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Research Article

TOXICOLOGICAL PROFILE ON *AYAPODI ELAGAM* - A SIDDHA HERBOMINERAL FORMULATION IN WISTER ALBINO RATS

A.Kalaivani[1]*, K.Jeevaraj[1], P.Shanmugapriya[2], R.Madhavan[3]

*[1]PG Scholar, [2]Guide and Lecturer, [3]HOD i/c, Dept. of. Nanju Maruthuvam, National Institute of Siddha, Chennai, Tamil nadu, India.

ABSTRACT

Ayapodi Elagam (A.E) was used in Siddha system of medicine for many years to treat *Pandu* (Anemia). This medicine contains *Nellikai, Keezhanelli*, *Karisalai* and *Ayam*. These herbs are helps to improve the blood to correct the anemia. This study was carried out to evaluate the acute and chronic toxic effect on *Ayapodi Elagam* and to determine the $LD_{50.}$ The toxicity study was done as per the guidelines of world health organization (WHO) guideline. As the herbs and *Ayam* were used for treating anemia by traditional practitioners for years together, the toxicity study was also proposed to study in both sexes. In acute study the animals were divided into two groups A.E was administered at 5000mg/kg orally and animals were observed for toxic sign at 0,5,1,4,24 hour and for 14 days. In chronic toxicity study A.E was administered at 450,900 and 1800 mg/kg body weight/day to 3 groups of animal, respectively. The distilled water was administered to control animals. The result showed that the acute toxicity study of A.E. at the dose level of 5000mg/kg does not produce any toxic sign and mortality among the experimental groups and the LD_{50} value of the drug was found to be more than 5000mg/kg bodyweight. The weight of rats, wellness parameters, mortality, hematological parameters, biochemical parameters and histological analysis of all vital organs were observed to know the chronic toxic effect of the drug. All the parameters of the study do not show the any significant chances between the control and experimental groups.

KEYWORDS: *Ayam*, Siddha system, Anemia, Toxicity study, *Pandu*.

INTRODUCTION

Anaemia is the most prevalent nutritional deficiency disorder in the world. It is a condition that occurs when the red blood cells do not carry enough oxygen to the tissues of the body. According to World Health Organization criteria, anemia is defined as blood hemoglobin (Hb) concentration <130 g/L (<13 g/dL) or hematocrit (Hct) <39% in adult males; Hb <120 g/L (<12 g/dL) or Hct<37% in adult females. Signs and symptoms of anaemia are varied, depending on the level of anaemia and the time course over which it developed. A physiologic approach to anaemia diagnosis is based on the understanding that a decrease in circulating RBCs can be related to either inadequate production of RBCs or increased RBC destruction or loss. Within the category of inadequate production, erythropoiesis can be either ineffective, due to an erythrocyte maturation defect, or hypoproliferative.[1]

Most of the anemia's are due to an inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid are also involved in the maintenance of Haemoglobin level. Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%) Prevalence of anaemia in all the groups is higher in India as compared to other developing countries. In India, anaemia affects an estimated 50% of the population. The problem becomes more severe as more women are affected by it as compared to men.[2]

In Siddha science anemia is called by the name *Pandu* (or) *Veluppu noi*. In *Veluppu noi* the appetite is lost. The food taken will not be digested well, which result in anemia and body weakness. Besides these *Anal pitham* and the *Ranjaga pitham* which gives the natural colour to our skin are affected in this disease. The body will be pale and loses its weight. It will increase the '*Azhal*' humour the increased *Azhal* humour also increases the other two humours and *Vyanan* which result in *Pandu* (anemia).[3]

Ayapodi Elagam is one among the *Elagam* preparation in Siddha system of medicine. The ingredient of the drug was *Ayam* (Iron), *Nellikai* (*Phyllanthus emblica)*, *Keezhanelli* (*Phyllanthus niruri)* and *Karisalai* (*Eclipta alba*). It is used for treating anaemia, jaundice. Iron is the most important metal used in Siddha system of medicine for treating anemia. The herbal drugs also help to reduce the *Azhal* humour. The medicine *Ayapodi Elagam* is used by many Siddha practitioners to treat anemic patients. But safety of the *Ayapodi Elagam* is not known. Hence I want to evaluate the toxicity of *Ayapodi Elagam* in animal model acute and long term toxicity study as per WHO guideline.

