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A Toxicity study on Ayakaandha Abraga Chendhuram

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Abstract

Metals play a vital role for the treatment of many diseases. Siddha medical system is efficacious because of the numerous mineral and metallic formulations. Because herbo metallic preparations like "*Parpam*", "*Chendhuram*", "*Kattu*" type of medicines having some advantages like include better stability, lower dosage, ease of storability and sustained availability. To Evaluate the acute toxicity study (as per OECD guidelines 423) and
To Evaluate the 28 days repeated dose oral toxicity study (as per OECD guidelines 407) of Ayakaandha abraga chendhuram.

Keywords: Metals, Ayakaandha abraga chendhuram., acute toxicity.

Introduction

Siddha system is one of the oldest conventional medical system in the world. The Siddha formulation of medicines are based on the theory of "*Arusuvai*" and "*Panchabhoodham*[1]" And the diseases diagnosed depends on the three vital humours *Vatham*, *Pitham* and *Kabham*. The usage of heavy metals in siddha system of medicine having some queries regarding the threatening effects of those metals which in use. Though metallic siddha medicinal formulations have high therapeutic potential when compared to allopathic drugs there is also a challenge to ensure its safety for global acceptance. Siddha herbo mineral formulation *Ayakaandha Abraga Chendhuram* indicated as a best choice of drug to treat diabetes.

Preparation of aya kaandha abraga chendhuram:

The purified *Ayam* powder, purified *Kaandham* powder and purified *Abragam* (*Abragam Navaneetham*) are all grinded by using *Aadutheendaapaalai* (*Aristolochia Bracteata*) juice for 7 days. Then it is made into small pellets. Then these pellet are subjected into a calcinations process called *putam* by using 150 cow dung cakes. Then it is mixed with *Paeyathi* decoction (ficus hispida) for 12 hours and then again subjected into *putam*. Then it is grinded with *Sithiramoola Vaerpattai* decoction(*Plumbago zeylanica*) and again it is subjected in to *putam* and it is grinded well. Now it is prepared as *Chendhuram*. Then it is placed in a iron made saucer. The *kaadi seyaneer* is to be poured on it for 10 hours and allowed to dry. And again it is mixed with kaadi seyaneer and made into a pellet and dry it and placed in a 7 layers mud sealed earthern saucer and subjected into heat for

12 hours and allowed cool for one night then next morning, it is placed and heat again and made into powder. Finally the ***Ayakaandha abraga chendhuram*** is prepared. Toxicological study are done to prove its efficacy. Here the obtained results of the studies are the following:

Results and Discussion

Table 1 Results of Siddha Standardization – Interpretation

S.NO	PARAMETER	RESULTS OF IDEAL *CHENDHURAM*	RESULTS OF AAC	INTERPRETATION
1.	Colour	Reddish	Brownish red	*Chendhuram* colour
2.	Floating on Water	Floats on water	Floats on water	Lightness of drug.
3.	Finger Print Test	Impinged in the furrow of fingers	Impinged in the furrow of fingers	Indicates fine particles of powder.
4.	Lustre	Lustreless	Lustreless	Change of specific metallic character of raw material after incineration.
5.	Taste	No specific taste	No specific taste	Change of specific metallic character of raw material after incineration.

Colour:

It is Brownish red in colour. The absence of shining indicates there is no free form of metals.

Floating on water:

Ayakaandha abraga chendhuram floats on water. It is due to its less specific gravity. So, it possess the property of *Chendhuram*.

Finger print test:

Ayakaandha abraga chendhuram impinged on the cervices of finger. This indicates the particles are fine and it is in micro size.

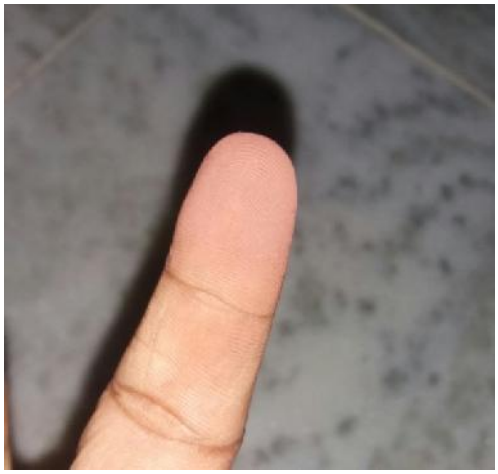


Image: Finger print test of AAC

Interpretation:

Specific gravity of *Ayakaandha abraga chendhuram* was less than the water. Thus, it indicates the lightness of the drug

Toxicological studies:

Acute Toxicity Study Table:

Table 2 Physical and behavioral examinations

Group no	Doses (mg/kg)	Observation sign	No of animal affected
Control	**Distilled water**	**Normal**	**0 of 3**
Group I	**5mg/kg**	**Normal**	**0 of 3**
GroupII	**50mg/kg**	**Normal**	**0 of 3**
GroupIII	**300mg/kg**	**Normal**	**0 of 3**
Group IV	**2000mg/kg**	**Normal**	**0 of 3**

Table 3 Showed the effect of AAC (control,5mg,50mg,300mg,1000mg/kg) on general behavior after single oral administration in mice

S.No	General Behaviour	Time of Observation after AAC(control,5mg,50mg,300mg,1000mg/kg) Administration		
		1st hr	3rd hr	4th hr
1	Sedation	-	-	-
2	Hypnosis	-	-	-
3	Convulsion	-	-	-
4	Ptosis	-	-	-
5	Analgesia	-	-	-
6	Stupar Reaction	-	-	-
7	Motor activity	-	-	-
8	Muscle Relaxant	-	-	-
9	CNS Stimulant	-	-	-
10	CNS Depressant	-	-	-
11	Pilo Erection	-	-	-
12	Skin Colour	-	-	-
13	Lacrimation	-	-	-
14	Stool Consistancy	-	-	-

'+ ' PRESENT & ' –' ABSENT

Table 4 Showed the effect of AAC (2000mg/kg) on general behavior after single oral administration in mice

