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Pharmacological Study

Toxicological evaluation of *Panchakola Avaleha*, an Ayurvedic classical formulation, in albino rats

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Abstract

The present study was carried out to assess the safety of standardized *Panchakola Avaleha* on albino rats (Wistar strain). Animals were administered three doses of *Panchakola Avaleha* by oral routes, viz. higher (500 mg/kg/day), middle (250 mg/kg/day), and therapeutic dose (50 mg/kg/day) for 28 consecutive days. Effects of the test drug on hematological, biochemical, and histopathologic parameters were evaluated. This study revealed normal behavior, no mortality, and no significant changes in hematological, biochemical, and histopathological examinations.

Key words: *Panchakola Avaleha*, toxicity study, Wistar rats

Introduction

Panchakola is one of the most popular formulations in Ayurveda, which is being used as general health tonic. It is used as antipyretic, analgesic, anti-inflammatory, appetizer, digestive, and carminative.[1] It is highly effective particularly in *Vatasleshmika Jvara*.[2] It is available in different pharmaceutical forms like *Churna* (powder), *Paka* or *Avaleha* (confection), and *Ghrita* (medicated fatty preperation). *Panchakola Avaleha* rejuvenates the female reproductive system, particularly in peurperium stage and helps in early involution of uterus. This formulation also helps to improve the quality and quantity of breast milk.[3] A recent clinical study showed that *Panchakola Siddha Yavagu* is effective as an appetizer.[4] This formulation is also being used for centuries in different conditions of *Sandhigata Vata* (Arthritis and rheumatism) as a supportive measure. Infact the toxicological study of *Panchakola Avaleha* has not been done and the present study is being carried out for toxicological evaluation. This article presents the results of a 28-day repeated dose oral toxicity study conducted to ensure the safety of the drug.

Materials and Methods

This study was conducted at National Research Institute of Ayurvedic Drug Development, Kolkata, a peripheral center under Central Council for Research in Ayurvedic Sciences, Department of AYUSH, Ministry of Health and Family Welfare, Government of India.

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Test animals and housing

The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (138A/CRI/2000-2002) dated 29.12.2004 and Registration No. 694/a/CPCSEA/03 dated 01.10.2002. The present study was conducted on inbred albino rats (Wistar strain) of both sexes, which were procured from NIN, Hyderabad.

Wistar rats of 6-7 weeks of age, weighing 140-250 g (20 males + 20 females = 40) were selected based on the body weight and distributed into four groups (5 males and 5 females in each group). Animals were acclimatized for 7 days and health examination was performed during acclimatization period. Rats were housed individually in polypropylene cages, were fed animal pellet diet and mineral water *ad libitum* during the entire study period.

The temperature was maintained at 26 ± 3°C and relative humidity at 60-70%, and illumination was controlled to give approximately a sequence of 12 h light and 12 h dark.

Test drug

The test drug, i.e., *Panchakola Avaleha* is composed of five major indigenous plant materials, viz. *Pippali* (*Piper longum* L.), *Pippalimula* (*Piper longum* L.), *Chavya* (*Piper chaba* Trel. and Yunck.), *Chitraka* (*Plumbago zeylanica* L.), and *Shunthi* (*Zingiber officinale* Roscoe), and *Guda* (jaggery), *Sarkara* (sugar) and *Ghrita* (ghee) were used for preparation of *Avaleha* following the method of preparation of Ayurvedic practice and principles[5] [Table 1].