MATERIALS AND METHODS

Collection and Authentication of Raw Drugs

Ayam was collected from Trichy river bed. And other herbal drugs were collected from vegetable market, Tambaram, Chennai.

The mineral drug *Ayam* were identified and authenticated by Lecturer, Department of Gunapadam, National Institute of Siddha, Chennai.

The herbal drugs *Nellikai* (*Phyllanthus emblica*), *Keezhanelli* (*Phyllanthus niruri*), *Karisalai* (*Eclipta alba*) were identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai.

Ingredients

Nellikai

Karisalai

Keezhanelli

Vellam

Honey

***Ayam* before Purification**

***Ayam* after Purification**

Preparation of the Test Drug

Purified *Ayapodi* (iron powder)-280gm, *Nellikai chaaru* (*Phyllanthus emblica* juice)-140gm, *Keezhanellic hamoolachaaru* (*Phyllanthus niruri* whole plant extract) - 140gm, *Karisalai chaaru* (*Eclipta alba* plant extract) -105gm, Purified honey - 87.5g, *Jaggery* (*Vellam*) – 70gm was taken. The extract were mixed with the *Ayapodi* in a mortar and grind it for twelve hours, the resultant product was put in an iron pot; mix it well in the remaining part of the extracts and heat the pot slowly. Stir well with an iron, honey and jaggery '*Vellam*'. When cool pour the paste into a porcelain vessel and keep it for use.[4]

Animals and Husbandary

Male and female Wistar albino rats weighing 150-750gm were used for the study. Animals were housed in groups (3-5/cage) in polypropylene cages in the well ventilated room under an ambient temperature of 22°C (±3°) and relative humidity 30–70% 12-h light/12-h dark cycle. They were provided with food and purified water *ad libitum.* All the animals were acclimatized at least for 7 days to the laboratory conditions prior to the study. The cages were labelled with group, weight of the animal and day of drug administration. Separate record was maintained for each animal in all the groups including control animals. Guidelines of CPCSEA, laboratory animal care were strictly followed throughout the study. This study was carried out with the approval of Institutional Animal Ethic Committee (IAEC), National Institute of Siddha. (NIS/IAEC-I/2016/10)[5]

Acute toxicity study

Acute toxicity study was performed as per the WHO guideline. Young adult Wistar albino rats male and female weighing 150-750gm were used for the study. One week after acclimatization, 20 animals were selected and divided into two groups control and test group. Control group received distal water; the test group was treated with A.E. at the dose level of 5000mg/kg b.wt by oral gavage. All the study animals were observed for their behavioral sign and mortality at 30 mints, 1,2,4 hours upto 24 hours and then followed for further 14 days. At the end of the 14th day, the overnight fasted animals were sacrificed by using excessive anesthesia and subjected to gross pathological examination.

Long term toxicity study

According to WHO guideline the chronic toxicity study was carried. The animals in both sexes were divided in four groups each group consists of 10 animals (5 male and 5 female).Control group received distal water. A.E was administered at 450,900 and 1800 mg/kg b.wt/day to 3 groups of animal, respectively. All the animals were treated once daily for 90 days. The sign of toxicity and mortality were monitored for 90 days. Body weight were Calculated weekly once. Feed & water intake were Calculated daily. The overnight fasted animals were sacrificed after 90 days except the high dose group four animals. The high dose group two male and two female animals were set as satellite group. The satellite groups were sacrificed on after 30 days. Blood samples were collected from abdominal aorta under excessive anaesthesia with and without anticoagulant and used for haematological and biochemical parameters.

The haematological parameters were analysed by using fully automated haematology analyser. Plasma was separated and used for the estimation of biochemical parameters.

Histopathology examination of organs

The organs included liver, kidney, spleen, brain, heart, lung, stomach and bone marrow of the animals were preserved and they were subjected to histopathological examination.