S.No	General Behaviour	Time of Observation after AAC (2000mg/kg) administration		
		1st hr	3rd hr	4th hr
1	Sedation	-	+	+
2	Hypnosis	-	-	-
3	Convulsion	-	-	-
4	Ptosis	-	-	-
5	Analgesia	-	-	-
6	Stupar Reaction	-	+	-
7	Motor activity	-	-	-
8	Muscle Relaxant	-	+	+
9	CNS Stimulant	-	-	-
10	CNS Depressant	-	-	-
11	Pilo Erection	-	-	-
12	Skin Colour	-	-	-
13	Lacrimation	-	-	-
14	Stool Consistancy	-	-	-

'+ ' PRESENT & ' –' ABSENT

Table 5 mortality

Group No	Doses (mg/kg)	Mortality
Control	**Distilled water**	**0 of 3**
Group I	**5(mg/kg)**	**0 of 3**
GroupII	**50(mg/kg)**	**0 of 3**
GroupIII	**300(mg/kg)**	**0 of 3**
GroupIV	**2000(mg/kg)**	**0 of 3**

28 Days repeated dose oral toxicity study:

Table 6 Effect of *Ayakaandha Abraga Chendhuram* on body weight during 28 days treatment in rats

GROUPS	DRUG TREATMENT	BODY WEIGHT (GMS)				
		1ST DAY	7TH DAY	14TH DAY	21ST DAY	28TH DAY
I	Control Distilled Water (1ml/kg, p.o)	155.36± 2.87	158.58± 2.46	162.65± 1.98	169.71± 1.90	180.54± 2.35
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	163.75± 2.72	168.74± 3.22	171.35± 4.45	178.36± 3.70	187.64± 2.96
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	158.66± 2.65	165.32± 2.22	169.46± 2.55	177.82± 3.89	185.90± 4.43

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 1 Effect of *Ayakaandha Abraga Chendhuram* on body weight during 28 days treatment in rats

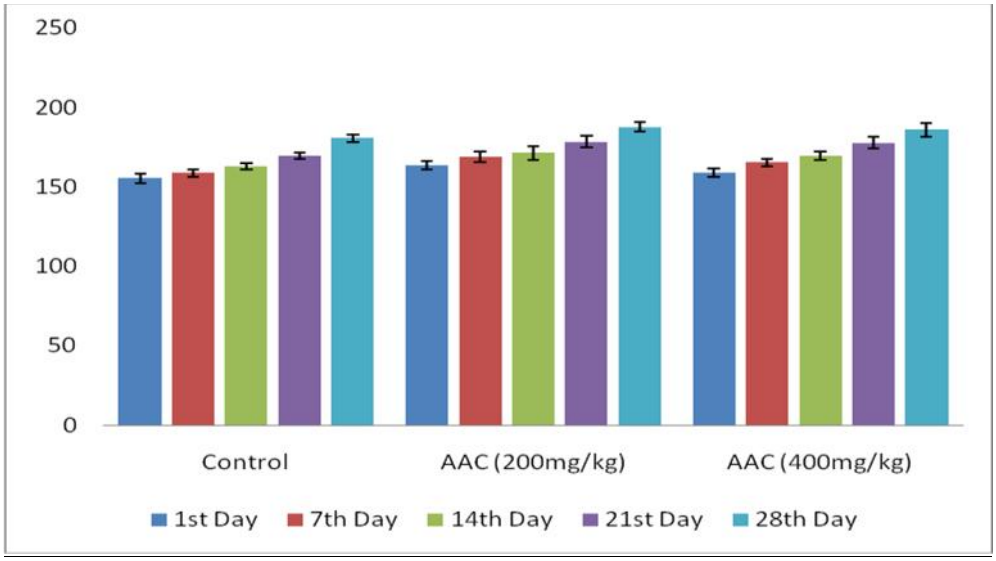


Table 7 Effect of *Ayakaandha Abraga Chendhuram* on food intake during 28 days treatment in rats

Groups	Drug Treatment	Food Intake (gms)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control Distilled Water (1ml/kg, p.o)	24.36± 1.23	23.66± 2.07	23.23± 2.23	24.32± 2.31	25.59± 2.06
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	26.32± 1.45	22.74± 1.87	25.87± 2.06	24.54± 2.23	24.90± 1.63
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	23.78± 2.09	26.89± 1.75	27.51± 2.05	26.85± 1.45	25.56± 2.05

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 2 Effect of *Ayakaandha Abraga Chendhuram* on food intake during 28 days treatment in rats

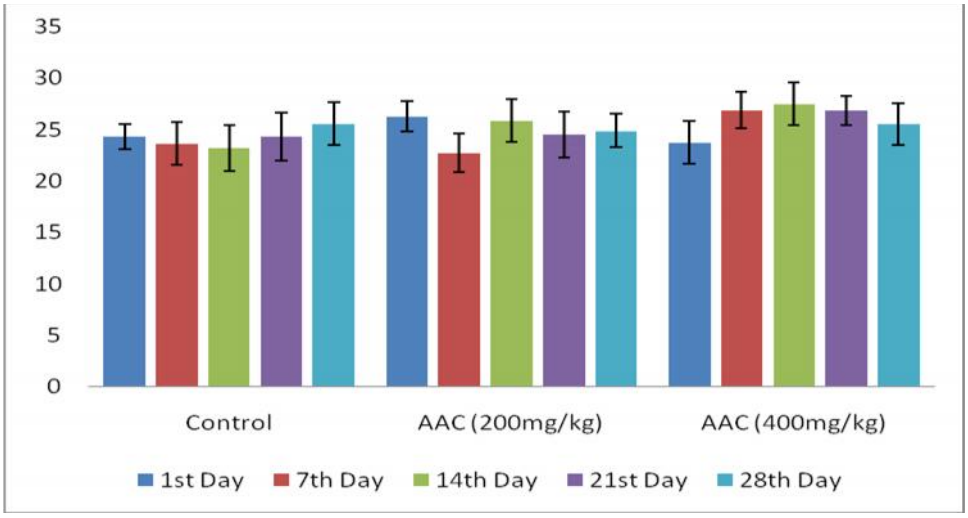


Table 8 Effect of *Ayakaandha Abraga Chendhuram* on water intake during 28 days treatment in rats