Experimental design

Twenty-eight days repeated dose oral toxicity study was carried out on 40 rats (both sexes) and the rats were divided into four groups consisting of 5 males and 5 females in each. The rats received daily vehicle control (honey:Deionized water –2:3 proportion) and 500, 250, and 50 mg/kg/day *Panchakola Avaleha* (dissolved in honey:Deionized water –2:3 proportion) by oral gavage, once daily for 28 consecutive days. Both the test and control groups received the same volume of drug or vehicle as per body weight. Animals were checked for mortality, and general clinical observations, viz. salivation, activity, irritability, fecal pellet condition, diarrhoea, eye ball movement, and external appearance, were made daily in the morning and evening. The body weight was taken before the start of the treatment in vehicle control as well as in treated groups. The weekly feed consumption and water consumption of rats were recorded by measuring the difference between the feed and water offered and feed and water left over the subsequent week. The weekly feed and water consumed per cage was calculated and presented. Blood sampling was performed on day 29, prior to euthanasia. Animals were fasted overnight, and blood samples were drawn from the retro-orbital plexus under diethylether-induced anesthesia. For hematological analysis, blood was collected in a silica-coated vial with anticoagulant containing Ethylene Di Amine Tetra Acetic acid (EDTA) to measure hemoglobin (Hb%), Total Erythrocyte Count (TEC), and Total Leukocyte Count (TLC). Blood was collected in a centrifuge tube without anticoagulant for collection of serum for clinical chemistry parameters including serum total bilirubin,[6] total protein, serum albumin,[7] Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT),[8] serum alkaline phosphatase,[9] and serum creatinine.[7] Necropsy was performed on day 29 of the study. Organs were observed, collected, weighed, and preserved in 10% neutral buffered formalin, viz. kidney, adrenal, heart, spleen, and liver. Tissues were then trimmed and dehydrated in ascending grades of alcohol. Finally the tissues were embedded in melted paraffin and blocks were prepared. The tissue sections were cut to 3-5 μm in microtome and stained with hematoxylin and eosin.[10] Finally, the sections were mounted with Distyrene Plasticizer and Xylene (DPX) and examined under microscope. Histopathologic examination was performed on all collected tissues from males and females of the control and 500 mg/kg/day group.

Table 1: Composition of *Panchakola Avaleha* in 10 g

Name of the ingredients	Botanical name	Part of use	Amount (mg)
Pippali	*Piper longum*	Fruit	435.0
Pippalimula	*Piper longum*	Root and stem	435.0
Chavya	*Piper chaba*	Stem	435.0
Chitraka	*Plumbago zeylanica*	Root	435.0
Shunthi	*Zingiber officinale*	Rhizome	435.0
Guda (jaggery)	–	–	2250.0
Sarkara (sugar)	–	–	5050.0
Ghrita (ghee)	–	–	525.0

Preservatives such as sodium methyl paraben (0.15%), propyl paraben (0.05%), sodium benzoate (0.5%), and antioxidant (0.1%) were used in the formulation

Statistical analysis

All the data were analyzed by Student's *t*-test followed by analysis of variance (ANOVA).

Observations and Results

No mortality and treatment-related clinical signs were observed throughout the study period. There was no significant change in treated animals at 2nd and 4th weeks, when compared with the initial values of the respective groups. However, the mean values of treated animals were higher when compared with controlled group and these changes were attributed to sporadic deviation within the groups and had no biological significance, hence not considered as drug-related adverse effect [Table 2]. All animals from *Panchakola Avaleha* treated groups consumed similar amount of food, compared with the corresponding control group. The observations of feed and water intake changes were not dose dependent and not observed consistently. Hence, it was not considered as a treatment-related effect. In case of treated animals in 50 mg/kg/day group, statistically significant increase of hemoglobin percentage on 28th day was observed; however, the value was within normal range [Table 3]. There were no significant changes in liver function tests, i.e., total serum bilirubin, SGOT, SGPT, serum albumin, total protein, alkaline phosphatase, and serum creatinine, on 28th day of study when compared with their values in the respective groups and control group, except for the levels of albumin and total protein in 500 mg/kg/day group and the level of total protein in 250 mg/kg/day group [Table 4]. There were also no significant changes in weights of vital organs, viz. kidney, adrenal, heart, spleen, and liver, on 28th day of study [Table 5]. Gross necropsy examination revealed no treatment-related lesions. There was significant increase in weight of liver and spleen in 500 mg/kg/day group, but in other groups no such change was observed [Table 5]. Hence, it is not considered as a treatment-related effect. However, histopathology evaluation of any of the organ did not reveal any treatment-related or dose-dependent changes [Figures 1-10].