The organ pieces (35µm thick) of all the animals (control, high dose and satellite group) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Bone Marrow Smear

About 0.2 ml of smear aspirated from the femur thigh bone of the experimental animal was dropped onto the glass slide and made in to thin smear allow the smear to dry. Dried smear was stained with leishman stain and washed. Followed by this cedar wood oil was placed on to the smear and was observed microscopically.[6]

Statistical analysis

Findings such as body weight changes, food consumption, water intake, hematology and biochemical analysis were subjected to One-way ANOVA Dennett's test using a computer software program followed by Graph Pad Instat-3.[7]

RESULTS

Acute toxicity study

Acute toxicity of *Ayapodi Elagam* does not produce any toxicity signs and mortality at the dose level of 5000mg/kg b.wt in the animal during 14 days of the study. Further the gross necropsy revealed no

abnormalities in the internal organs of the experimental animals.

Long term toxicity study

In Chronic toxicity study there was no behavioural abnormality and mortality throughout the study period. Bodyweight was gradually gained in A.E. administered rats when compared with the vehicle treated rats but it is not statistically significant. No significant different in food and water consumption was observed in all the animals throughout the study. The haematological parameter shows the elevated lymphocyte count seen in the low and high dose group animals but it become normal in post retrieval group animals. In lipid profile the mid dose and high dose group animal's shows significance in Total cholesterol and LDL level it was normal in post retrieval group animals.

Figure 1: Body weight (g) changes of albino rats (male and female) exposed to *Ayapodi Elagam* in chronic toxicity study

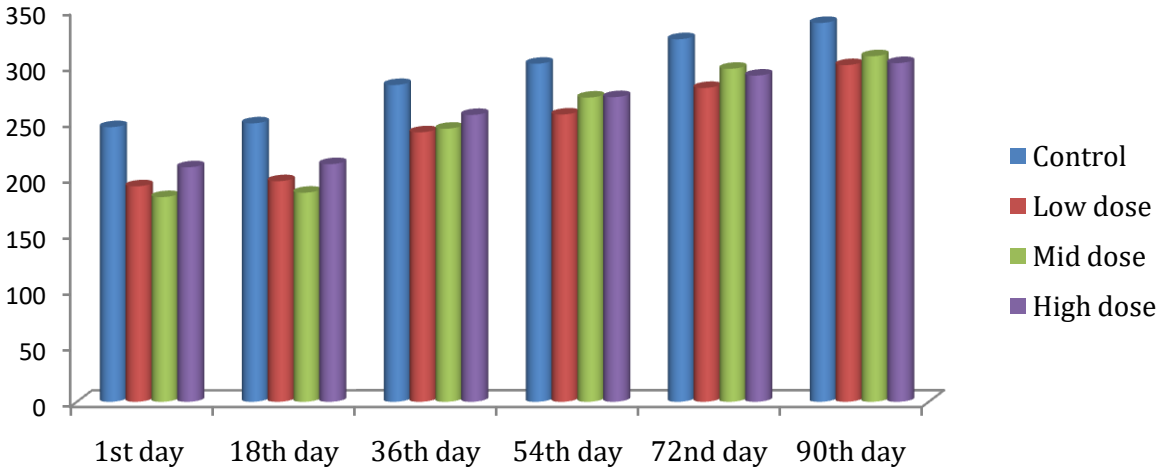


Table 1: Effect of *Ayapodi Elagam* on Hepatic Parameters

Dose (mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
Total Bilirubin (mg/dl)	0.4±0.10	0.44±0.32	0.5±0.30	0.6±0.40	0.45±0.13
SGOT(U/L)	86.44±16.6	94.7±21.95	94±21.62	108.83±22.9	137±37.12**
SGPT(U/L)	28.7±7.8	30.9±5.7	32±9.2	33.7±5.5	31.5±7.6

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Table 2: Effect of *Ayapodi Elagam* on Haematological Parameters

Parameters	Control	Low dose	Mid dose	High dose	Satellite group
RBC (×10^6µl)	7.4±0.4	7.6±0.30	7.43±1.34	7.13±1.18	6.6±1.05
WBC (×10^3µl)	9.2 ±2.05	8.92±1.2	8.89±2.16	9.15±2.17	7.9±0.9
PLT (×10^3µl)	797±108.3	725.±127.3	757.1±152.6	628.83±100.3	735.5±107.2
HGB (g/dl)	13.58±2.32	13.48±2.01	13.4±1.51	12.61±1.9	14.9±2.3
Neutrophils (10^3/mm^3)	1.74±0.54	1.92±0.7	1.75±0.45	1.58±0.3	1.72±0.4
Lymphocyte (%)	73.25±9.08	88.74±10.86**	78.88±8.55	89.35±7.34**	74.8±9.8
Monocyte (%)	3.39±1.7	3.64±1.6	2.4±1.21	3.68±1.49	3.52±0.7
Eosinophil's (%)	1.32±0.26	1.35±0.33	1.36±0.3	1.45±0.23	1.6±0.35
Basophils (%)	-	1	1	-	0
MCH (pg)	20.05±3.01	17.93±3.8	18.65±4.2	19.23±3.70	17.85±3.15
MCV (fl)	58.84±5.13	56.43±5.75	57.96±5.4	59.01±5.03	56.9±4.9