Groups	Drug Treatment	Water Intake (ml)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control Distilled Water (1ml/kg, p.o)	66.37± 2.45	69.41± 3.09	72.55± 4.33	70.23± 3.39	75.58± 4.98
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	70.63± 2.90	69.94± 3.05	80.22± 4.80	65.07± 4.57	75.77± 2.88
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	89.72± 4.62	76.69± 5.32	75.55± 5.25	80.62± 4.90	79.93± 5.53

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 3 Effect of *Ayakaandha Abraga Chendhuram* on water intake during 28 days treatment in rats

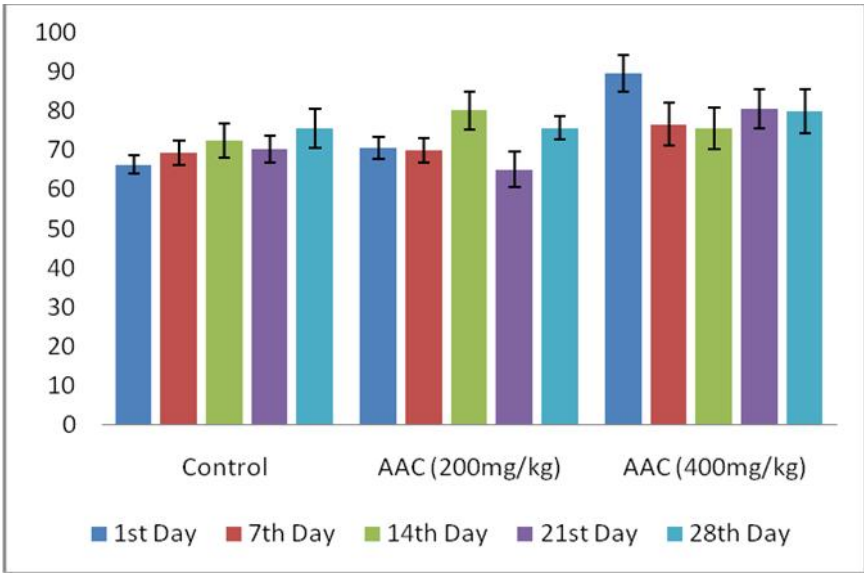


Table 9. Shows the effect of *Ayakaandha Abraga Chendhuram* on RBC, WBC and Hb in rats after 28 days treatment

Groups	Drug Treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %
I	Control Distilled Water (1ml/kg, p.o)	4.21 ± 0.40	8696.81 ± 67.32	14.40± 0.59
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	5.01 ± 0.34	8698.24 ± 85.41	15.55 ± 0.48
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	5.03 ± 0.43	8792.33 ± 69.54	15.28 ± 1.06

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 4 Shows the effect of *Ayakaandha Abraga Chendhuram* on RBC in rats after 28 days treatment

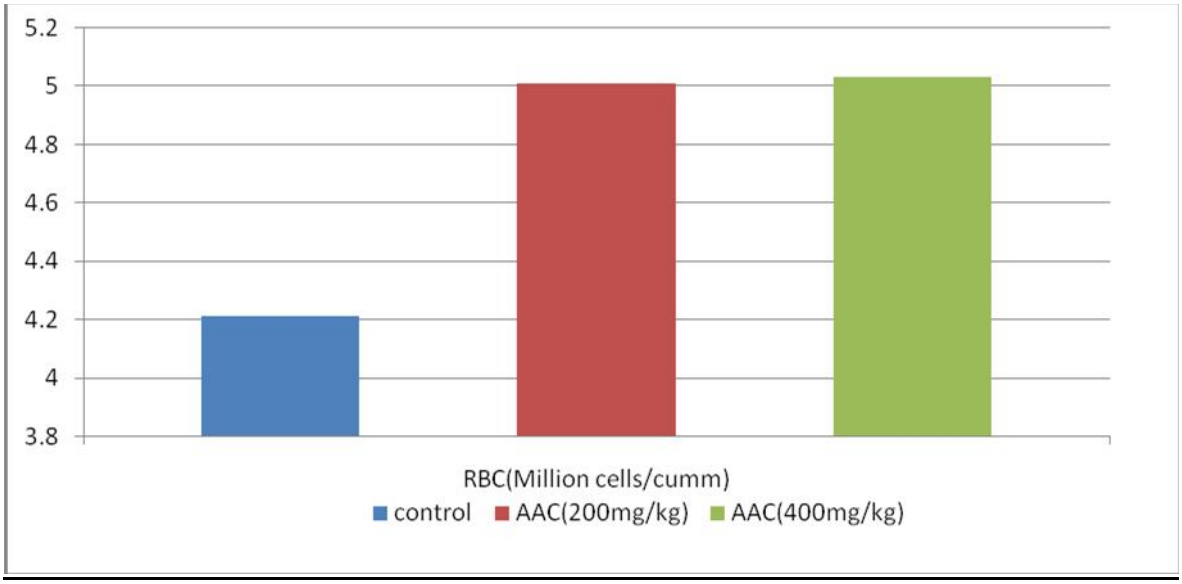


Chart 5 Shows the effect of *Ayakaandha Abraga Chendhuram* on Hb in rats after 28 days treatment

