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RED BLOOD CELL PICTURE AND TOTAL LEUCOCYTIC COUNT IN ALBINO MICE INTOXICATED WITH ALLIUM SATIVUM L.

(With 6 Tables & 8 Figs.)

By

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دراسات دموية على الجرزان البيضاء المتسممة بنبات الثـــــوم

محمد كـرام ، عـادل شحاتـه ، ثابت عبدالمنعـم ، منال عبد اللطيـف

تمت هذه الدراسة على عدد ٣٠٠ من الجرزان البيضاء (٢٠ ـ ٣٥ جم) لمدة ثماني أسابيع متواصلة بعد حقنها يوميا بإستخدام أنبوبة اللى المعدى بعشر الجرعة المميته المستخلصة من نبات الثوم بإستخدام الهيكسان ثم عمود الكروماتوجراف للفصل . وتم أخذ العينات اسبوعيا . وأوضحت النتائج أن صورة الدم قد تأثرت كثيرا في حيوانات التجربة والمعرضة لعشر الجرعة المميته الأمر الذى أدى إلى حدوث إنخفاض معنوى في العدد الكلى للكرات الدموية الحمراء (RBCs) لازمه إنخفاضا معنويا في نسبة الهيموجلوبين (Hb) ، مصحوبا بإنخفاض معنوى في الحجم الكلى للخلايا المضغوطة (PCV) وانخفاض معنوى لمتوسط المحتوى الخلوى للهيموجلوبين (MCV) والمتلازم مع إرتفاع معنوى في متوسط تركيز الهيموجلوبين بالخلية (MCHC) ومن هذا أستنتج أن نوع الأنيميا .

Macrocytic hypochromic anemia.

كما لوحظ أيضا أن العدد الكلى للخلايا البيضاء قد سجل إرتفاعا معنويا طوال فترة التجربة مما سبق أستخلص الباحثون ضرورة عدم الإفراط في تناول كميات كبيرة من الثوم ولوبجرعات صغيرة على فترات طويلة ومنتظمة سواء في العلاج أو أغراض التغذية والذى انعكس بدوره على صورة الدم مما يجعل لزاما علينا النصح بضرورة عمل صورة كاملة للدم بين الحين والآخر منعا للأضرار الجانبية لهذه العلاجـــــات .

SUMMARY

Haemogram findings during garlic toxicity were studied on 300 albino mice (20-53 gm). The mice were given daily 1/10 LD_{50} of crude hexane extract and its three fractions (A,B,C) for 8 weeks.

haematological results revealed a marked decrease in the total R.B.Cs, Hb concentration, PCV and MCHC, while there was a highly significant elevation in the MCV which indicates a case of macorocytic hypochromic anaemia. Also Heinz bodies were demonstrated in stained blood films of toxicated mice. Leucocytosis was evident along the period of the experiment.

INTRODUCTION

Allium species are seldom thought to have toxic effects in animals, although it has been reported that cultivated Allium cepa are toxic to cattle and horses under certain condition. (JAMES and BINNS, 1966).

Also, Allium cepa poisoning in cattle as, reported in India, had been chracterised by anemia, haemolytic effect besides the action of n-propyle disulphide which was already established (JUBB and KENNEDY, 1970). ATSUSHI, et al. (1984) stated that anemia was obvious sign in rats during general toxicity with garlic. Poisoning occured when the LD_{50} was more than 30 ml/kg and subcutaneously injected. While STEVENS (1984) recorded haemolytic anemia and deaths among sheep grazing leaves of wild garlic (Allium ursinum).

THROP and HARSHFIELD (1939) recorded anemia associated with increase MCH% in mare with Allium cepa poisoning. They revealed that the majority of the erythrocytes had been injured. Heinz bodies appeared as round, deep purple projections on the margin of the injured erythrocytes. JAMES and BINNS (1966) observed decreased packed cell volume (PCV), haemoglobin content, anorexia and marked loss of weight in sheep fed Allium validum. PIERCE and JOYCE (1972) recorded gradual decrease in PCV% started at the 4th day in horse fed Allium cepa either naturally or experimentally. KIRK and BULGIN (1979) observed low RBCs count, normal haematocrit, and increased MCV for Allium cepa-fed ewes. SMITH (1980) reported that when ruminant animals were fed on plants containing S-methylecystine sulphoxide (SMCO), severe haemolytic anaemia with decreased haemoglobin values was evident.

The present work aimed to study the haemogram of Albino mice toxicated with garlic (Allium Sativum L.).

MATERIAL and METHODS

Materials:

A total number of 300 male albino mice (20-35 grams) were used in the present study. Animals were devided into to 5 equal groups, 80 in each. The first four groups were daily inoculated by 1/10 LD_{50} of garlic crude extract (35 mg/kg. body weight), fraction A (31.25 mg/kg body weight), fraction B (25.5 mg/kg. b.w, and fraction C (22.5 mg/kg. b.w) respectively for eight weeks. The 5th group was used as control. The clinical signs were recorded. Blood samples were collected every week for a period of 8 weeks. Anticoagulated blood samples for haematological investigation (RBCs, Hb, PCV and WBCs count) were collected from the tail vein in clean, dry; sterile containers containing EDTA (1 mg/1 ml fresh blood).