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Table 3: Effect of *Ayapodi Elagam* on Renal Parameters

Dose(mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
BUN(mg/dl)	15.9±3.01	15.7±4.8	15.8±5.0	14.33±6.74	16.2±3.9
Creatinine (mg/dl)	0.7±0.12	0.8±0.11	0.7±0.2	0.8±0.15	0.75±0.21

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01

Table 4: Effect of *Ayapodi Elagam* on Biochemical parameters

Dose (mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
Total cholesterol (mg/dl)	137.6±14.20	129.7±10.9	119.09±14.70*	135.8±19.4	148.32±19.45
HDL (mg/dl)	59.4±8.53	56.9±5.30	60.9±8.3	61.2±8.08	59.75±8.6
LDL (mg/dl)	62.5±9.64	53.9±9.70	42.7±11.6**	56.33±15.6	69.5±21.64
VLDL (mg/dl)	15.6±2.50	18.91±4.93	15.5±3.5	18.33±3.7	19.07±2.14
Triglycerides (mg/dl)	38.5±10.34	36.8±8.40	32.1±9.3	41.33±8.9	36.25±3.8

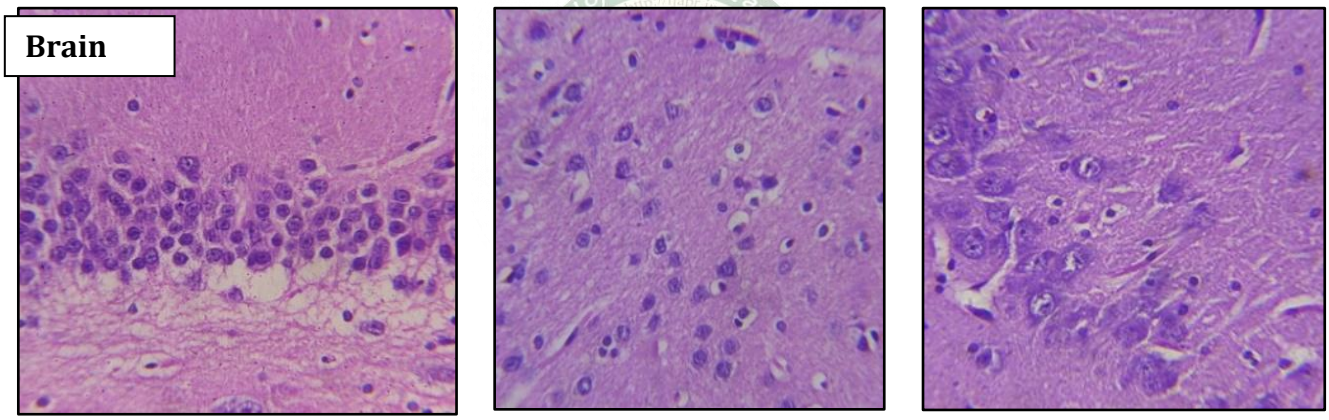
Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01.

Histopathological study

The histopathological study the organ such as brain, heart, kidney, liver, lungs, spleen, stomach, uterus, ovary and testis were taken. There are no pathological changes observed in all the group of animals.

