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Toxicity studies on Amukkarac curanam - A Siddha poly herbal drug

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Abstract

Objective: The effect of Amukkarac curanam (AC), a widely used Siddha medicine was evaluated for acute and chronic toxicity in rats. Material and methods: Amukkarac is botanically equated to *Withania somnifera* Dunal. Acute dosages were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 g/kg body weight while the chronic dosage was 500 mg/kg body weight /day. All external morphological, hematological, serum parameters, histopathological, in addition to body weight and vital organ weights were recorded. Results and conclusion: During this investigation no significant mortality was observed. The results showed that AC at the given dose of 500 mg/kg body weight did not produce any significant toxic effect in rats during 90 days period of the treatment.

Keywords: Chronic toxicity, Hematology; Marker enzymes, Lipid profile.

1. Introduction

Amukkarac curanam (AC) is a popular drug in Siddha system of medicine containing amukkarac root as the major ingredients. Amukkarac is botanically equated to *Withania somnifera* Dunal. It is used in the treatment of colic, hiccup, tumours, chlorosis and spermatorrhoea. It is also used in rheumatic diseases, sexual insufficiency and insomnia. It is considered to be a general tonic and anabolic [1, 2]. The ingredients of amukkarac curanam are kirampu (*Syzygium aromaticum* Linn, flower buds, 1 part); cirunakappu (*Cinnamomum wightii* Meiss, flower buds, 2 parts); elam (*Elettaria cardamomum* Maton. Fruits, 4 parts); milaku (*Piper nigrum* Linn, fruits, 8 parts); tippili (*Piper longum* Linn, root, 16 parts); cukku (*Zingiber officinale* Rosc, rhizome, 32 parts); amukkarac (*Withania somnifera* Dunal, tuberous roots, 64 parts) and cane sugar (128 parts), all the above ingredients are powdered and mixed with sugar [2]. As there is no report on the toxicological effects of AC, the present investigation was taken up to evaluate the toxicity profile of the drug in albino rats.

2. Materials and Methods

2.1 Preparation of Amukkarac curanam

Amukkarac curanam (AC) was prepared according to the method given in The Siddha Formulary of India [2].

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2.2 Animal stock

Adult, healthy Swiss albino mice (18-20 g) and Wistar albino rats (150-200 g) of either sex were used. The animals were fed on standard diet with free access to water under standard laboratory conditions of light (12 h light/dark), humidity and temperature. The study was approved by the Institutional Animal Ethical Committee of CSMDRIA, Chennai, India (IAEC/CSMDRIA/02/2005).

2.3 Acute toxicity

Acute toxicity study of AC was carried out in Swiss albino mice of either sex [3]. The animals were made to fast for 4 h, but allowed free access to water throughout. The fasted mice were divided into eight groups of 10 animals each. AC prepared in distilled water was administered orally in varying doses 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 g/kg respectively. Negative control received a similar volume of water. They were continuously observed for 1-2 h to detect changes in the behavioral, neurological and autonomic responses, viz. awareness, irritability, spontaneous activity, convulsions, righting reflex, corneal reflex, urination, salivation, piloerection [4]. The experimental animals were observed for further 72 h for any toxic symptoms and mortality. On the basis of the above study the dose levels of 500 mg/kg of AC was chosen for further experiments.

2.4 Chronic toxicity

Albino rats of either sex were randomly divided into two groups of 10 animals each. Group I animals received normal diet and served as control. Group II animals received 500 mg/kg/day AC orally. The treatment was continued for a period of 90 days [5]. The animals were observed for all external general symptoms of toxicity, body weight changes and mortality. The average pre-and post-treatment body weights and vital organ weights of the chronically-treated animals were compared with those of the control group.

Animals were sacrificed twenty-four hours after the last administration (91st day of the experiment). Blood samples were collected for investigation. Haematological parameters such as red blood cell (RBC), hematocrit (%), hemoglobin (Hb), white blood cell (WBC), neutrophil (N %), lymphocyte (L %), eosinophil (E %), monocyte (M %), basophil (B %), erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) [6] were determined.

Biochemical analysis of serum was performed using a Semi autoanalyser (E-Merck model 200). Biochemical parameters measured were alkaline phosphatase (ALP), acid phosphatase (ACP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid. Serum lipids like total cholesterol, total lipid, triglyceride, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL) were also estimated. Serum levels of sodium, potassium and calcium were estimated using Digital Flame Photometer TDF - 35.

