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Safety and Toxicological Evaluation of Siddha Anti HIV Medicine *Deva Choornam*

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Abstract:*Deva choornam (DC) is a renowned Siddha herbal formulation, which is clinically acclaimed for its efficacy in treating Acquired Immuno Deficiency Syndrome (AIDS) patients. The formulation was assessed for its safety and toxicological profile using Animal model. Our study aims to validate the safety profile of the test drug DC using Albino wistar rat model. Acute and chronic toxicity studies were carried out as per the WHO guidelines. Oral Acute toxicity study was done at the limit dose level of 2000 mg/kg body weight of the animal. For the chronic toxicity evaluation, animals were divided into 3 groups of 3 animals each. The drug was administered at two dose levels (Mid dose and High dose), with one group kept as control. The treated animals survived throughout the study period and did not reveal any observable signs of toxicity. On necropsy, no abnormalities were observed. For the chronic toxicity, the treated animals did not shown any abnormal findings at both test dose levels. The values of haematological and biochemical parameters were within the normal limits, indicating that the drug exerted nil impact on the parameters. The necropsy studies showed no remarkable changes.In histo pathological studies, both mid dose and high dose treated rats shown no significant abnormalities. Based on toxicity studies, the trial drug Deva Choornam was found to be nontoxic when studied on animal Models.*

Keywords:*Siddha medicine, Anti HIV Formulation, Deva Choornam, Toxicity studies*

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I. Introduction

Acquired Immuno Deficiency Syndrome (AIDS) is one of the leadingcauses of mortality that is affecting globally. [1] Time tested Siddha formulations are available in practice that are potent to be used in infectious conditions associated with AIDS. As a holistic approach, most of the medicines act in a broad-spectrum manner, either to improve the quality of life, to arrest viral multiplication, to improve the overall metabolism and vitality, to prevent the occurrence of secondary symptoms or diseases associated with it. Deva Choornam (DC) is a popular herbal blend in the form of choornam (Powder) which is specific for its role as a complete health provider to HIV sufferers. [1,2] Many of the clinical, preclinical researches carried out on the drug yielded fruitful results in terms of safety and efficacy. [3]Till the drug has not undergone animal toxicity studies, which is a key mark to justify the safety profile of the drug. DC were studied for its acute toxicity and chronic toxicity evaluation using albino wistar rats.

II. Material And Methods

II. a*Preparation of the drug*

All the raw drugs were procured from the authentic country shops, prepared as per the standards of choornam mentioned in the Siddha formulary of India. [4](Table 1)

Table 1 Ingredients of Deva Choornam

S.No	Tamil Name	Botanical Name	Part Used	Quantity
1	*Devadaru*	*Cedrusdeodara*	Hard wood	100 g
2	*Chittarathai*	*Alpinia officinarum*	Rhizome	100 g
3	*Ilavangapathri*	*Cinnamomum tamala*	Leaf	100 g

II. b*Toxicity Studies* [5-7]

Animals

Sexually matured adult albino rats,both male and females were obtained from the concerned approved facility in Chennai. Both the acute and chronic studies were performed with the consent of Institutional Animal Ethical Committee (IAEC), National Institute of Siddha, Chennai (IAEC approved Number: NIS/IAEC- VI/24042018/03).The procedures were carried out as per the WHO and OECD guidelines for animal toxicity studies. [5] The preprocedures is illustrated in Table. 1

Table no 1: Acute and Chronic Toxicity studies of Deva Choornam (DC)		
1.	**Test Drug**	*Deva Choornam* (DC)
	Nature of the Trial drug	Choornam (Powder)
	Selection of Animal Species	❖ Wistar albino rat ❖ 6 to 8 weeks old ❖ Female nulliparous and nonpregnant ❖ Average weight: 150 ± 20g
	Total No: Animals for the study	Acute: 4 males + 4 females Chronic: 9 females
2.	**Housing and Feeding Conditions**	❖ Animals were housed under standard laboratory conditions of National Institute of Siddha ❖ They were maintained in a ventilated room. ❖ Room temperature: 220 (± 30). ❖ The relative humidity: 50% - 60%. ❖ Lighting: artificial; maintained at 12h light/dark cycle. ❖ Animals were kept in a clean polypropylene cage with bedding of husk.. ❖ Rats were fed with standard pellet diet (VRK'S scientist's Choice Laboratory Animal feed,Sangli Maharashtra) and water ad libitum.
	Preparation of animals	The animals were randomly selected, marked on its fur to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions
	Preparation for Acute Toxicity Studies	
	a) Grouping (Table 2)	Animals were group-caged (4 males + 4 females)
	b) Identification of animals	By cage number, animal number and individual marking by using Picric acid.
	c) Route of drug administration	Oral
	d) Duration of the study	14 days
	Preparation for Chronic Toxicity Studies	
	a) Grouping (Table 3)	Animals were group-caged (9 females)
	b) Identification of animals	By cage number, animal number and individual marking by using Picric acid.
	c) Route of drug administration	Oral
	d) Duration of the study	90 days