Figure 3: Histopathology

a) Control (40X) b) High dose (40X) c) Satellite group (40X)



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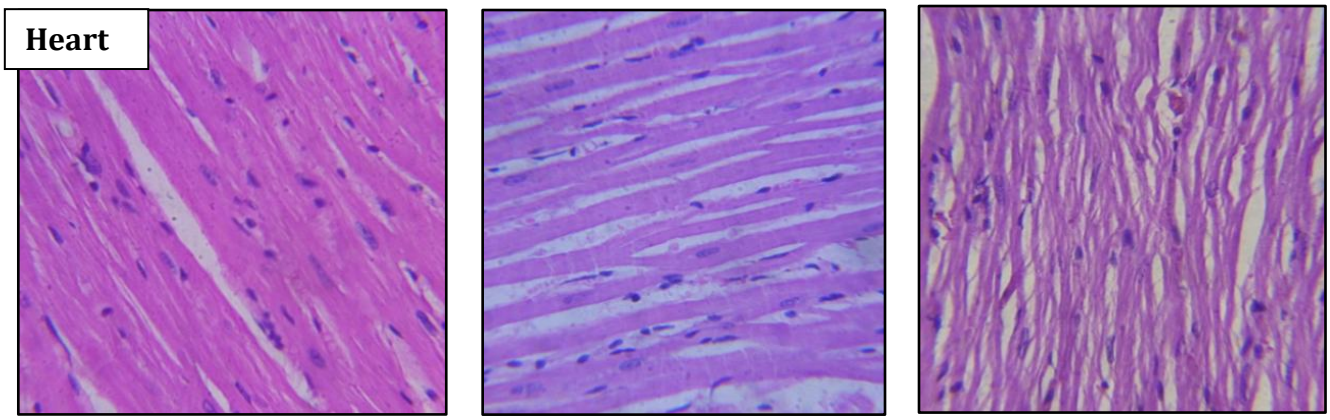