Methods:

RBCs ($x10^6/mm^3$), WBCs ($x10^3/MM^3$) count and Hb content (gm%) were determined using electronic blood cell counter Cx310 (COLES, 1980).

Packed Cell Volume (PCV%) was caried out using Jantzki Microhaematocrit Centrifuge (SCHALM, et al. 1975).

Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated mathematically (COLES, 1980).

Blood films were stained by Giemsa as the technique described by SCHALM, et al. (1975) and examined microscopically.

The oral LD_{50} of crude hexan exctract and its fractions A, B, and C of garlic (Allium sativum L) against male albino mice were determined previously by ABDEL-MOHSEN (1988).

The obtained data were statistically analysed according to SNEDECOR & COCHRAN (1974).

RESULTS

The bulb of Allium sativum L. was successively extracted. The crude hexane extract was fractioned into three fractions (A, B and C) using column chromatography and screened by thin layer chromatography. No clinical signs were observed during the whole period (8 weeks) of experiments in the four compounds (crude hexane and its three fractions A, B and C). Haemtological findings in albino mice toxicated with 1/10 LD_{50} of crue extract (Allium sativum L) and its fractions (A, B and C) were presented in tables (1:6) and Figures (1:8).

DISCUSSION

Our choice of studying the toxic effect of long term toxicity for 8 weeks was dependent on the nature of exposure of livestock to garlic in agricultural field, and the possibilities of using it as a medicinal treatment for a long period.

Haematological findings of the toxicated albino mice by 1/10 LD_{50} of crude hexane extract and its fractions (A, B and C) revealed a highly significant decrease ($P/ 0.01$) of erythrocytic count (Erythropenia)-beginning at the 3rd week in case of crude extract and fraction A, and at the 4th week for both B and C fractions-till the end of experiment (8 weeks). The lowest count of erythrocyte (reached 2.89 millions/mm^3) comparing with the control (7.10 millions/mm^3) for fraction A was exsisted at the last week of experiment and 3.45 millions/mm^3 for fraction C at the 7th week of the experiments. Such results are in agreement with THORP and HARSHFIELD (1939) in mare toxicated with Allium cepa; STALLBAVMER (1980), in dog toxicated with Allium cepa and ATSUSHI, et al. (1984) who recorded a decrease of erythrocytic count in rat submited to subacute and chronic toxicity by garlic extract.

With regard to packed cell volume (PCV%) and HB concentration (gm%) in toxicated ablino mice results results revealed highly significant decrease ($P/ 0.01$) which was

correlated with the erythrocytic count all over the period of experiment. The lowest amount of packed cell volume in fraction A reached 31.50% at the 8th weeks.

The decreased Hb values beganat 5th week for fraction B and 4th week for fraction C till the end of the experiment. The same results were previously obtained by JAMES and BINNS (1966) in ewes toxicated with Allium validum, SMITH (1980) in cattle and sheep intoxicated with SMCO and ATSUSHI, et al. (1984) in rat toxicated with garlic extract.

The mean corpuscular volume (MCV/FL) revealed a highly significant increase (P/ 0.01) all over the whole period of the experiments. The highest volume of the corpuscles was 105.30 (FL) in blood of albino mice toxicated by fraction C in the 7th week (63.6 u^3 for control).

Mean corpuscular haemoglobin concentration (MCHC gm/dl) values revealed highly significant decrease (P/ 0.01) for both fraction B and C at the beginning of the 4th week of the experiment till the end of it. The lowest concentration observed in the 7th week (24.49%).

The characterestic observation during microscopic examination of blood smear was the presence of refractile stainable granules within the RBCs, the so called Heinz-Ehrilich bodies (Fig. 7 & 8). Previous authors recorded the presence of Heinz bodies in case of Allium species toxicities (THORP and HARSHFIELD, 1939; NATT and HERRICK, 1952 and SMITH, 1980).

The WBCs picture revealed a highly significant elevation in total counts (Leucocytosis), began at the first week for all components and extended till the 3rd week of the experiments. These results agreed with those previously recorded by KIRK and BULGIN (1979) in ewes toxicated by Allium cepa.

From the obtained results it appeared that with Allium sativum L. toxicity, there was a clear picture of macrocytic hypochromic anaemia that predominated in most animals specially in the last five weeks of experiments.

The persistance usage or administration of smallest dosage of garlic for treatment or for any purpose must be followed by continuous analysis of blood from time to time to avoid its bad sideeffects.

REFERENCES

Atsushi, K.; Shizutoshi, N.; Hiromichi, S.; Koji, M.; Heroshi, H.; Satorus, N.; Satoshi, D.; and Tohru, F. (1984): General toxicity of agarlic extract preparation containing vitamins (kyoleopin) Oyo yakuri, 27 (55), 909-29.

Coles, E.H. (1980): Veterinary Clinical Pathology. W.B. Saunders Comp. Philadelphia and London.