Administration of Doses in Acute and Chronic Toxicity studies

1. ***Acute toxicity level dosing***

*Deva chooranam*was suspended in sterile water + Honey and administered to the test group of Wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle (honey). (Table. 2) Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. The dose level of 2000 mg/kg body weight of the animal was administered orally to each animals of the test group.After the substance has been administered, food was withheld for a further 3 - 4 hours. The principle of laboratory animal care was followed. [6]

Table no 2 Grouping of Animals (Acute Toxicity studies of DC)	
Groups	**No: Animals**
Control (Vehicle Group) - Honey	2 Males + 2 Females
Test Group (DC)	2 Males + 2 Females

Justification of Dose selection

Since inference from safety profile tests of DC and its clinical outcome in patients prove to be a completely nontoxic drug, a limit test dose level of 2000 mg/kg, will be carried out with 4 animals (2 males and 2 females) on the test group

Observations

Observations were made and recorded systematically and continuously as per the guidelines after substance administration. Animals were observed individually after dosing at during the first 30 minutes, every 1-hour, 2^{nd} hour, 4^{th} hour, 8^{th} hour and 24^{th} hour. The observation of mortality is continued for a total of 14 days.

Gross necropsy

All animals were subjected to gross necropsy. Gross necropsy included the examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

2. ***Chronic toxicity study level dosing***

Nine female albino wistar rats were used for the study, divided into 3 groups. The control animals (Group I) were administered vehicle (Honey) only. Groups II, III (Mid dose and high dose level) were administered with DCtwice daily for 90 consecutive days at doses 150 mg and 300 mg/kg body weight (p.o) respectively. (Table 3)

Table 3 Grouping of Animals (Chronic Toxicity studies of DC)	
Groups	**No: Animals**
Control (Vehicle Group) – Honey	3 Females
Test Group (DC) Mid dose (150 mg/kg)	3 Females
Test Group (DC) High dose (300 mg/kg)	3 Females

Observations and Investigations

Experimental animals were kept under observation throughout the course of Study. All the animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded. All animals were observed twice daily for mortality during entire course of study. At the end of the study, all the animals were sacrificed by excessive anesthesia on day 91. Necropsy of all the animals was carried out. Hematologic and biochemical investigations were done as per the guidelines. One animal of high dose group and one animal from control group were subjected to histopathological analysis.

III. Results and observations

Interpretation of Acute toxicity Studies

The acute oral toxicity potentials of DC in Wistar albino rats were studied effectively. From the maximum tolerable dose, 2000 mg/kg of Deva choornam, the treated animals (2M+2F)were observed for mortality, untoward clinical/toxic signs, and necropsy findings during the study. The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels. Morphological characters like changes in skin, eyes, fur, and nose appeared normal. The rats did not reveal any observable signs of toxicity (Table. 4). On necropsy, no abnormalities were observed.

Table 4: Grouping of Animals (Acute Toxicity studies of DC) and results of cage side observations

Grouping			Cage side Observational results															
No	Group	No: Animals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Control (Vehicle)	2M+2F	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
	Test group	2M+2F	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-

1. Alertness 2.Aggressiveness3.Piloerection4.Grooming5. Gripping 6.Touch Response 7.Decreased Motor Activity 8.Tremors 9.Convulsions 10. Muscle Spasm 11. Muscle relaxation 12. Hypnosis 13.Lacrimation 14.Diarrhoea 15. Writhing 16. Respiration **(+ Present, - Absent)**

