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Effect of Ethanolic Extract of the Seeds of *Annona squamosa* on Mammalian Blood

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Abstract: *Annona squamosa* Linn. family Annonaceae, shows various medicinal effects like insecticidal, antiovulatory and abortifacient. The present study was designed to elucidate the biochemical effects of ethanolic extract of seeds of *A. squamosa* on hematological and serum parameters in adult male albino rats. The male rats were gavaged ethanolic extract of *A. squamosa* seeds at the dose level of 200 and 300 mg/rat/day for 28 days. A significant decrease ($p<0.05$) in lymphocyte, RBC, hemoglobin and blood glucose was observed after treating the rats with ethanolic extract of seeds of *A. squamosa* whereas a significant increase ($p<0.05$) was observed in neutrophils, WBC, SGPT, SGOT and serum cholesterol.

Key words: *Annona squamosa* · Rat · Seeds · Blood Biochemistry

INTRODUCTION

Annona squamosa Linn. From Annonaceae family, is commonly known as "Custard apple" and is a native of West Indies and South America and is cultivated throughout India, mainly for its edible fruits [1]. This plant recently came under intense scrutiny for potential source of potent biologically active annonaceous acetogenins isolated from seeds and bark. This plant has significant medicinal properties, which include anti-fertility, anti-tumour and anti-diabetic activities in mice and rats. *A. squamosa* also possesses anti-spermatogenic activity [2-4]. The present study was undertaken to evaluate alterations in blood biochemistry of albino rats after exposure to ethanolic extract of the seeds of *A. squamosa*.

MATERIAL AND METHODS

Plant Material and Extract Preparation: Fresh fruits of *A. squamosa* were collected from Gorakhpur district of U.P. (India) and were verified in the department of Botany, University of Gorakhpur. Seeds collected from fruits were dried under shade, pulverized by a mechanical grinder and passed through a 50 mesh and stored in airtight containers. The powdered seeds (50g) were extracted with ethanol for 48 hours. This ethanolic extract was dried at controlled temperature (40-50°C) to yield solid powder that was further used for experiments.

Animal Model and Experimental Procedure: Healthy colony bred male albino rats weighting 120-150 grams were used for the experiments. The animals were housed in polypropylene cages and these cages were cleaned regularly to avoid rat smell and to maintain under standard hygienic conditions (12h light / 12h dark cycles and 25°C ± 5°C at room temperature). The rats were acclimatized to laboratory conditions for 10 days and fed with standard diet and water was provided *ad libitum*. The protocol for these experiments was approved by the departmental ethical Committee of D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India, 273009.

The animals were divided into three groups containing 16 animals in each group. A group of rats was administered 200mg/rat/day of the ethanolic extract of *A. squamosa* seeds (EEAS) and other group was administered 300mg/rat/day every morning for 28 days. The remaining control group was fed with vehicle of similar dilution without test material. After every 7^{th}, 14^{th}, 21^{st} and 28^{th} days both control and treated groups were autopsied under light chloroform anesthesia.

Autopsy Schedule: Four animals of each three groups were autopsied for every 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Blood was collected through cardiac puncture.

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Blood Analysis: The blood samples were collected carefully for blood biochemistry and hematology. Heparinized blood samples were used for the determination of red blood cell (RBC) and white blood cell (WBC) count, hemoglobin, lymphocyte and neutrophils [5]. Non-heparinized samples were used for the estimation of serum parameters like Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), serum cholesterol and glucose. Blood glucose, serum cholesterol, SGPT and SGOT were evaluated by the use of a Biochemical Analyzer Transasia ERA CHEM-5 PLUS (India) [6].

Statistical Analysis: The data is expressed as mean ± S.E. of four replicates. Student ´t´ test and two way ANOVA were applied for measurement of variation between control and treated groups.

RESULTS

Hemoglobin: The mean value of hemoglobin was 11.55±0.07, 11.50±0.08, 11.40±0.07 and 11.32±0.05 (gm%) after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of hemoglobin was 11.50±0.06, 11.43±0.06, 11.30±0.06 and 10.67±0.2 after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS. While in control rats the mean value of hemoglobin was 14.48±0.19, 14.55±0.07, 14.60±0.71 and 14.65±0.61 (gm%) after 7,14,21 and 28 days, respectively (Table 1).