Histological evaluation of the internal organs like brain, liver, heart, kidney, lung, spleen, adrenal gland, stomach, testis in male rats or ovary and uterus in female rats of both treated and control groups was performed. Fresh tissues were excised and then fixed in 10% formalin for routine histoprocessing by paraffin embedding technique [7].

Table 1. Quantitative data on the body weight and organ weights (g) (per100 g body weight) in rats after chronic treatment with Amukkarac curanam.

Treatment and dose (500mg/ kg/day for 90 days)	Pre-treatment (average body weight ± S.E.M)	Post-treatment (average body weight ± S.E.M)	Average organ weight per 100 g body weight ± S.E.M										
			Brain	Heart	Lungs	Liver	Kidney	Spleen	Stomach	Adrenal	Testis	Uterus	ovary
Control	115.90 ± 4.50	246.10 ± 13.49	1.10 ± 0.01	0.31 ± 0.05	0.56 ± 0.03	5.82 ± 0.14	0.81 ± 0.03	0.18 ± 0.02	0.49 ± 0.02	0.04 ± 0.01	1.26 ± 0.03	0.17 ± 0.02	0.04 ± 0.01
AC	116.40 ± 3.31	304.20 ± 11.16**	1.13 ± 0.01	0.47 ± 0.03**	0.63 ± 0.01	5.82 ± 0.12	0.82 ± 0.02	0.20 ± 0.03	0.48 ± 0.02	0.03 ± 0.01	1.23 ± 0.02	0.17 ± 0.01	0.03 ± 0.01

AC = Amukkarac curanam. The values are expressed as mean ± S.E.M; * P<0.05, **P<0.01, *** P<0.001 compared to control group; n = 10.

Table 2. Hematological studies on Amukkarac curanam (AC) after chronic treatment in rats.

Treatment and dose (500mg/ kg/day for 90 days)	RBC (x10^6 cells/mm^3)	Hema tocrit (%)	Haemoglo bin (g/dl)	WBC (x 10^3 cells/mm^3)	Differential count (%)					ESR (mm/hr)	MCV (μm^3/red cell)	MCH (μm^3/red cell)	MCHC (μm^3/red cell)
					N	L	E	M	B				
Control	8.32 ± 0.01	47.47 ± 0.02	14.54 ± 0.01	5.81 ± 0.08	30.75 ± 0.04	61.68 ± 0.07	2.46 ± 0.06	3.65 ± 0.01	1.52 ± 0.05	1.81 ± 0.03	57.05 ± 0.11	17.47 ± 0.04	30.62 ± 0.04
AC	8.42 ± 0.03**	47.51 ± 0.11	14.64 ± 0.04	5.71 ± 0.08	30.79 ± 0.04	61.49 ± 0.06	2.53 ± 0.05	3.48 ± 0.06**	1.59 ± 0.05	1.73 ± 0.04	56.43 ± 0.31**	17.39 ± 0.09	30.82 ± 0.14

AC = Amukkarac curanam. RBC = red blood cell. WBC = white blood cell. N = neutrophil. L = lymphocyte. E = eosinophil. M = monocyte. B = basophil. ESR = erythrocyte sedimentation rate. MCV = mean cell volume. MCH = mean corpuscular hemoglobin. MCHC = mean corpuscular hemoglobin concentration. The values are expressed as mean ± S.E.M; * P<0.05, **P<0.01, *** P<0.001 compared to control group; n = 10.

Table 3. Effect of Amukkarac curanam on the serum biochemical values after chronic treatment.