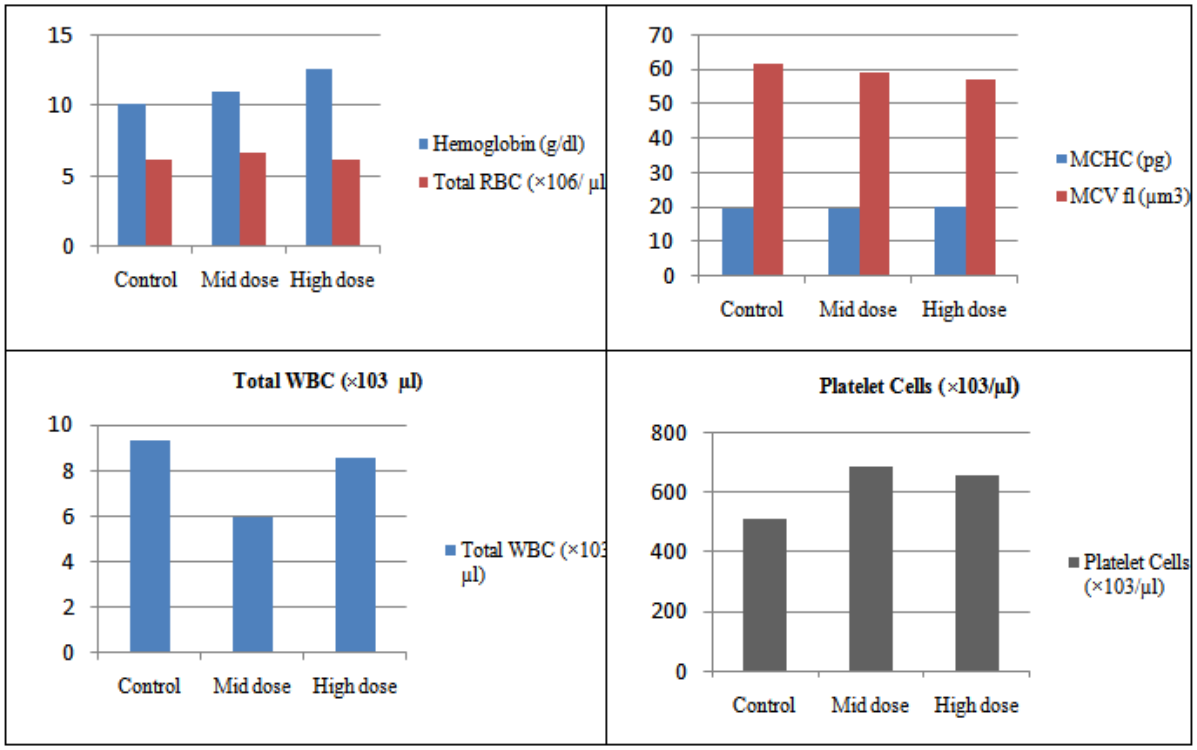
Interpretation of Chronic toxicity Studies

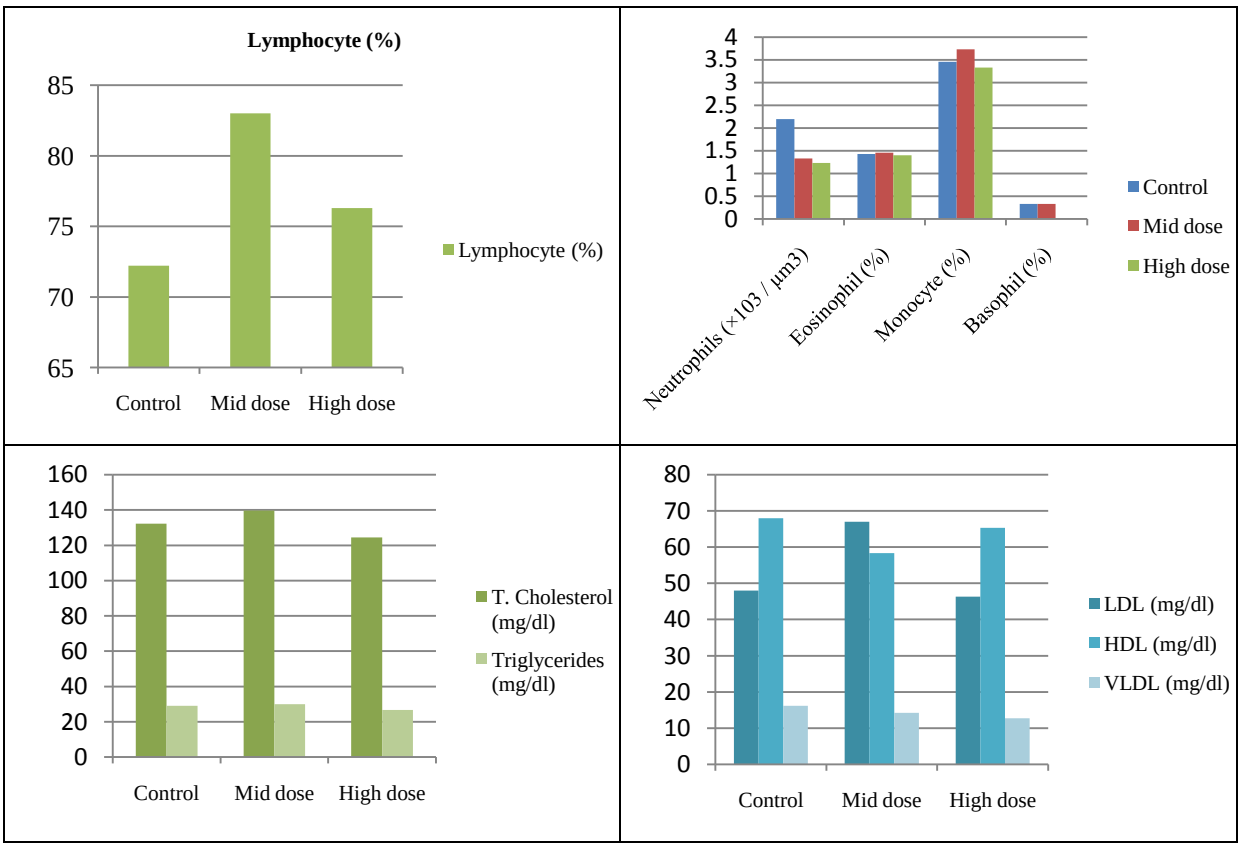
The repeated oral toxicity potentials of *Deva Choornam* in Wistar albino rats were studied effectively. In the sighting study, the test substance was administered in two level doses and adjuvant to control group respectively. The treated animals were observed for mortality, untoward clinical/toxic signs, and necropsy findings during the study. The treated animals survived throughout the study period of 90 days and did not reveal any treatment related major abnormal clinical signs at the test dose levels. Hematological and other biochemical parameters were within the reference range (Table. 5 and Fig A). Morphological characters of organs (skin, eyes, fur, and nose) appeared normal at the end of the study. Histopathological studies were carried out in one animal from control and one from high dose group and did not revealed any signs of toxicity (Fig. B and Table. 6).