RBC Count: The mean value of red blood cell (RBC) was 8.22±0.04, 8.10±0.23, 8.003±0.28 and 7.90±0.30million/mm^3 after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of R.B.C. was 7.90±0.34, 7.68±0.20, 7.63±0.20 and 7.51±0.20 million/mm^3 after 7, 14, 21 and 28 days, respectively when treated with 100mg/kg body weight of EEAS. While in control rats the mean value of R.B.C. was 8.30±0.10, 8.40±0.11, 8.53±0.07 and 8.80±0.13million/mm^3 after 7, 14, 21 and 28 days, respectively (Table 1).

WBC Count: The mean value of white blood cell (WBC) was 8.92±0.34, 9.13±0.25, 9.33±0.02 and 10.10±0.30thousand/ mm^3after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of W.B.C. was 10.45±0.23, 11.10±0.34, 11.60±0.26and 12.86±0.17thousand/ mm^3after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS while in control rats the mean value of W.B.C. was 8.47±0.08, 8.49±0.09, 8.81±0.17and 8.90±0.32 thousand/ mm^3after 7, 14, 21 and 28 days, respectively (Table 1).

Lymphocyte: The mean value of lymphocyte was 85.29±0.73, 82.65±0.40, 81.15±1.45 and79.48±0.28 (%)after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of lymphocyte was 78.29±0.73, 78.95±0.32, 75.07±0.27 and 72.91±0.37 (%)after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS. While in control rats the mean value of was lymphocyte 86.10±0.18, 86.07±0.36, 85.73±0.01and 85.66±0.01 (%) after 7, 14, 21 and 28 days, respectively (Table 1).

Neutrophils: The mean value of neutrophils was 13.80±0.19, 19.06±0.45, 22.20±0.97 and 23.94±0.42(%) after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of neutrophils was 21.03±0.31, 23.33±0.14, 29.94±0.78 and 34.05±0.42(%) after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS. While in control rats the mean value of neutrophils was 10.32±0.02, 11.29±0.15, 11.64±0.23 and 12.41±0.007(%) after 7, 14, 21 and 28 days, respectively (Table 1).

SGPT: The mean value of SGPT was 66.69±0.33, 70.17±0.20, 84.01±0.64and 90.67±0.45 (IU/L) after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of SGPT was 72.21±0.15, 76.90±0.70, 115.10±1.39 and 130.06±2.23 (IU/L) after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS while in control rats the mean value of SGPT was 55.03±1.49, 56.35±0.86, 60.16±0.94and 62.24±0.61(IU/L) after 7, 14, 21 and 28 days, respectively (Table 2).

SGOT: The mean value of SGOT was 89.64±0.107, 91.93±0.42, 93.09±0.12and 94.44±0.21(IU/L) after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of SGOT was 105.89±1.87, 115.37±0.08, 123.56±0.42and 127.56±0.10 (IU/L) after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS. While in control rats the mean value of SGOT was 83.14±0.24, 82.62±0.43, 83.92±0.35 and 85.80±0.31(IU/L) after 7, 14, 21 and 28 days, respectively (Table 2).

Table 1: Effect of different doses of ethanolic extract of seeds of *A. squamosa* on blood parameters.