Treatment and dose (500mg/ kg/day for 90 days)	ALP (μ mol of phenol liberated mg/ protein/min)	ACP (μ mol of phenol liberated mg/protein min^{-1})	ALT (μ mol of pyruvate liberated mg/protein/ min)	AST (μ mol of pyruvate liberated mg/protein/ min)	Total Protein (g/dL)	Albumin (g/dL)	Bilirubin (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)	Uric acid (mg/dL)
Control	50.38 ± 3.18	25.95 ± 1.89	28.14 ± 2.13	81.74 ± 2.27	6.98 ± 0.08	4.94 ± 0.03	0.69 ± 0.03	21.35 ± 0.17	0.74 ± 0.023	154.75 ± 2.35	2.21 ± 0.06
AC	49.52 ± 3.55	24.67 ± 1.25	29.99 ± 1.56	83.11 ± 3.53	7.10 ± 0.04	4.99 ± 0.03	0.73 ± 0.01	21.81 ± 0.36	0.75 ± 0.03	152.98 ± 4.36	2.27 ± 0.04

AC = Amukkarac curanam. ALP = alkaline phosphatase. ACP = acid phosphatase. ALT = alanine aminotransferase. AST = aspartate aminotransferase. BUN = blood urea nitrogen. The values are expressed as mean ± S.E.M; * P<0.05, **P<0.01, *** P<0.001 compared to control group; n = 10.

Table 4. Effect of Amukkarac curanam on the serum lipids and electrolyte values after chronic treatment.

Treatment and dose (500mg/ kg/day for 90 days)	Total cholesterol (mg/dL)	Total Lipids (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (mmol/L)
Control	83.87 ± 3.60	158.03 ± 0.46	3.47 ± 0.04	29.87 ± 1.87	60.54 ± 3.66	135.46 ± 1.71	3.39 ± 0.06	2.13 ± 0.02
AC	80.05 ± 3.91	157.97 ± 0.55	3.40 ± 0.05	23.35 ± 0.58**	53.69 ± 1.96	132.82 ± 2.41	3.65 ± 0.23	2.15 ± 0.01

AC = Amukkarac curanam. LDL = Low density lipoprotein cholesterol. HDL = high density lipoprotein cholesterol. The values are expressed as mean ± S.E.M; * P<0.05, **P<0.01, *** P<0.001 compared to control group; n = 10.