Table 5: Results of Sub Chronic Toxicity studies - Hematological and Serological Values (mean) of control, mid dose and high dose group treated with *Deva Choornam*

S.no	Category	Control Group (n=3)	Mid dose group (n=3)	High dose Group (n=3)
I.	**HEMATOLOGICAL**			
1	HEMOGLOBIN (G/DL)	10.1 ± 0.1	10.9 ± 1.38	12.56 ± 1.36
2	TOTAL RBC ($\times 10^6$/ µL)	6.2 ± 0.87	6.6 ± 0.45	6.2 ± 0.26
3	MCH (PG)	19.7 ± 2.25	19.6 ± 2.34	19.96 ± 2.41
4	MCV (μM^3)	61.66 ± 6.97	58.9 ± 6.25	56.83 ± 6.60
5	TOTAL WBC ($\times 10^3$ µL)	9.3 ± 0.43	5.96 ± 1.50	8.53 ± 1.93
6	PLATELET CELLS ($\times 10^3$/µL)	508 ± 39.23	681 ± 170.76	652.3 ± 211
7	LYMPHOCYTE (%)	72.22 ± 4.49	83 ± 3.32	76.3 ± 12.59
8	NEUTROPHILS ($\times 10^3$ / μM^3)	2.2 ± 0.43	1.33 ± 0.20	1.23 ± 0.11
9	EOSINOPHIL (%)	1.43 ± 0.28	1.46 ± 0.40	1.4 ± 0.36
10	MONOCYTE (%)	3.46 ± 0.05	3.73 ± 1.53	3.33 ± 1
11	BASOPHIL (%)	0.33 ± 0.57	0.33 ± 0.57	0
II.	**LIPID PROFILE**			
1	T. CHOLESTEROL (MG/DL)	132.2 ± 12.71	139.6 ± 10.1	124.4 ±5.2
2	TRIGLYCERIDES (MG/DL)	29 ± 4.35	30 ± 2	26.6 ± 6.35
3	LDL (MG/DL)	48 ± 8.88	67 ± 12.2	46.3 ± 4.93
4	HDL (MG/DL)	68 ± 3.46	58.3 ± 4.9	65.3 ± 9.81
5	VLDL (MG/DL)	16.2 ± 3.24	14.2 ± 0.86	12.73 ± 0.75
III.	**LIVER FUNCTION TESTS**			