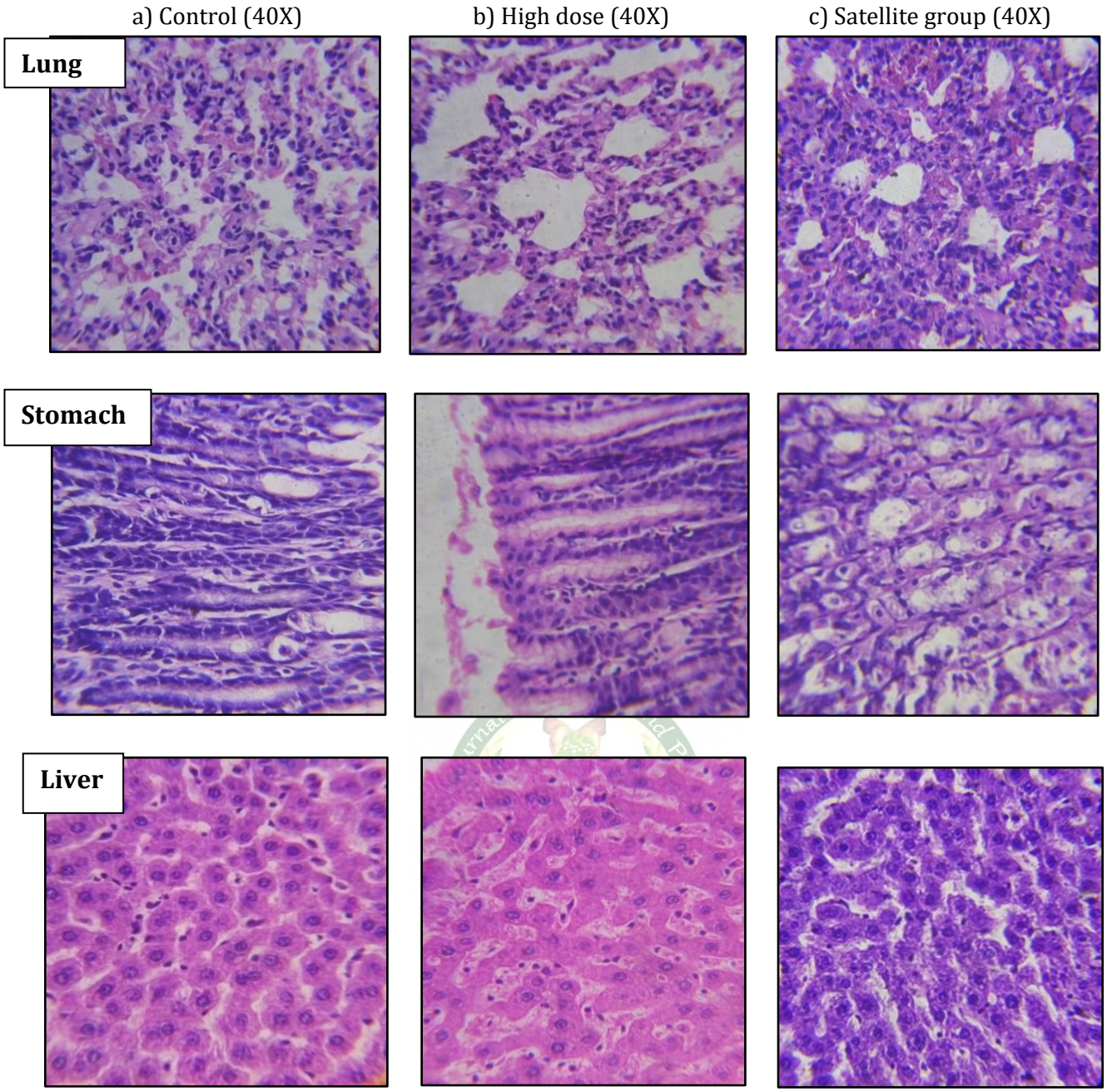
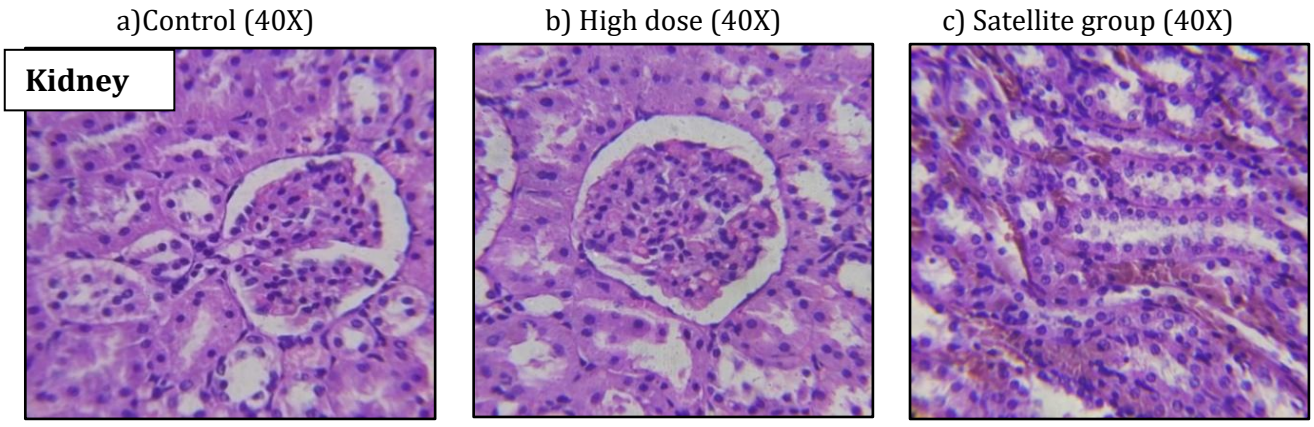
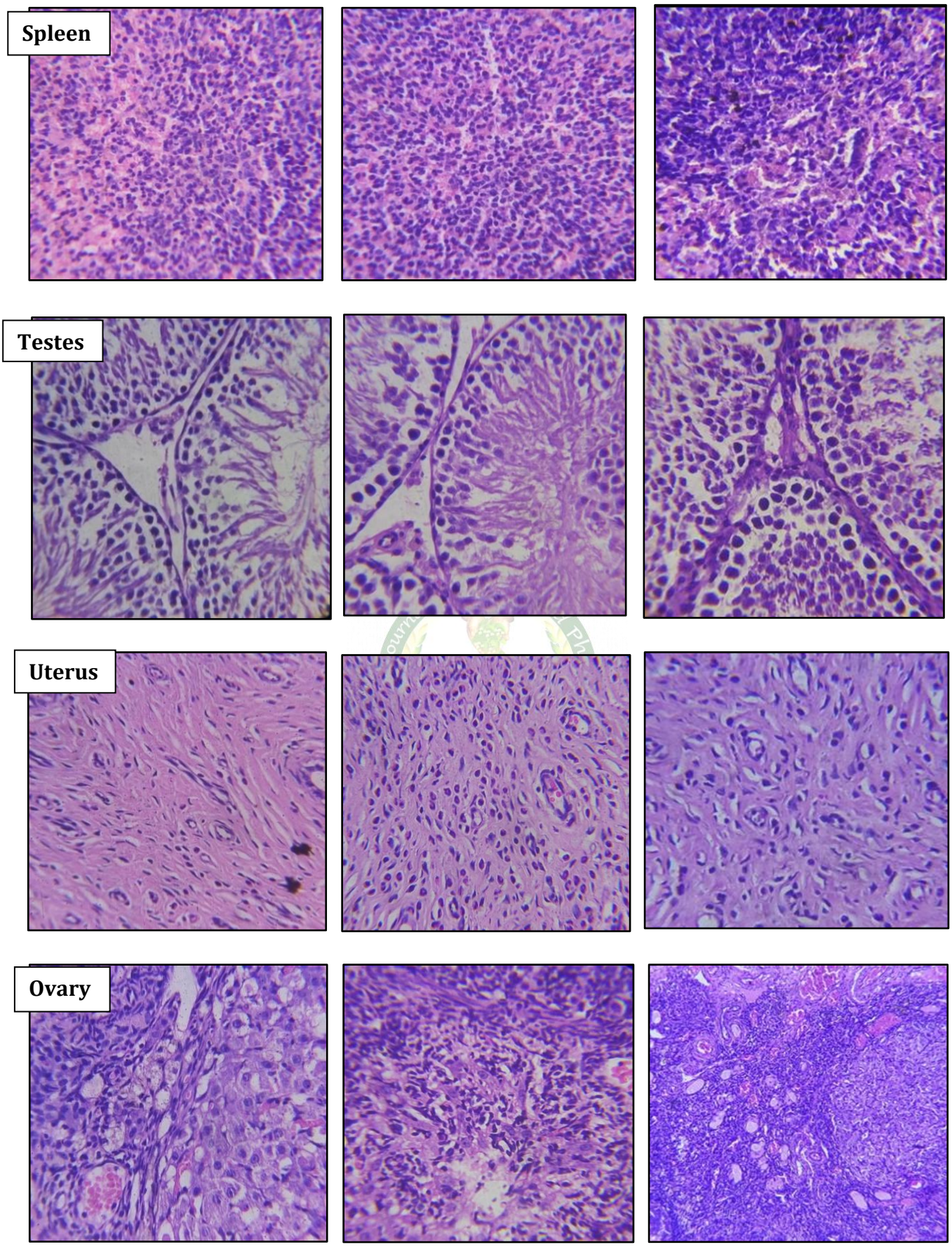