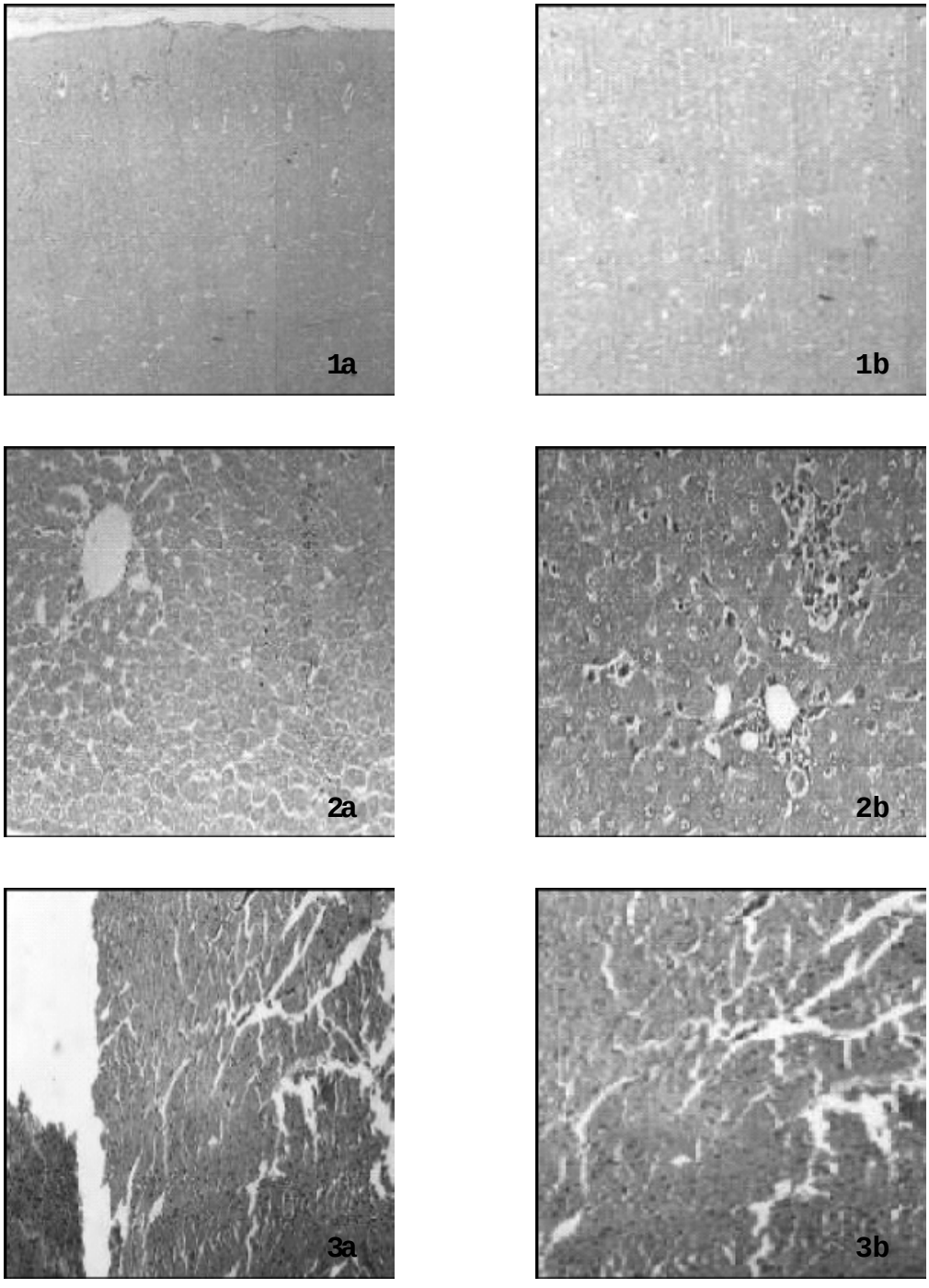
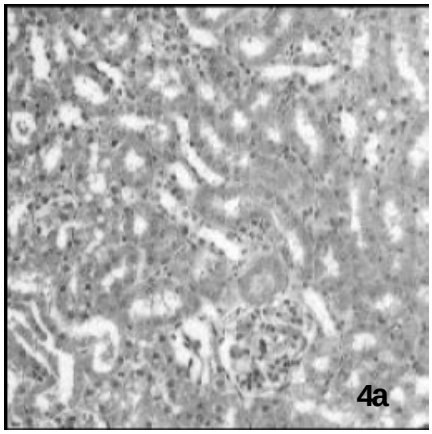
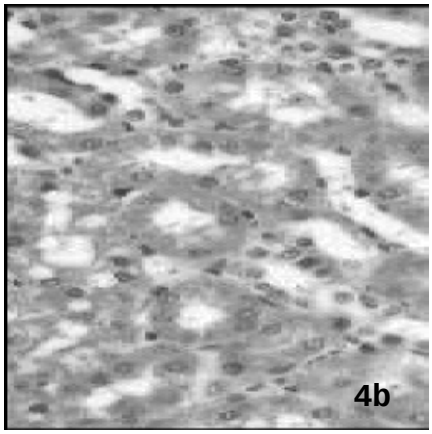
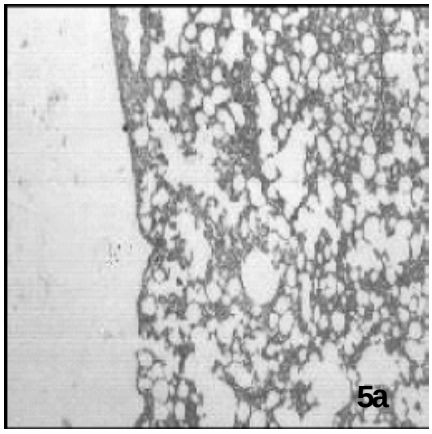
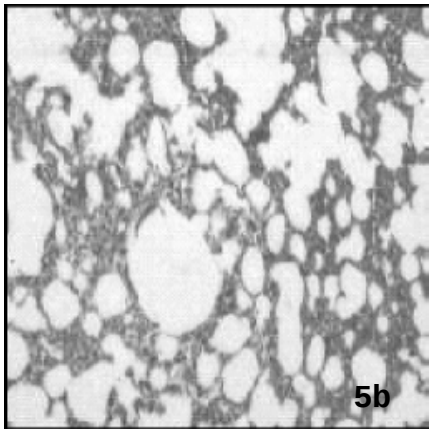