Discussion

Among the variety of Ayurvedic medicines, drugs are being used to treat various diseases according to Ayurvedic pharmacology. Since this traditional treatment is based on extensive knowledge gathered from applications of natural resources to humans, people have usually assumed that the treatment is safe.

Table 2: Effect of *Panchakola Avaleha* on body weight (g) measured weekly in rats at different doses for 28 days (*n*=10)

Body weight	Control (vehicle) group	Test drug groups-PKA (mg/kg)		
		500	250	50
Initial wt.	170.0±05.16	183.0±11.75	181.0±09.48	172.0±07.72
1st week	177.0±09.20	195.0±11.18	180.0±10.86	179.0±09.00
2nd week	186.0±09.45	203.0±14.92	197.0±12.75	190.0±12.21
3rd week	187.0±09.19	208.0±26.03	198.0±24.02	194.0±21.64
4th week	198.0±04.91	226.0±28.99	198.6±25.48	200.0±29.23

Data represented as mean±SEM; PKA - *Panchakola Avaleha*

Table 3: Investigation of hematology of rat blood treated with *Panchakola Avaleha* on 28th day (*n*=10)

Hematological parameters	Control (vehicle) group	Test drug groups-PKA (mg/kg)		
		500	250	50
Hb (g% /dl)	16.20±1.43	18.62±2.19	19.58±1.48	20.90±1.52*
WBC (10^3/mm^3)	06.62±0.18	06.42±0.32	06.80±0.54	07.20±0.25
RBC (10^6/mm^3)	06.04±0.34	05.48±0.64	06.60±0.51	07.30±0.46
Leukocyte	61.00±1.67	66.40±1.75	58.20±0.80	63.00±1.90
Neutrophil	32.60±1.43	28.40±1.50	33.20±1.71	31.60±1.29
Monocyte	01.00±0.31	00.60±0.24	02.60±1.07	00.40±0.24
Eosinophil	05.00±0.31	04.40±0.40	05.80±0.37	05.00±0.77
Basophil	00.42±0.24	00.20±0.20	00.20±0.20	00.00±0.00

Data are represented as mean±SEM; PKA - *Panchakola Avaleha*; *P<0.05 (control *vs.* treated)

Table 4: Investigations of blood biochemistry of rat treated with *Panchakola Avaleha* on 28th day (*n*=10)

Bio-chemical parameters	Control (vehicle) group	Test drug groups-PKA (mg/kg)		
		500	250	50
Total bilirubin (mg/dl)	1.08±0.35	1.17±0.30	1.28±0.35	1.01±0.28
SGOT (U/L)	144.80±2.79	147.60±17.58	141.20±7.02	138.40±15.83
SGPT (U/L)	46.40±4.23	52.40±6.68	49.80±4.81	62.80±8.66
Albumin (g/dl)	2.90±0.44	4.30±0.24*	3.40±0.27	3.42±0.54
Serum creatinine (mg/dl)	0.67±0.09	0.58±0.05	0.65±0.05	0.55±0.08
Alkaline phospatase (U/L)	189.80±31.44	260.60±55.18	216.20±42.03	163.40±61.76
Total protein (g/dl)	6.86±0.71	8.40±0.24*	8.92±0.20*	7.94±0.65

Data are represented as mean±SEM; PKA - *Panchakola Avaleha*; *P<0.05 (control *vs.* treated)

Table 5: The weight of vital organs (g) treated with *Panchakola Avaleha* on 28th day (*n*=10)