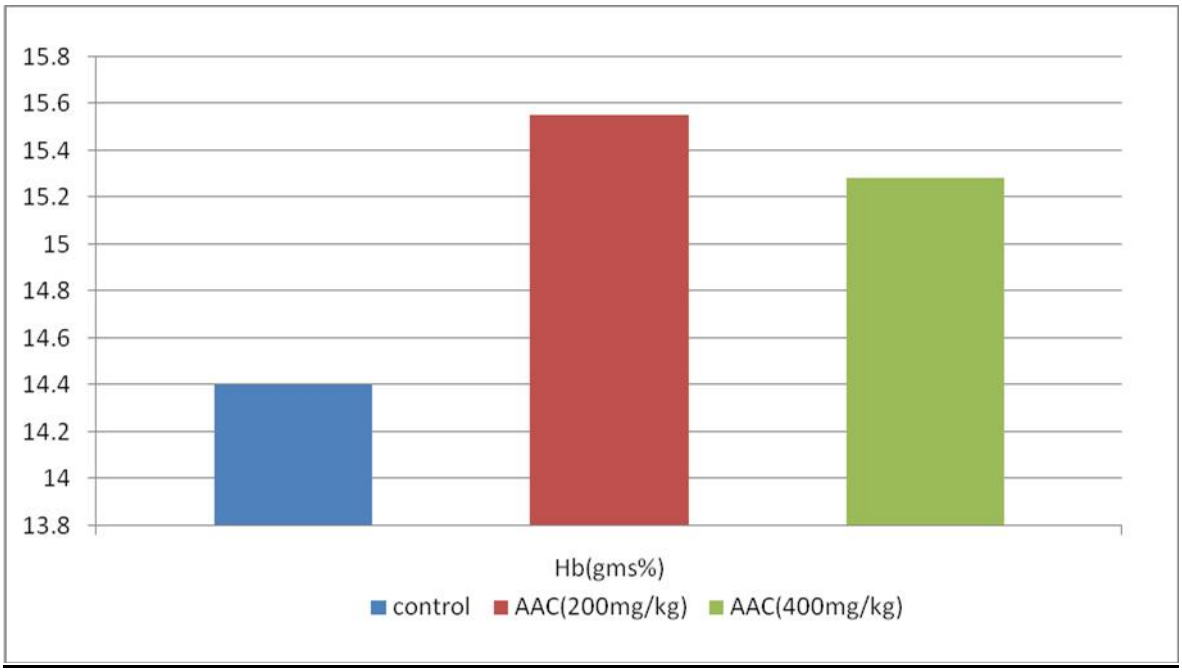


Chart 6 Shows the effect of *Ayakaandha Abraga Chendhuram* on WBC in rats after 28 days treatment

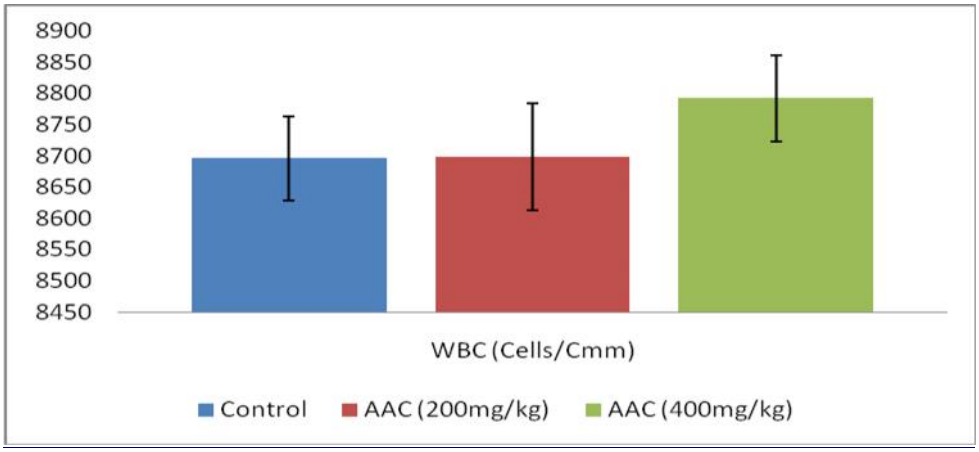


Table 10 Shows the effect of *Ayakaandha Abraga Chendhuram* on Differential Count in rats after 28 days treatment

Groups	Drug Treatment	Differential Count %			
		Neutophils	***Eosinophils***	***Monocyte***	***Lympocyte***
I	Control Distilled Water (1ml/kg, p.o)	31.72± 1.60	1.93 ± 0.15	3.89 ± 0.19	63.17 ± 3.76
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	32.81 ± 1.40	1.90 ± 0.13	3.98 ± 0.28	63.33 ± 4.53
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	31.93 ± 1.42	1.98 ± 0.10	3.74 ± 0.18	63.61 ± 3.90

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 7 Shows the effect of *Ayakaandha Abraga Chendhuram* on Differential Counts in rats after 28 days treatment

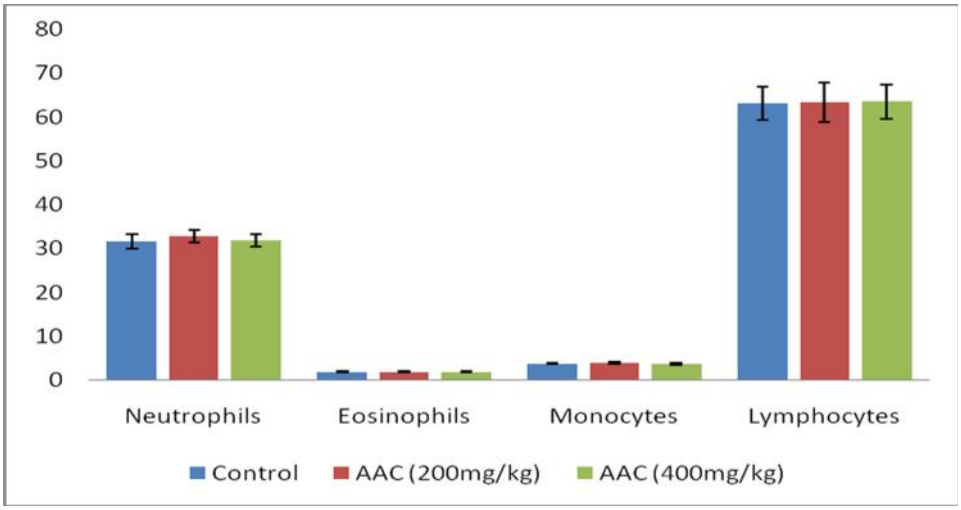


Table 11 Shows the effect of *Ayakaandha Abraga Chendhuram* on Hepatic Functions (SGPT, SGOT and ALP) in rats after 28 days treatment.