Figure 3: Histopathology

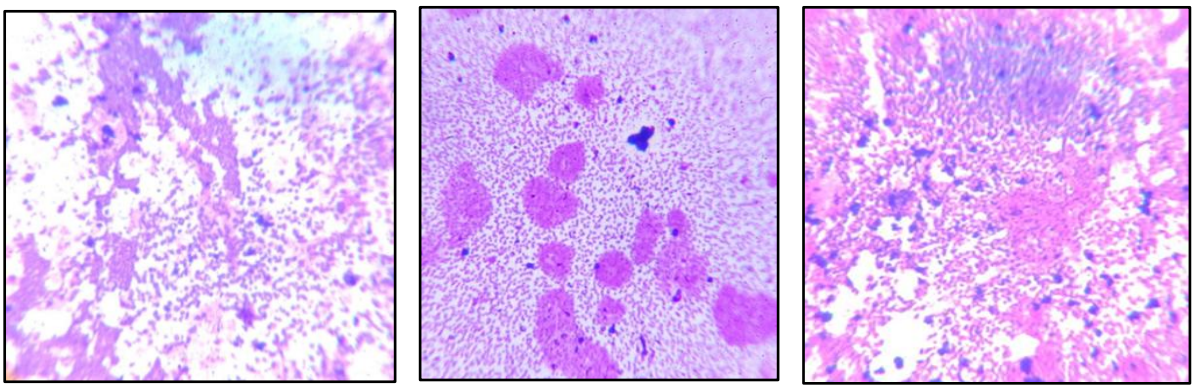




Bone marrow study

The smear study shows increased network of erythroblastic islets were observed in mid dose level and high dose level.

Fig 2: Microscopic View of Bone Marrow Smear of Rats Low Dose Group mild Dose Group high Dose Group



In the bone marrow study Bulky prominent marrow with dense cellular portions were observed and active zones of erythropoiesis were observed in sample belong to HD.