Vital organs	Control (vehicle) group	Test drug groups-PKA (mg/kg)		
		500	250	50
Heart	0.66±0.03	0.78±0.08	0.72±0.05	0.83±0.13
Kidney	1.32±0.05	1.53±0.18	1.43±0.18	1.61±0.22
Adrenal	0.056±0.006	0.057±0.01	0.065±0.01	0.073±0.02
Spleen	0.42±0.01	0.51±0.04*	0.46±0.02	0.51±0.05
Liver	6.53±0.33	8.91±1.09*	7.32±0.73	7.79±1.34

Data are represented as mean±SEM; PKA - *Panchakola Avaleha*; *P<0.05 (control *vs.* treated)

However, as Oriental remedies have been rapidly growing in popularity worldwide, consumers are paying as much attention to safety issues as to their therapeutic efficacy. However, few studies have therefore been conducted to evaluate the safety of indigenous medicines or the associated risk of adverse effects. In this context, the toxicological study of *Panchakola Avaleha* in experimental animals has been carried out following the OECD guideline to assure the safety of drug use for global aspect.

Panchakola Avaleha is being widely used by the Ayurvedic physicians which consists of five active ingredients, viz. *Pippali*, *Pippalimula*, *Chavya*, *Chitraka*, and *Shunthi* in the form of a confection which is pharmaceutically prepared by adding jaggery, sugar, and ghee. This formulation is being used for centuries in certain conditions, particularly for rejuvenation of body by mechanism of *Agnideepana* (appetizer and carminative). As per the Ayurvedic principles, it has *Katu Rasa* (pungent taste) predominance and *Ushna Virya* (hot prooperty) which potentiates the drug effect.[11] However, *Ushna Virya* and *Katu Rasa* of the drug may cause *Rukshana* (emaciation) if it is used for a long term. Moreover, there has been no such study of safety of this formulation with reference to toxicological study. The present study was initiated for evaluation of toxicological aspect of *Panchakola Avaleha* to revalidate the Ayurvedic pharmaceutical practices in drug development. In 28 days repeated dose toxicity, 5 male and 5 female animals were studied into 4 different groups. It was observed that there were no such changes in food intake, water consumption and their behaviour in the treated groups. Values of body weight of animals and weight of the vital organs of treated groups were insignificant when compared with control group. Even, there were no significant changes in liver function tests and routine haematological investigations. Moreover, treated animal in 50 mg/Kg/day group, the Hb% was increased. In the necroscopy study, there were no treatment related lesions in vital organ which revealed the safety of the test drug.

Conclusion

It is revealed from the study that there was no mortality of the experimental animals. There were no morphological, haematological and biochemical changes in the treated animals when compared with control animals. It may be concluded that *Panchakola Avaleha* is safe in animal models.

Acknowledgment

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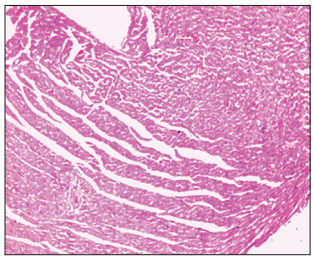