1	T. BILIRUBIN (MG/DL)	0.43 ± 0.11	0.36 ± 0.15	0.36 ± 0.05
2	SGOT/AST (IU/ML)	91 ± 6	93.3 ± 4.93	87 ± 12.16
3	SGPT/ALT (IU/ML)	25 ± 8.18	20 ± 3.46	25 ± 5.19
IV.	**RENAL FUNCTION TESTS**			
1	BUN (mg/dl)	15.3 ± 1.52	16.3 ± 4.72	16.3 ± 2
2	CREATININE (mg/dl)	0.56 ± 0.40	0.6 ± 0.1	0.23 ± 0.15





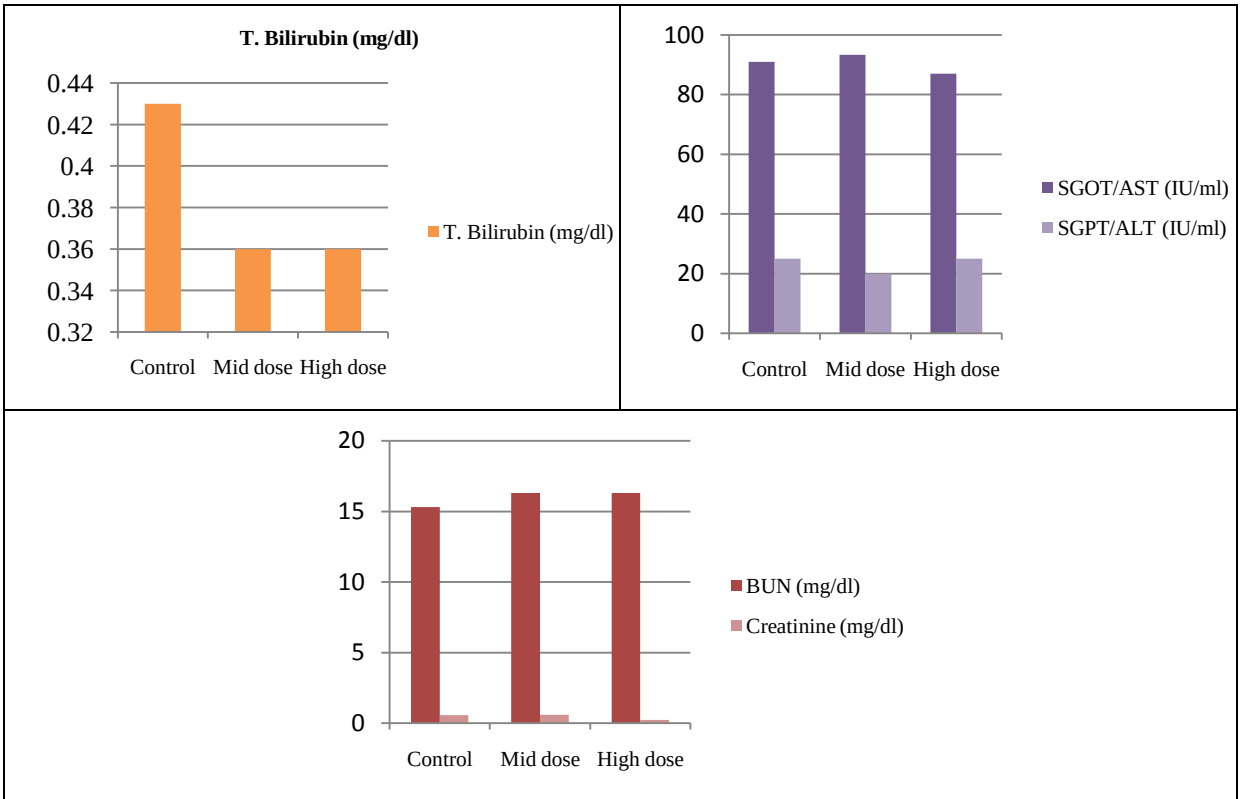
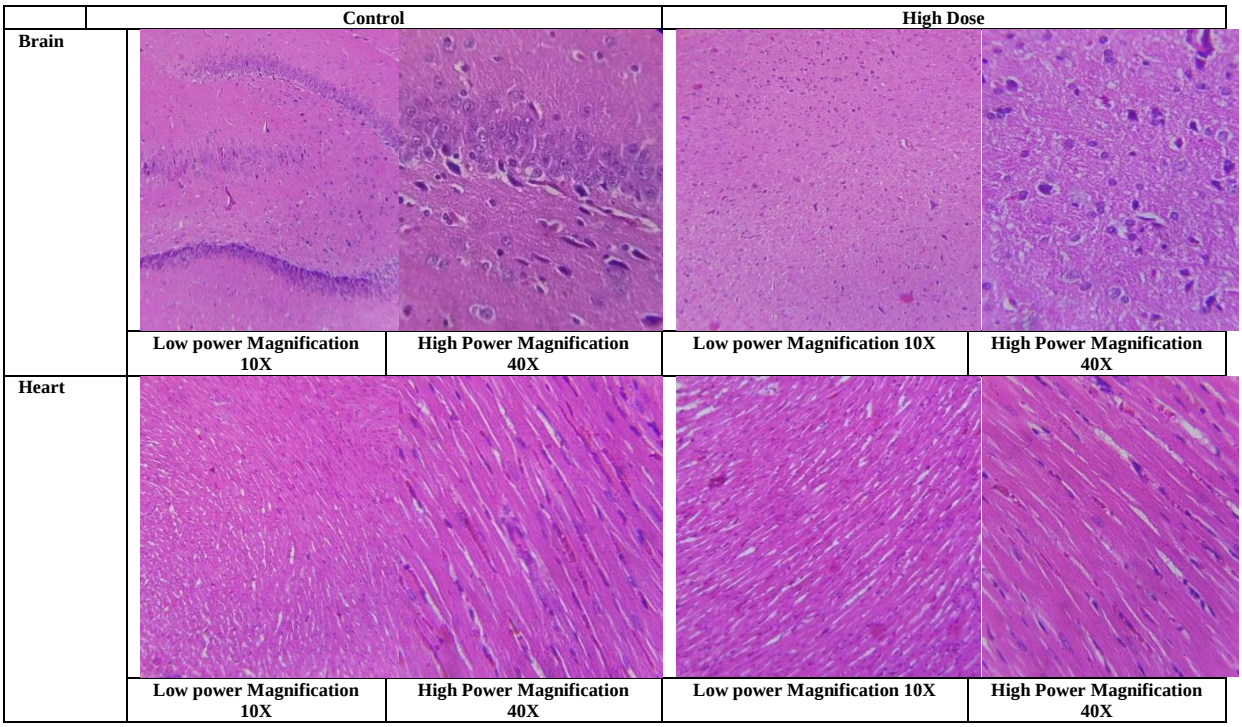
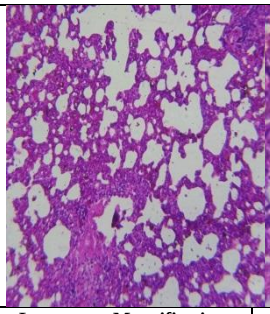
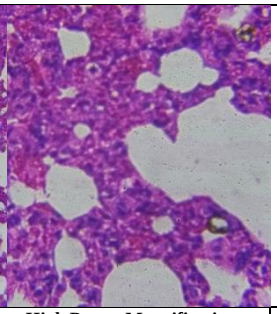
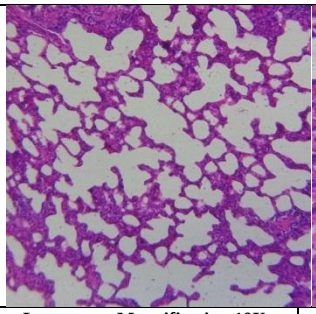
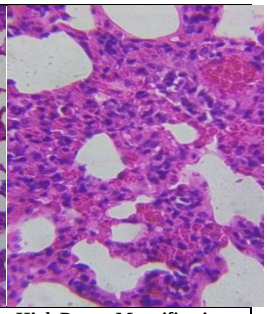