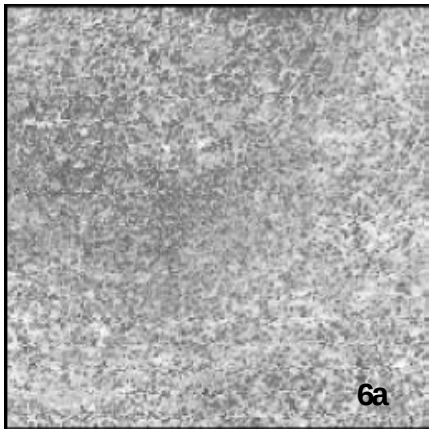
Fig. 1-3 - Histological examination of brain, liver and heart tissue sections in control and experimental animals (Hematoxylin and eosin, 80x). Section of brain tissue from (1a) control rat showing normal architecture; (1b) AC treated rat showing apparently normal architecture with no pathological changes. Section of liver tissue from (2a) control rat showing normal architecture; (2b) AC treated rat showing mild mononuclear cell infiltration and fatty changes. Section of heart tissue from (3a) control rat showing normal architecture; (3b) AC treated rat showing normal architecture with no pathological changes.











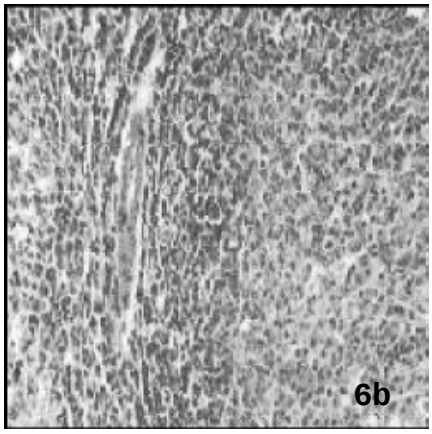


Fig. 4-6 - Histological examination of kidney, lung and spleen tissue sections in control and experimental animals (Hematoxylin and eosin, 80x). Section of kidney tissue from (4a) control rat showing normal architecture; (4b) AC treated rat showing slight inclusions. Section of lung tissue from (5a) control rat showing normal architecture; (5b) AC treated rat showing normal architecture with no pathological changes. Section of spleen tissue from (6a) control rat showing normal architecture; (6b) AC treated rat showing mild lymphoid depletion.