Figure 1: Heart of control rat. H and E, ×100

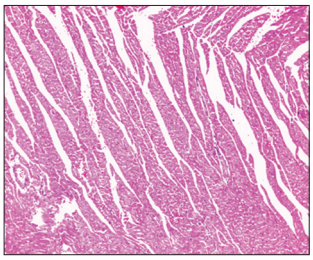

Figure 2: Heart of rat, 225 mg/ kg/body weight H and E, ×100

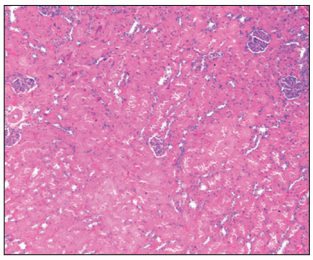

Figure 3: Kidney of control rat. H and E, ×100

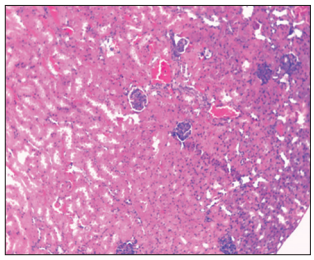

Figure 4: . Kidney of rat, 225 mg/kg/body weight dose group H and E, ×100

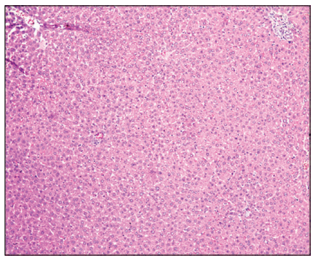

Figure 5: . Liver of control rat. H and E, ×100

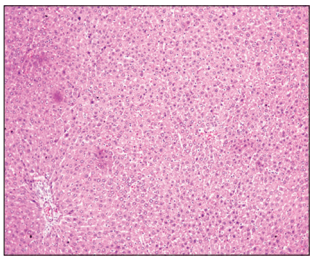

Figure 6: . Liver of rat, 225 mg/ kg/ body weight dose group H and E, ×100

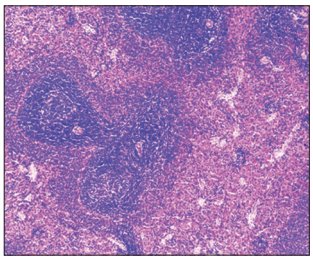

Figure 7: Spleen of control rat. H and E, ×100

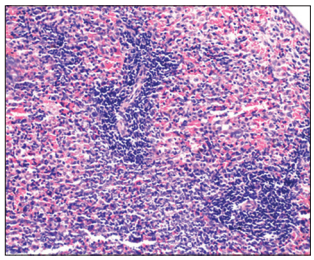

Figure 8: Spleen of rat, 225 mg/ kg/ body weight dose group H and E, ×100

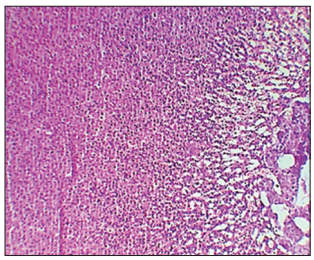

Figure 9: Adrenal of control rat. H and E, ×100

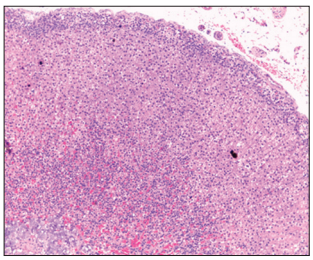

Figure 10: Adrenal of rat, 225 mg/kg/ body weight dose group H and E, ×100

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हिन्दी सारांश

शास्त्रीय आयुर्वेदिक औषध योग–पंचकोल अवलेह के विषाक्तता प्रभाव का प्रायोगिक अध्ययन

राजेन्द्र कुमार सिंह, रिता बनर्जी, सच्चिदानंद उपाध्याय, अचिन्त्य मित्रा, जयराम हाजरा

मानकीकृत पंचकोल अवलेह के विषाक्तता प्रभाव का सुरक्षात्मक मूल्यांकन चूहों पर किया गया । मुख मार्ग से पंचकोल अवलेह औषध की तीन प्रकार की मात्रा का चूहों पर अध्ययन – उच्च (५०० मि.ग्रा./कि.ग्रा./दिन), मध्य (२५० मि.ग्रा./कि.ग्रा./दिन) और निम्न (५० मि.ग्रा./कि.ग्रा./दिन) लगातार २८ दिनों तक दिया गया । इस औषध का प्रभाव, रक्त एवं विभिन्न अंगों पर प्रभाव का हिस्टोपैथोलाजी अध्ययन किया गया । इस अध्ययन में सभी प्राणियों में सामान्य व्यवहार, मृत्यु दर एवं उसका रक्त और विभिन्न अंगों पर कोई विषाक्त प्रभाव नहीं पाया गया ।