Fig A Results of Sub Chronic Toxicity studies - Hematological and Serological Values (mean) of control, mid dose and high dose group treated with *Deva Choornam*



Lung				
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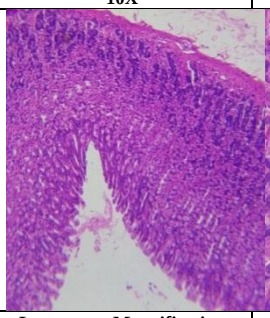
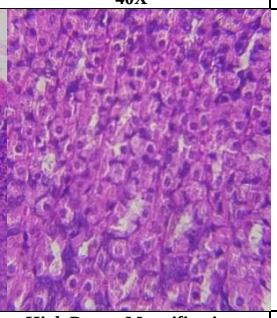
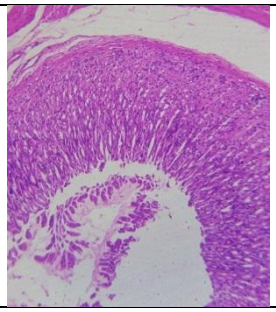
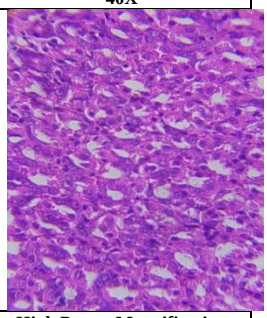
Stomach				
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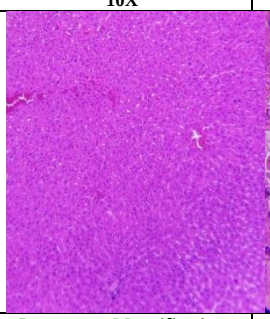
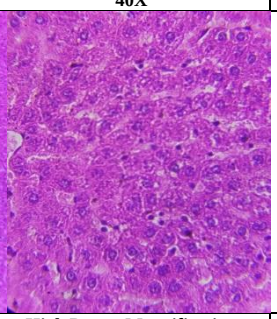
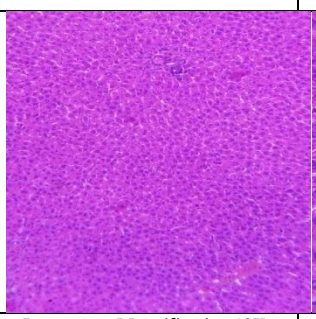