			Treated rats		Change in %	
Parameters	Days	Control Rats Mean±SE	200mg/kg Body Weight Mean± SE	300mg/kg Body Weight Mean± SE	200mg/kg Body Weight Mean± SE	300mg/kg Body Weight Mean± SE
Hemoglobin (gm %)	7	14.48 ±0.19	11.55* ±0.07	11.50* ±0.06	20.24↓	20.60↓
	14	14.55 ±0.07	11.50* ±0.08	11.43* ±0.06	20.76↓	21.44↓
	21	14.60 ±071	11.40* ±0.07	11.30* ±0.06	22.03↓	22.71↓
	28	14.65 ±0.61	11.32* ±0.05	10.67* ±0.2	22.73↓	27.17↓
RBC count (million/mm^3)	7	8.30 ±0.103	8.22* ±0.04	7.9* ±0.34	1.0↓	4.82↓
	14	8.40 ±0.11	8.10* ±0.23	7.68** ±0.2	3.6↓	8.6↓
	21	8.53 ±0.07	8.003* ±0.28	7.63* ±0.2	6.2↓	10.55↓
	28	8.8 ±0.13	7.9* ±0.3	7.51* ±0.2	10.23↓	14.70↓
WBC count (thousand/ mm^3)	7	8.47 ±0.084	8.925* ±0.34	10.45* ±0.23	5.37↓	23.37↓
	14	8.49 ±0.09	9.135* ±0.254	11.10* ±0.34	7.59↓	30.74↓
	21	8.81 ±0.17	9.33* ±0.02	11.60* ±0.26	5.90↓	31.36↓
	28	8.90 ±0.322	10.10* ±0.30	12.86* ±0.17	13.48↓	44.49↓
Lymphocyte (%)	7	86.10 ±0.18	85.29 ±0.73	78.29** ±0.73	0.94↓	9.07↓
	14	86.07 ±0.363	82.65** ±0.405	78.95** ±0.320	3.97↓	8.27↓
	21	85.73 ±0.016	81.15** ±1.45	75.07** ±0.275	5.35↓	12.43↓
	28	85.66 ±0.015	79.48** ±0.281	72.91** ±0.371	7.22↓	14.88↓
Neutrophils (%)	7	10.32 ±0.027	13.80* ±0.193	21.03* ±0.311	33.65↓	103.72↓
	14	11.29 ±0.150	19.06* ±0.450	23.33* ±0.146	68.82↓	106.64↓
	21	11.64 ±0.231	22.20* ±0.97	29.94* ±0.784	90.66↓	157.10↓
	28	12.41 ±0.007	23.94* ±0.42	34.05* ±0.425	92.79↓	174.22↓

*Indicates significant (p<0.05) and ** indicates significant (p<0.01).

Serum Cholesterol: The mean value of Serum cholesterol was 34.92±0.27, 35.47±0.23, 36.39±0.12 and36.90±0.25 (mg/dl)after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of Serum cholesterol was 41.04±0.52, 43.91±0.71, 47.40±0.07 and 53.00±0.47 (mg/dl)after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS. While in control rats the mean value of Serum cholesterol was 28.83±0.27, 30.78±0.55, 31.04±0.51 and 31.58±0.40 (mg/dl)after 7, 14, 21 and 28 days, respectively (Table 2).

Table 2. Effect of different doses of ethanolic extract of seeds of *A. squamosa* on Serum parameters.

			Treated rats		Change in %	
Parameters	Days	Control Rats Mean±SE	200mg/kg Body Weight Mean± SE	300mg/kg Body Weight Mean± SE	200mg/kg Body Weight Mean± SE	300mg/kg Body Weight Mean± SE
SGPT (IU/L)	7	55.03 ±1.49	66.69* ±0.33	72.21* ±0.15	21.18↓	31.21↓
	14	56.35 ±0.86	70.17* ±0.20	76.90* ±0.70	24.52↓	36.46↓
	21	60.16 ±0.94	84.01* ±0.64	115.1* ±1.39	39.64↓	91.32↓
	28	62.24 ±0.61	90.67* ±0.45	130.06* ±2.23	45.67↓	108.96↓
SGOT ((IU/L)	7	83.14 ±0.24	89.64* ±0.10	105.89* ±1.87	7.81↓	27.35↓
	14	82.62 ±0.24	91.93* ±0.42	115.37* ±0.08	11.26↓	39.63↓
	21	83.92 ±0.35	93.09* ±0.12	123.56* ±0.42	10.92↓	47.23↓
	28	85.80 ±0.31	94.44* ±0.21	127.56* ±0.10	10.06↓	48.66↓
Serum cholesterol (mg/dl)	7	28.83 ±0.27	34.92* ±0.40	41.04* ±0.52	21.12↓	42.35↓1
	14	30.78 ±0.55	35.47* ±0.23	43.91* ±0.71	15.23↓	42.65↓
	21	31.04 ±0.51	36.39* ±0.12	47.40* ±0.07	17.23↓	52.70↓
	28	31.58 ±0.40	36.90* ±0.25	53.01* ±0.47	16.84↓	67.85↓
Blood glucose (mg/dl)	7	118.73 ±0.52	110.01* ±0.16	102.41* ±0.54	7.83↓	15.93↓
	14	119.70 ±0.64	109.93* ±0.20	99.10* ±0.26	8.89↓	20.78↓
	21	122.14 ±0.63	108.10* ±0.27	97.73* ±0.32	12.98↓	24.97↓
	28	123.04 ±0.80	105.83* ±0.12	97.39* ±0.15	16.26↓	26.33↓

*Indicates significant (p<0.05) and ** indicates significant (p<0.01).