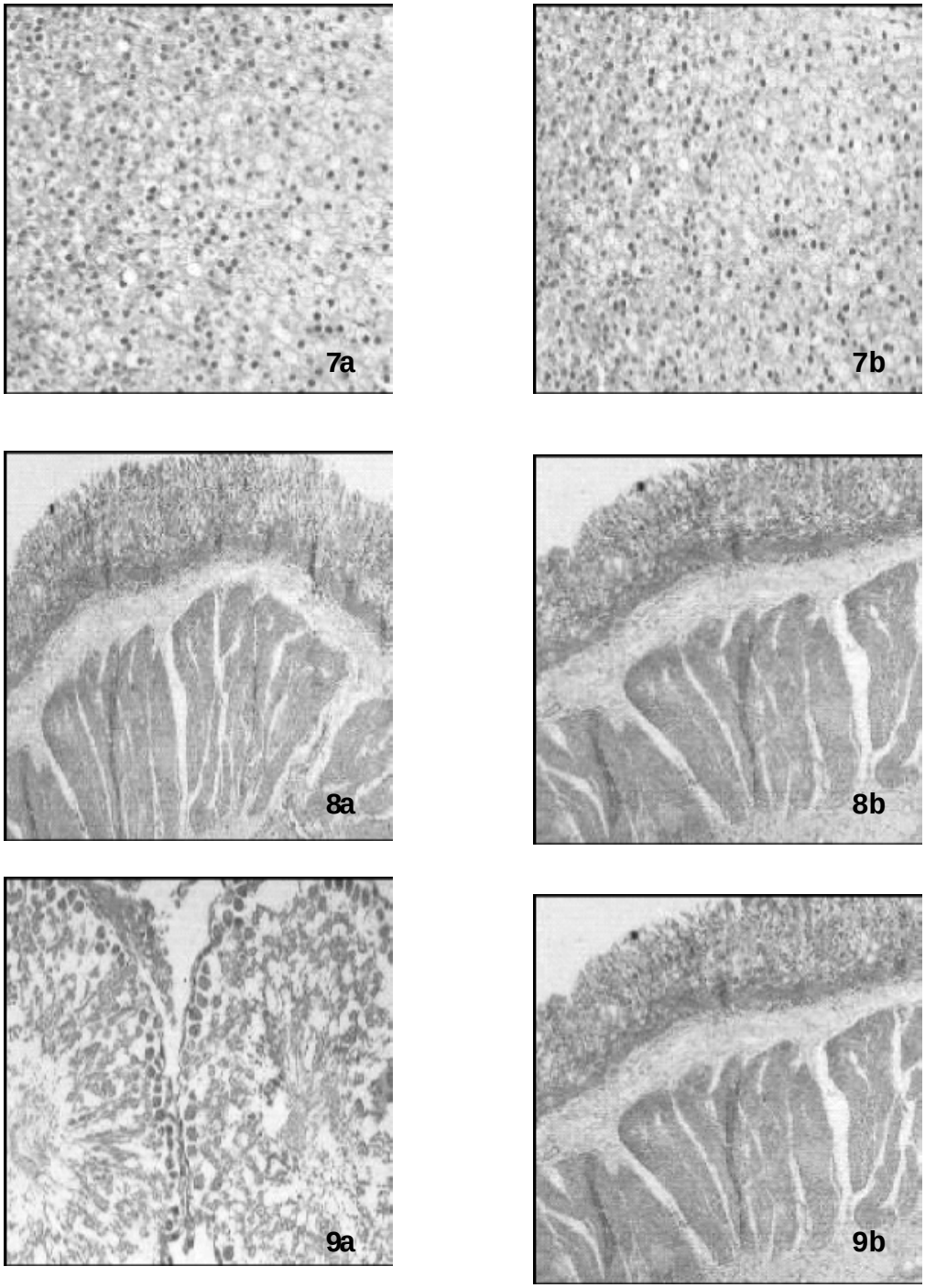


Fig. 7-9 - Histological examination of adrenal gland, stomach and testis sections in control and experimental animals (Hematoxylin and eosin, 80x). Section of adrenal gland tissue from (7a) control rat showing normal architecture; (7b) AC treated rat showing normal architecture with no pathological changes. Section of stomach tissue from (8a) control rat showing normal architecture; (8b) AC treated rat showing apparently normal architecture with no pathological changes. Section of testis tissue from (9a) control rat showing normal architecture; (9b) AC treated rat showing normal architecture.