DISCUSSION

The *Ayapodi Elagam* is used for the treatment of *Pitha Pandu, Pitha Vettai*, and *Kamalai* etc., under the Siddha system of medicine. One of the ingredients of this medicine was Iron (*Ayam*), has a long history in the treatment of anaemia among Siddha doctors in the Tamilnadu.

Karisalai have the Hepatoprotective effect[8], Analgesic[9], Anti-inflammatory activity[10] and Anticancer activity[11] *Amla* is powerful food for the brain. Studies show that *Amla* helps lower cholesterol. *Amla* also helps maintain the functioning of the liver, increases hemoglobin, red blood cell count.[12] *Keezhanelli* have Anti-amnesic[13] Hepato-protective[14], Diuretic and Immunomodulator effect.[15] Honey have Wound healing property[16] and Anti-proliferative Effect.[17]

Literature reviews reveal that there was no such research has been done on *Ayapodi Elagam*. So I would like to study the safety profile of the drug. Its safety has been confirmed through acute and long-term oral toxicity study as per WHO guideline.

In acute toxicity study carried out as per WHO guidelines there was no treatment related death or significance of toxicity developed in albino rats at dosage level 5000mg/kg/b.wt throughout the study period. (Table 1) Further no gross pathological changes have been seen in the internal organs of both control and treated groups. Thus the LD_{50} valve was found to be greater than 5000mg/kg/b.wt.

To ensure the safety of *Ayapodi Elagam* long-term toxicity study was carried out as per WHO guideline. Except for hyperactivity at the time of drug administration, no other signs of toxicity were noted. After the blood collection all the animals were euthanized for gross pathological examination of all major internal organs. The blood sample was sent to a lab for haematology and biochemical analysis. The organs were preserved in 10% buffered formalin solution before sending for histopathological study. All the reports were statistically analyzed.

Ayapodi Elagam significant elevation in the body weight (Fig 1) and water intake in the test group animals when compared with the control group during the study period but they were within the physiological limit, and this study reveals that it does not adversely affect the basal metabolic process of the experimental animals.

The hemopoietic system serves as an important target for the toxic chemicals and a sensitive index for pathological condition both in humans and animals. In haematological parameters (Table 2), it had been observed that lymphocyte count was elevated after the administration of *Ayapodi Elagam* at the low dose and high dose group animals when compared to control group animals but the lymphocyte count was normal in post retrieval animals after one month of the drug with drawl. The total cholesterol level was reduced in animal treated in the *Ayapodi Elagam* in the mid-dose group but they were in the normal limit. Transaminases (SGOT and SGPT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs. Any elevation pertaining to these enzymes indicate their outflow into the bloodstream due to damage in liver parenchymal cells. But, there was a marked increase in SGOT (Table 1) in Post Retrieval group treated animals when compared to control group but it was also in the normal range in another group. In the present study there was no treatment related abnormality in renal functions at all the animals (Table 3) and other biochemical parameters (Table 4) of *Ayapodi Elagam* treated animals were normal when it is administered at higher dose level (1800mg/kg).

The histopathological study the organ such as brain, heart, kidney, liver, lungs, spleen, stomach,

uterus, ovary and testis were taken. There is no pathological change observed in all the group of animals. (Fig 3)

The smear study shows an increased network of erythroblastic islets was observed in mid-dose level. (Fig 2)

CONCLUSION

Acute and Long-term toxicity study shows that the test drug can be used up to the dose of 5000mg/kg/b.wt. *Ayapodi Elagam* produces no notable abnormalities were observed in all the group of animals. Hence we conclude that the dosage of *Ayapodi Elagam* 2.5 – 5 gm. twice a day narrated in *Anubogavaithiya Navaneetham* is a safer therapeutic dose for uses of the human. The smear study shows increase erythroblastic islets were observed, so it also helps to increase Haemoglobin level for the anaemia patient. The author hopes that this study will be a food print to further research of pharmacological activity, teratogenicity and clinical trials regarding *Ayapodi Elagam*.

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***Address for correspondence**
Dr.A.Kalaivani
PG Scholar,
Dept. of. Nanju Maruthuvam,
National Institute of Siddha,
Chennai-47.
Email: arulkalaivani096@gmail.com