GROUPS	DRUG TREATMENT	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
I	Control Distilled Water (1ml/kg, p.o)	82.14±3.06	148.28±4.71	287.52±11.76
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	83.05±3.29	148.64±3.92	288.81±12.18
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	82.84±4.83	151.31±6.09	292.97±10.77

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 8. Shows the effect of *Ayakaandha Abraga Chendhuram* on Hepatic Functions in rats after 28 days treatment

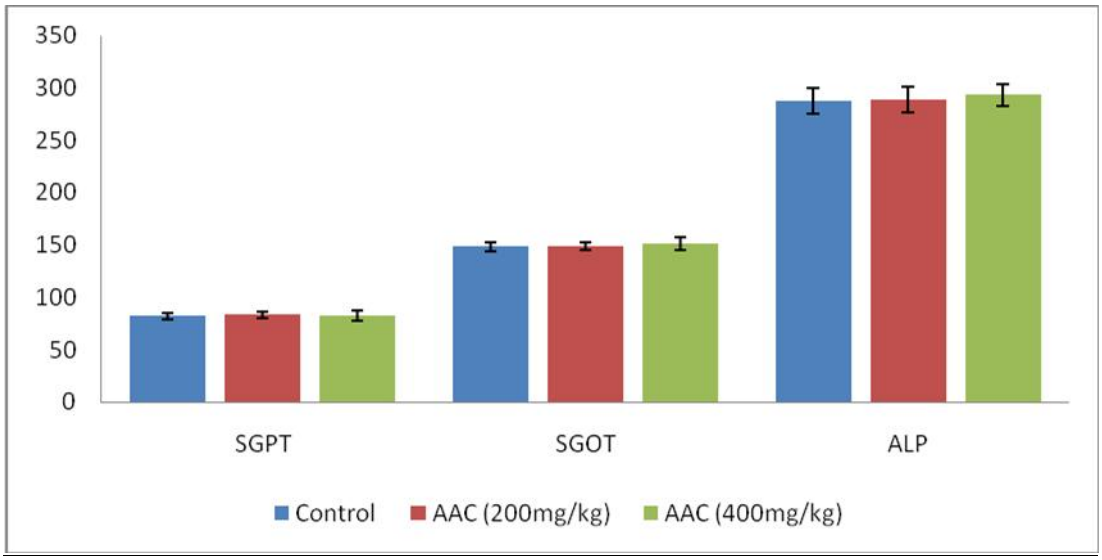


Table 12 Shows the effect of *Ayakaandha Abraga Chendhuram* on Kidney Functions in rats after 28 days treatment

Groups	Drug Treatment	Urea (mg/dl)	Creatinine (mg/dl)
I	Control Distilled Water (1ml/kg, p.o)	39.79±3.0	0.94±0.03
II	*Ayakaandha Abraga Chendhuram* (200mg/kg, p.o)	36.08±1.71	0.98±0.07
III	*Ayakaandha Abraga Chendhuram* (400mg/kg, p.o)	36.57±2.42	0.96±0.06

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Chart 9 Shows the effect of *Ayakaandha Abraga Chendhuram* on Kidney Functions (Urea) in rats after 28 days treatment

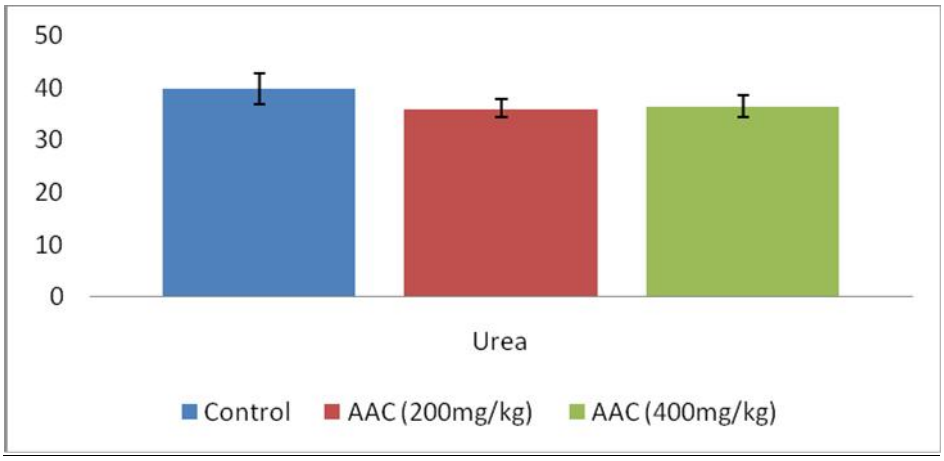


Chart 10 Shows the effect of *Ayakaandha Abraga Chendhuram* on Kidney Functions (Creatinine) in rats after 28 days treatment