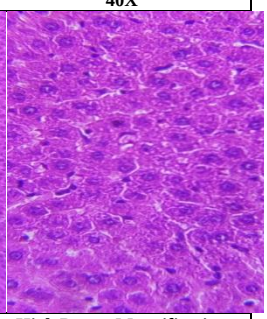
Liver				
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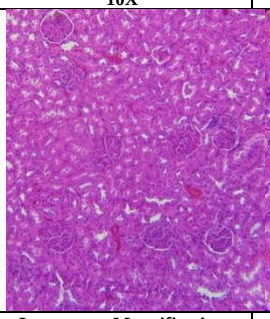
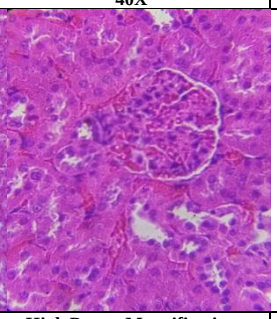
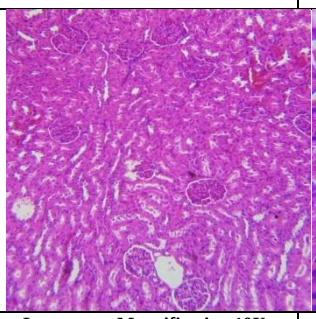
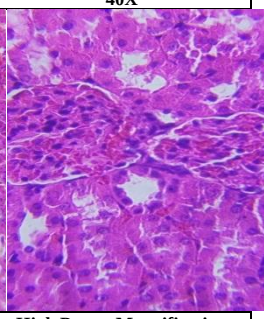
Kidney				
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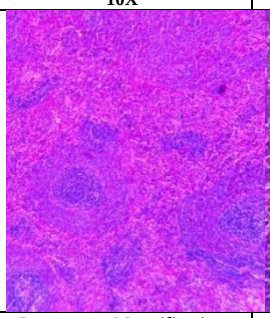
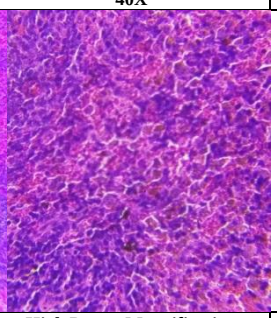
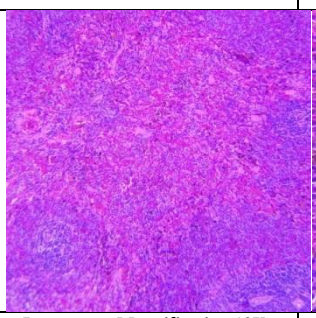
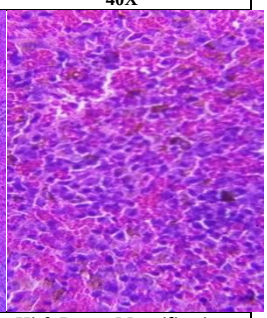
Spleen				
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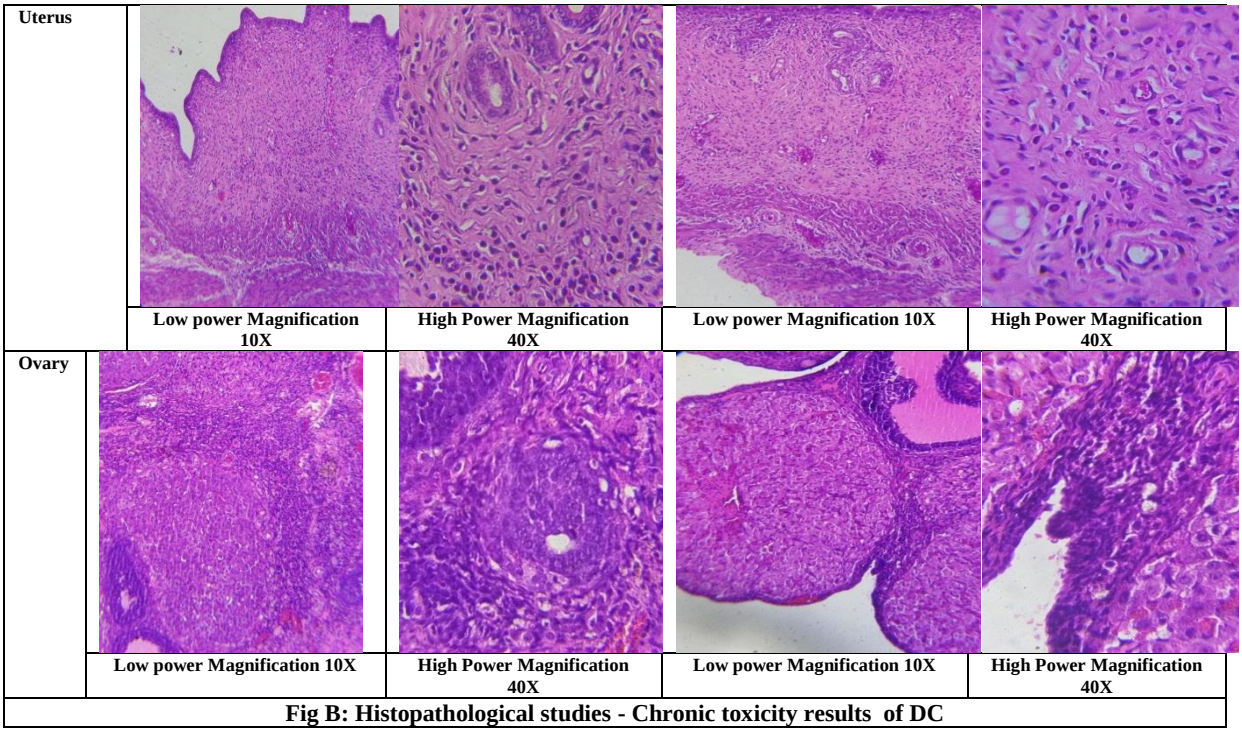