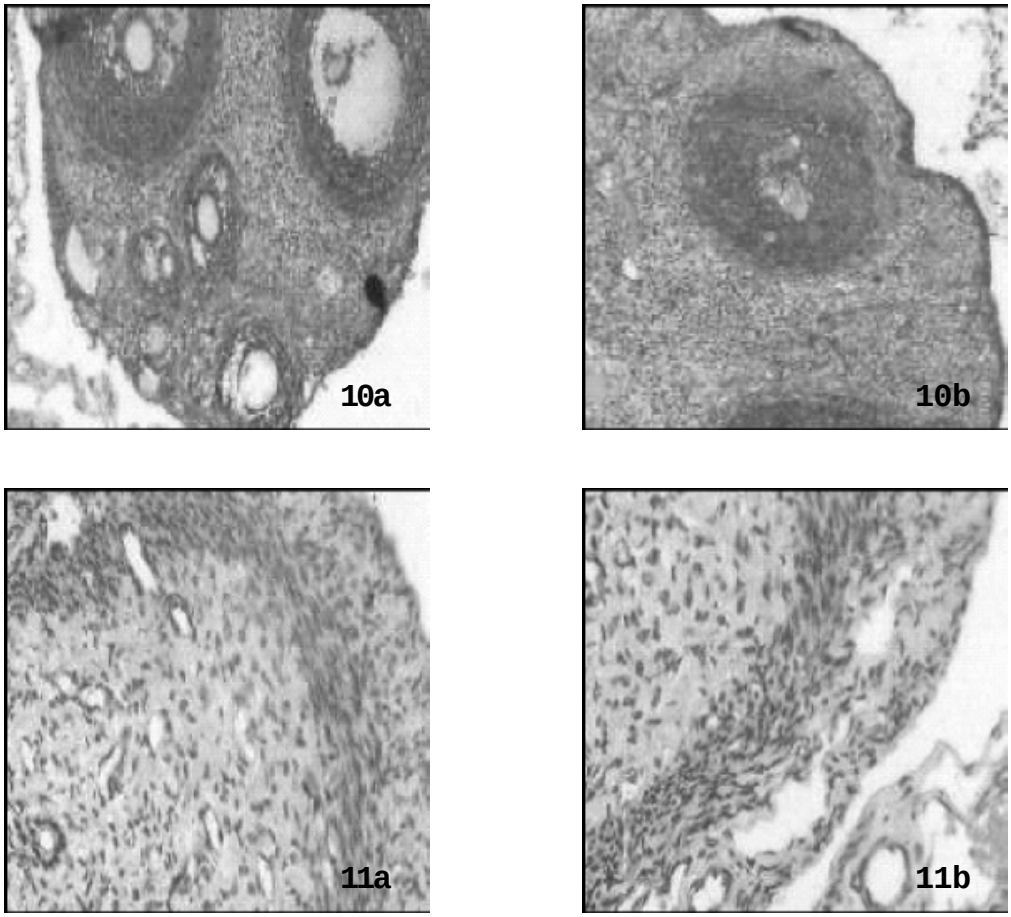


Fig. 10-11 - Histological examination of ovary and uterus sections in control and experimental animals (Hematoxylin and eosin, 80x). Section of ovary tissue from (10a) control rat showing normal architecture; (10b) AC treated rat showing normal architecture with no pathological changes. Section of uterus tissue from (11a) control rat showing normal architecture; (11b) AC treated rat showing normal architecture with no changes.

2.5 Statistical analysis

Data were statistically evaluated using ANOVA, expressed as mean±S.E.M followed by Post Hoc Dunnett T3 multiple comparisons test using the 7.5 version of SPSS computer software. Results were considered as significant when $P< 0.05$.

3. Results and Discussion

3.1 Acute toxicity study

Treated animals did not exhibit any casualties after 72 h of treatment upto 5.0g/kg dose level. Results did not show any significant changes in the behavioral or autonomic responses during the experimental period.

3.2 Chronic toxicity

3.2.1 Effect of AC on body weight and organ weights

During chronic treatment with AC both male and female rats were healthy till the end of the treatment and no mortality was observed when compared to the control groups. There was a

significant weight gain in the test as well as in the control groups. AC treatment induced a significant ($P<0.01$) change in heart weight upon chronic treatment. Whereas no difference in the average weights of other vital organs was found when compared to the control group (Table 1).

3.2.2 Effect of AC on hematological parameters

The hematological studies revealed no difference in the number of hematocrit, haemoglobin, white blood cells, %N, %L, %E, %M, %B, ESR including MCH and MCHC between AC-treated groups and control groups (Table 2). Whereas the levels of red blood cells ($P<0.01$) were significantly higher and the levels of MCV ($P<0.01$) were lower than that of control group. However, these changes were still within the normal range [8, 9].

3.2.3 Effect of AC on serum parameters

In treated rats, no differences in the serum levels of ALP, ACP, ALT, total protein, albumin, BUN, creatinine, glucose, uric acid were found between AC-treated groups and the control groups. It was found that the levels of serum AST and bilirubin were slightly higher than those of the control groups (Table 3), however this change was still within normal range [8, 9].

3.2.4 Effect of AC on serum lipid profile and electrolyte levels

No significant difference in serum levels of total lipid, triglyceride, sodium, potassium, calcium were found between AC-treated groups and the control group. It was found that the levels of total cholesterol, LDL, HDL were slightly lower than those of the control group (Table 4), however the values were still within the normal range [8, 9].

3.2.5 Effect of AC on histopathology of internal organs

Histological examination of the liver showed mild mononuclear cell infiltration and fatty change (fig. 2b). Similarly a slight inclusion in kidney (fig. 4b) and mild lymphoid depletion in spleen (fig. 6b) were found in treated group. Whereas microscopic examination of brain, heart, lung, adrenal gland, stomach, testis in male rats or ovary and uterus in female rats of treated and control groups showed normal architecture suggesting no detrimental changes and morphological disturbances were caused by the daily oral administration of AC at a dose of 500 mg/kg for 90 days (figs. 1-11). In conclusion, the results indicate that amukkarac curanam given orally at the dose level of 500 mg/kg/day, did not produce any sign of toxicity in rats during the 90 days administration period.

4. Acknowledgement

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