validated tests for toxicological properties before being introduced for widespread consumption [17].

In safety evaluation of test substances, acute oral toxicity study is considered as the preliminary step and facilitates classification and labeling of investigational agents. In the present study, single acute oral administration of APHP to female wistar rats at the dose level of 2000 mg/kg b.w. did not cause any mortality and the median lethal dose was found to be more than 2000 mg/kg b.w. Therefore, the findings resulted in classifying APHP in category 5 criteria according to the Globally Harmonised System.

Repeated dose oral toxicity studies are carried out to assess the adverse effects of a substance used for a prolonged period of time and to obtain information about the potential health hazards that may likely to occur from continuous exposure including information about target organ toxicity, possibilities of cumulative effects, and an estimate of the dose at which there is no observed adverse effect. As commonly recommended by Regulatory guidelines for subchronic toxicity testing, a 14-day dose range finding study was performed to select appropriate dose levels for the 90-day oral toxicity study. On administration of APHP at, 250, 500, and 1000 mg/kg for a period of 14 days, no deaths, treatment-related abnormal clinical or behavioural signs, alterations in body weight gain, and gross pathological observations were recorded till the end of the experiment.

Based on the results of the dose range finding study, three proportionate dose levels of 250, 500, and 1000 mg/kg b.w. were selected for the subchronic oral toxicity study in rats. Treatment with APHP orally for consecutive 90 days at and up to the dose level of 1000 mg/kg b.w. did not cause any mortality or toxicity signs during the dosing in test groups. The results and the functional observation examinations of treated animals also confirmed the normal physiological responses that were similar to the findings of control group of animals.

CONCLUSION: In conclusion, our study demonstrated that APHP prepared from the selected plants of interest has no signs of toxicity. The results of the various phytochemical tests indicated that the APHP was found to be rich in various biologically active compounds which could serve as potential source of the crude drugs.

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CONFLICT OF INTEREST: The author has no conflicts of interest.

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