Fig B: Histopathological studies - Chronic toxicity results of DC

Table 6: Histopathological Report: Chronic Toxicity studies of Deva Choornam

Specimen	Control	High dose
Brain	Dentate gyrus and CA3 pyramidal cells of the hippocampus appears normal	Regular marginal alignment on the neurons with promising histology were observed
Heart	Appearance of fibrils and cross striations are equidistant	Nucleus appears prominent with regular arrangement of fibres. No evidence of pyknotic nucleus.
Lung	Showing normal alveoli and collagen fibres	Opening of lumen of blood vessels appears regular with no invasion of inflammatory cells
Stomach	Regular arrangement of muscularisexterna and outer longitudinal muscle were observed.	Gastric epithelium and mucosa appears normal
Liver	Section of liver showing normal, homogenous, intact hepatic parenchyma; hepatic lobules, with normal central vein.	Normal hepatocytes with no signs of necrosis
Spleen	Erythropoietic cells (EP) are scattered throughout the red pulp. No abnormalities found in lymph node .	Normal cytoarchitecture with No signs of immunological activities.
Kidney	Appearance of glomerular basement membrane was normal.	Mild change in tubular basement membrane with hypertrophic tubules.
Uterus	Endometrial gland, epithelium and blood vessels appears normal.	Endometrial stroma; G, gland; M, myometrium; P, perimetrium; L, lumen exhibits normal histological aspect of endometrium and myometrium.
Ovary	Section of ovary showing well follicular development , Pre-ovulatory follicle surrounded by granulosa cells with normal zonapellucida and theca interna and externa.	Sequential arrangement of granulosa cells around oocyte was normal and regular.

IV. Conclusion

Both the acute and chronic toxicity evaluation carried out in Deva Choornam proves the nontoxic nature of this efficient formulation. Further clinical trials will be monitored by the researchers to establish the drug as a safe and efficient Anti HIV Siddha drug.

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