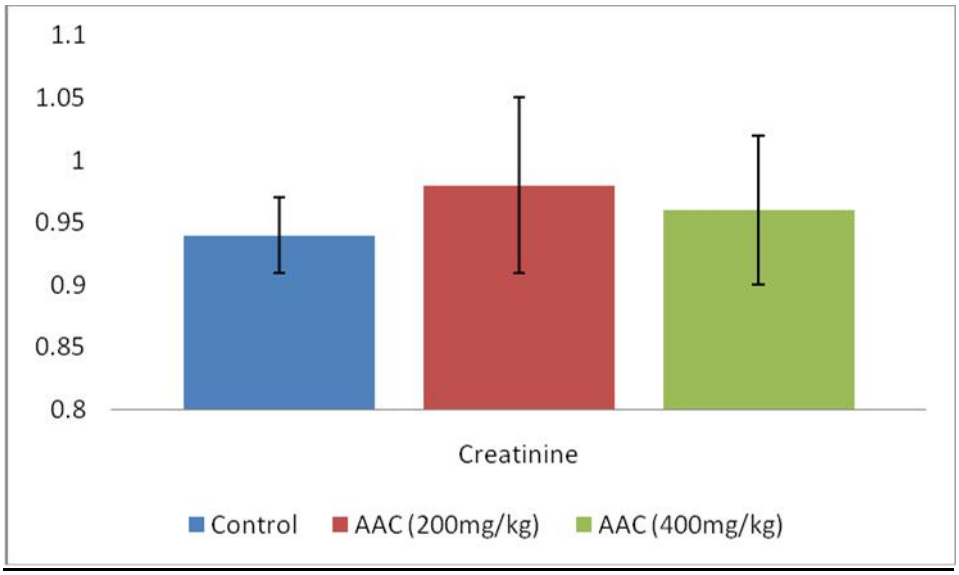
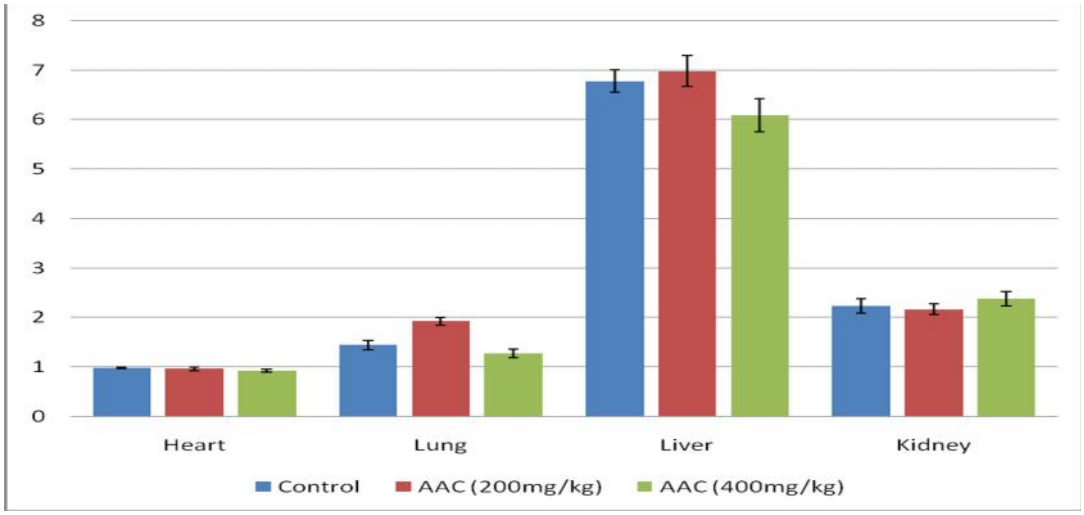


Table 13 Effect of Ayakaandha Abraga Chendhuram on organ weight after 28 days treatment in rats

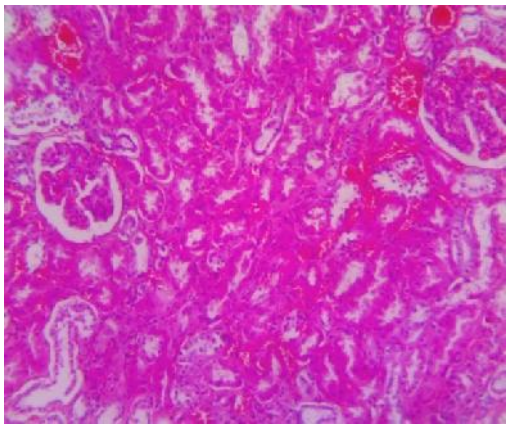
Groups	Drug Treatment	Organ Weight (gms)			
		Heart	Lungs	Liver	Kidney
I	Control Distilled Water (1ml/kg, p.o)	0.98±0.02	1.44±0.09	6.78±0.23	2.23±0.15
II	Ayakaandha Abraga Chendhuram (200mg/kg, p.o)	0.96±0.04	1.92±0.08	6.98±0.31	2.16±0.11
III	Ayakaandha Abraga Chendhuram (400mg/kg, p.o)	0.92±0.03	1.27±0.09	6.09±0.34	2.38±0.15

Values are in mean ± SEM (n=6)
*P<0.05, **P<0.01, ***P<0.001 Vs Control

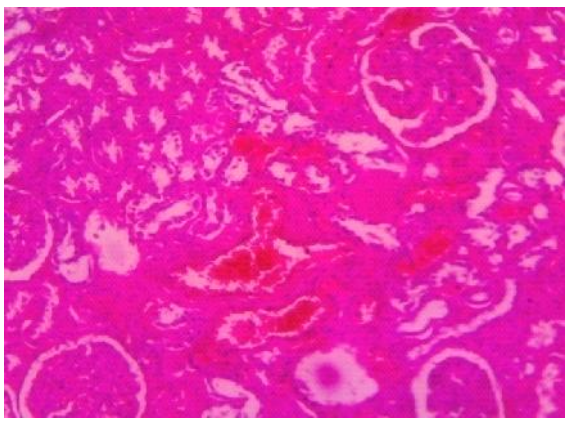
Chart 11 Effect of Ayakaandha Abraga Chendhuram on organ weight after 28 days treatment in rats

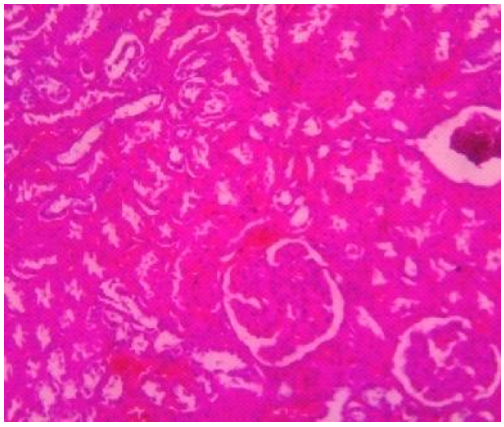


HISTOPATHOLOGICAL STUDIES:



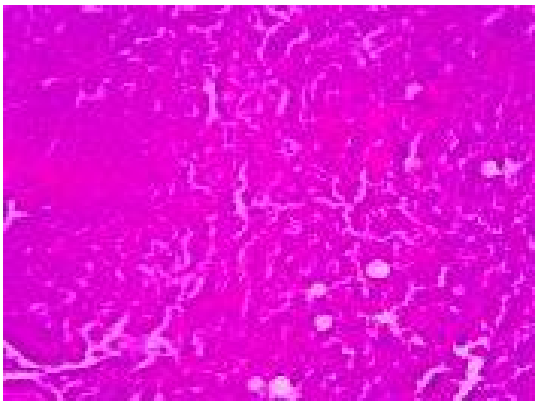
Control Group: Kidney



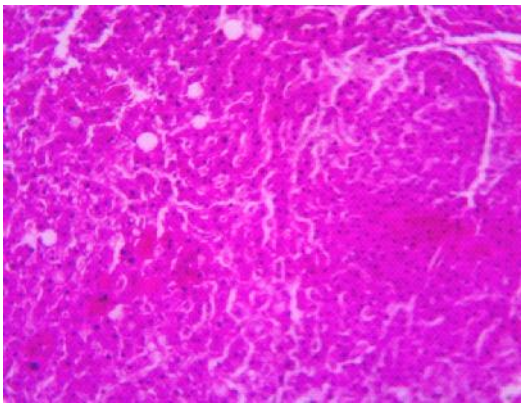


Low dose 200mg /kg High dose 400mg/kg

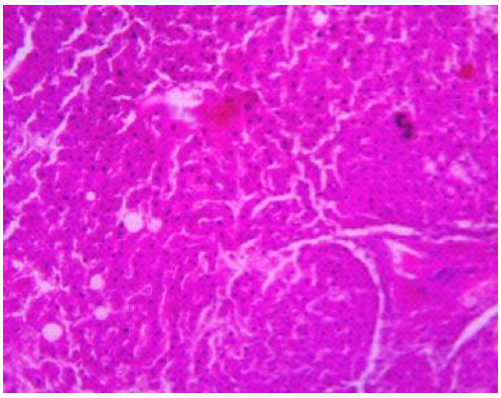
Results : Section shows kidney with normal histology



Control Group: Liver

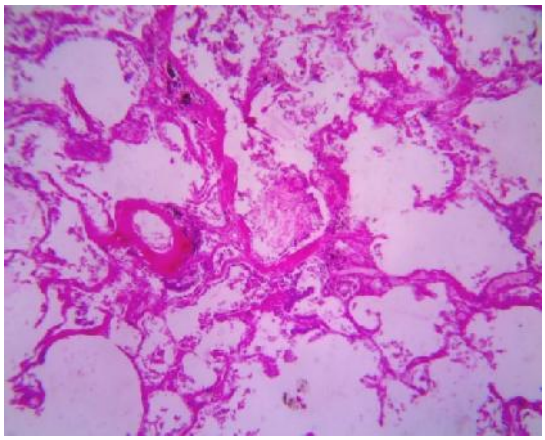


Low dose 200mg/kg

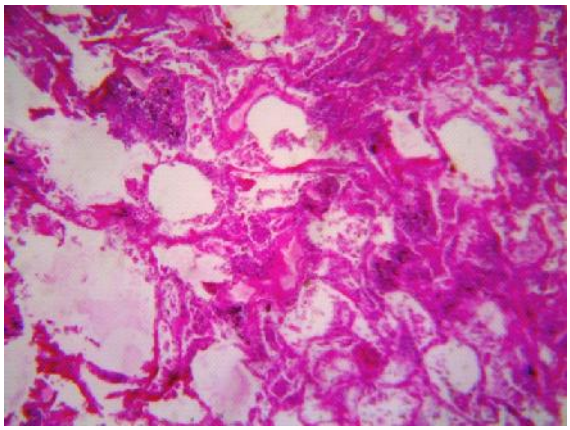


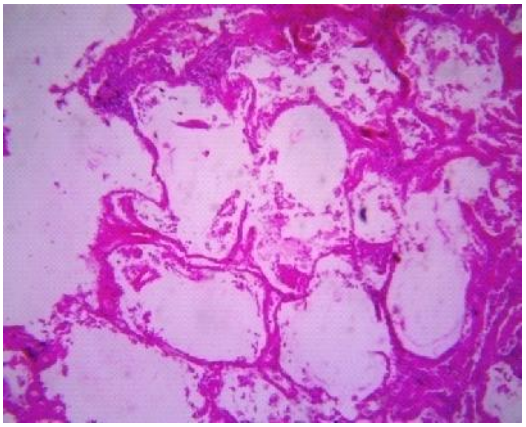
High dose 400mg/kg

Results :Section studied shows liver with normal histomorphology.