Blood Glucose: The mean value of Blood glucose was 110±0.16, 109.93±0.20, 108.10±0.27 and 105.83±0.12 (mg/dl) after 7, 14, 21 and 28 days, respectively when treated with 200mg/kg body weight of EEAS. The mean value of Blood glucose was 102.41±0.54, 99.10±0.26, 97.73±0.32 and 97.39±0.15(mg/dl) after 7, 14, 21 and 28 days, respectively when treated with 300mg/kg body weight of EEAS while in control rats the mean value of Blood glucose was 118.73±0.52, 119.70±0.64, 122.14±0.63 and 123.04±0.80 (mg/dl) after 7, 14, 21 and 28 days, respectively (Table 2).

DISCUSSION

The present study was undertaken to evaluate the biochemical effects of ethanolic extract of seeds of *A. squamosa*. Hematological parameters like hemoglobin content, blood cell counts (RBC and WBC), lymphocyte and neutrophils revealed significant changes due to the treatment. The lymphocyte percent decreased while the neutrophil count increased revealing the toxic nature of the extract. A significant decrease in erythrocyte (RBC) count and hemoglobin percent observed can be attributed to defective hemopoisis [7]. The decrease in hemoglobin content and RBC count can be correlated with paling of the animals, weakness and morbidity [7-9].

Significant increase in WBC count and neutrophils of treated rats can be attributed to the stimulation of immune system [10]. Mammalian neutrophils are responsible for phagocytosis and disposal of foreign materials or debris of damage tissues. The increase neutrophils may account for the removal of dead and damaged cell debris from the tissues under toxic stress of the compounds present in ethanolic extract of the seeds *A. squamosa*. An increase in the WBC count is also reported by various workers after chemical stress [11- 14].

Blood is the main pathway for xenobiotic substance after their entry into animal body, alterations in the amount of carbohydrate, blood particles and enzymes in it are of diagnostic use in a number of pathological conditions. If the cells are damaged or rendered more permeable due to toxicant, the cellular enzymes are released into the interstitial fluid and then into the blood [15]. Glutamic-oxaloacetic and glutamic-pyruvic transaminase (GOT and GPT respectively) are the two main enzyme which catalyze the transfer of an amino functional group from glutamic acid to either oxaloacetate or pyruvic acid. Release of large amount of the active enzymes into the blood stream can be detected on destruction and necrosis of liver, myocardium and kidney parenchyma. This detection is used as a diagnostic tool for tissue dysfunction [16]. Transaminase allows interplay between carbohydrate, fat and protein metabolism, the activities that occur for energy production, under stress condition [17].

An increase in SGOT is common in myocardial infraction [18], tissue damage mainly in kidney, liver and heart [19] and to increased synthesis or decreased catabolism of aminotransferase [16, 20-22]. Activity of SGPT and SGOT increases after pesticidal stress. This increase is reported to be due to some pathological changes such as necrosis of hepatocytes in liver, which causes increase in permeability of cell membrane resulting in release of transaminase in blood stream [23-25].

Cholesterol being the precursor for the steroid hormones constitutes an important physiological parameter, which may be utilized to assess metabolic effects of the pollutants. An increase in blood cholesterol level of rats on exposure to EEAS may be due to the accumulation of cholesterol as it is associated with retarded oxidative breakdown of sugar under stressed condition [26], it might be due to non-utilization of cholesterol for the synthesis of steroid hormones, it leads to its accumulation in tissues. This increase is also reported under chemical stress [27].

Carbohydrates play very crucial role in animal physiology due to its structural and metabolic activities. Carbohydrates are the main constituent of animal food and tissues and glucose is the most important carbohydrates in animal biochemistry because almost all the carbohydrates get converted into glucose for further metabolism. In blood also carbohydrates found in the form of glucose and it is the major fuel of the animal tissues. A decrease level of glucose in treated rats was due to the less consumption of food and loss of absorptive action of GIT with toxic symptoms that spend blood glucose during their action. Under stress condition animal requires more energy for metabolic as well as physiological activities.

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