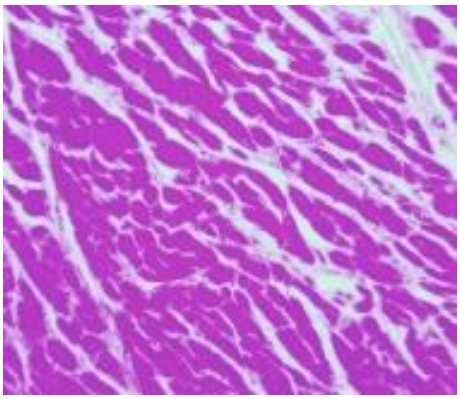
Control Group: Lungs



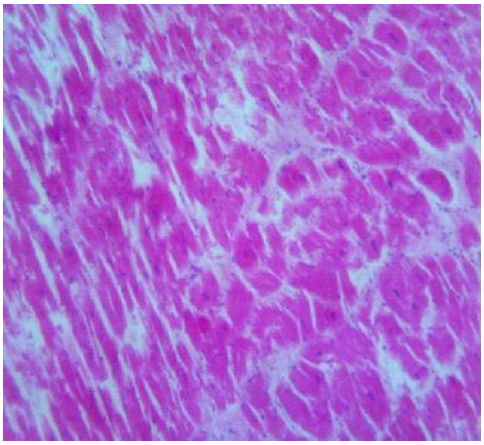


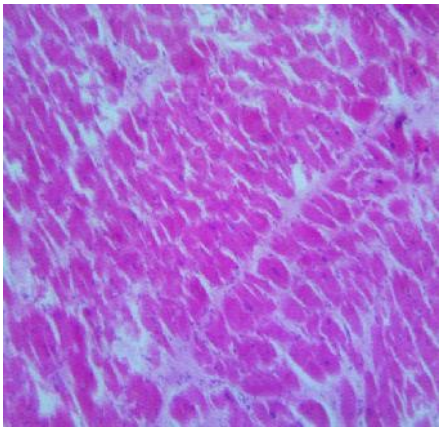
Low dose 200mg/g High dose 400mg/kg

Results : Section shows lung with normal alveoli



Control Group: Heart





Low Dose 200mg/kg High Dose 400mg/kg

Results : Section from the heart shows normal myofibers

Discussion

The results of acute toxicity study of AAC were shown on table 2-5. There was no mortality with the AAC after 3rd & 4th hrs even at higher dose of 2000mg/kg. The AAC did not alter the general behavior after 1hour of oral administration. After 3rd hrs of AAC oral administration, it showed mild sedation, stupar reaction and muscle relaxant property without altering other general behavior. After 4th hrs, AAC showed mild sedation and muscle relaxant property without altering other general behaviours. It did not show any lethality or toxic reactions during and after the study.

From acute toxicity study, 1/10 and 2/10 of maximum tolerated dose ie, 200 & 400mg/kg, were selected for further sub-acute toxicity study.

In sub-acute toxicity study, body weight, food intake and water intake were observed on 1st, 7th, 14th 21st and 28th day of *Ayakaandha Abraga Chendhuram* administration.

The effect of *Ayakaandha Abraga Chendhuram* on body weight during 28 days treatment in rats was given in table 6 and chart 1. There was no significant change in the body weight compared to control with both the doses of *Ayakaandha Abraga Chendhuram* during 28 days treatment.

The effect of *Ayakaandha Abraga Chendhuram* on food intake during 28 days treatment in rats was given in table 7 and chart 2. *Ayakaandha Abraga Chendhuram* did not alter the food intake at both the dose levels as compared to control during the 28 days treatment. It indicates that it does not influence food intake.

The effect of *Ayakaandha Abraga Chendhuram* on water intake during 28 days treatment in rats was given in table 8 and chart 3. *Ayakaandha Abraga Chendhuram* did not alter the water intake at both the dose levels as compared to control during the 28 days treatment. There was no significant change in water intake as compared to control.

Table 9, chart4,5 and 6 shows the effect of *Ayakaandha Abraga Chendhuram* on haematological parameters like RBC, WBC and Hb in rats after 28 days treatment. Both the doses of *Ayakaandha Abraga Chendhuram* did not produce any significant change in RBC, WBC and Hb compared to control.

The effect of *Ayakaandha Abraga Chendhuram* on Differential Count in rats after 28 days treatment was shown on table 10 and chart 7. Both the doses of *Ayakaandha Abraga Chendhuram* did not show any significant change in differential counts like Neutrophils, Eosinophils, Monocyte and Lymphocytes. From the effect of *Ayakaandha Abraga Chendhuram* on hematological parameters it was found that it does not produce any toxicity in haemopoietic system.

The effect of *Ayakaandha Abraga Chendhuram* on hepatic functions in rats after 28 days treatment was shown on table 11 and chart 8. The hepatic enzymes (SGPT, SGOT and ALP) were remain normal with both the doses of *Ayakaandha Abraga Chendhuram* and the values were similar as that of control group which received distilled water. From the result of hepatic enzymes it was found the *Ayakaandha Abraga Chendhuram* did not produce any toxic effects on liver in rats.

The effect of *Ayakaandha Abraga Chendhuram* on renal functions in rats after 28 days treatment was shown on table 12 and chart 9 & 10. Both the doses of *Ayakaandha Abraga Chendhuram* does not showed any significant change in urea and creatinine after 28 days treatment compared to control which indicates, *Ayakaandha Abraga Chendhuram* was free form renal toxicity. The effect of Ayakaandha abraga chendhuram on organ weight in rats after 28 days treatment shown on table 13 chart 11.

Ayakaandha Abraga Chendhuram was studied for its acute and sub-acue toxicity studies using laboratory animals. In acute toxicity study, *Ayakaandha Abraga Chendhuram* did not produce any specific toxicity and mortality even at the dose of 2000mg/kg in mice. In sub-acute toxicity study, 200 and 400mg/kg of *Ayakaandha Abraga Chendhuram* was used and it was administered once daily for 28 days through oral route. *Ayakaandha Abraga Chendhuram* did not alter the body weight, food intake and water intake during the study period. After 28 days the blood was subjected to liver and kidney function test. Both the doses of *Ayakaandha Abraga Chendhuram*, did not showed any significant change the functional parameters of liver and kidney. Form the study it was concluded that, *Ayakaandha Abraga Chendhuram* was found to be safe in laboratory animals.

Conclusion

The acute toxicity study confirms that the drug didn't show any mortality atthe dose level of 5mg, 50mg, 300mg, 2000mg. According to OECD guidelines thedrug has no acute toxicity effects.

In sub-acute toxicity study, 200 and 400mg/kg of *Ayakaandha AbragaChendhuram* was used and it was administered once daily for 28 days through oralroute. There is no abnormal changes in organ weight, blood parameters, urineparameters, histopathological findings. From the study it is concluded that,Ayakaandha abraga chendhuram is found to be safe in long term administration inclinical practice upto the dose of 400mg. The histomorphology of liver, kidney, heart,lungs shows normal features after 28 days of drug administration.So, through the toxicological study, there is no toxic effects absorbed.So the drug is very safe for clinical use.

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