James, L.F. and Binns, W. (1966): Effect of feeding wild onions (Allium validum) to bred ewes. J. Am. Vet. Med. Ass. 149, (5): 512-514.

Jubb, K.V.F. and Kennedy, P.C. (1970): Pathology of Domestic Animals Vol. L. 2nd Academic Press, New York, London.

Kirk, J.H. and Bulgin, M.S. (1979): Effects of feeding cull domestic onion (Allium cepa) to sheep. Am. J. Vet. Res, 40 (3): 397-399.

Manal, A. Abdel Mohsen (1988): Some Toxicological Studies of Allium Sativum L. on Allbino Mice. M.V.Sc. Thesis Faculty of Vet. Med., Assiut Univ.

Natt, M.P. and Herrick, C.A. (1952): A new blood diluent for counting the erythrocytes of chicken. Poult. Sci., 31, 735.

Pierce, K.R. and Joyce, J.R. (1972): Acute haemolytic anemia caused by wild onion poisoning in horse. J. Am. Vet. Med. Ass. 160 (3): 323-325.

Schalm, O.W.; Jain, N.C. and Carroll, E.J. (1975): Veterinary Hematology 3rd ed. Lee & Febiger, Pheladelphia.

Smith, R.H. (1980): Kale poisoning: The Brassica anemia Factor Vet. Rec., 5, 12-15.

Snedecor, G.W. and Cochran, W.C. (1974): Statistical Methods. 6th Ed. Ames, Iowa, State University Press, U.S.A.

Stallbavmer, M.M. (1980): Onion poisoning on a dog. Vet. Rec. 108 (24): 523-524.

Stevens, H. (1984): Suspected wild garlic poisoning in sheep. Vet. Rec. 115 (14): 363.

Throp, F. and Harshfield, G.S. (1939): Onion poisoning in horse J. Am. Vet. Med. Ass. 94: 52-53.

Table (1): Mean value ±S.E. of erythrocytic count (Millions/ml3) of albino mice post-intubation by daily 1/10 LD50 of Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	5.50 ± 1.10	* 5.30 ± 0.30	** 4.40 ± 0.90	** 4.10 ± 0.50	* 5.25 ± 0.26	* 5.52 ± 0.45	* 5.70 ± 1.77	5.90 ± 0.49
Fraction (A)	5.95 ± 0.34	5.35 ± 0.36	** 3.57 ± 0.12	** 4.20 ± 0.29	** 5,07 ± 0.22	** 4.85 ± 0.33	** 3.97 ± 0.27	** 2.89 ± 0.27
Fraction (B)	6.27 ± 0.79	6.28 ± 0.45	* 5.28 ± 0.40	** 4.87 ± 0.31	** 4.85 ± 0.29	** 4.92 ± 0.22	** 4.89 ± 0.45	** 4.45 ± 0.19
Fraction (C)	5.56 ± 1.00	* 5.14 ± 0.86	* 5.26 ± 0.48	** 4.22 ± 0.72	** 4.52 ± 0.78	** 4.80 ± 0.51	** 3.45 ± 0.46	** 4.80 ± 0.68
Control	7.27 ± 1.06	7.40 ± 1.16	7.30 ± 1.09	7.50 ± 1.20	7.26 ± 1.00	7.30 ± 1.12	6.99 ± 0.95	7,10 ± 1.15

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. : Week

Table (2): Mean value ±S.E. of packed cell volume (%) of albino mice post-intubation by daily 1/10 LD50 of Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	40.00 ± 2.16	42.50 ± 1.73	** 37.33 ± 1.15	** 36.50 ± 1.91	** 37.50 ± 0.91	** 38.50 ± 0.70	** 38.00 ± 1.63	* 39.00 ± 1.63
Fraction (A)	43.00 ± 2.64	42.50 ± 0.58	** 33.00 ± 0.82	** 36.50 ± 2.08	** 37.50 ± 2.89	** 36.50 ± 2.38	** 32.00 ± 1.41	** 31.50 ± 1.00
Fraction (B)	42.40 ± 5.73	44.75 ± 0.96	42.00 ± 1.83	40.75 ± 0.50	** 37.25 ± 2.63	** 37.50 ± 0.71	** 36.33 ± 1.53	** 35.00 ± 0.00
Fraction (C)	** 39.00 ± 1.02	** 38.00 ± 0.42	** 38.50 ± 0.80	** 38.00 ± 0.65	** 39.00 ± 1.00	** 38.33 ± 1.15	** 36.33 ± 1.15	** 36.80 ± 2.68
Control	44.1 ± 2.22	45.02 ± 2.28	45.56 ± 2.18	44.00 ± 2.10	44.20 ± 1.95	44.00 ± 2.00	44.50 ± 2.12	45.18 ± 2.20

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. : Week

Table (3): Mean value ±S.E. of haemoglobin concentration (gm %) of albino mice post-intubation by 1/10 LD50 OF Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	16.75 ± 2.71	16.90 ± 2.83	13.30 ± 0.82	13.15 ± 1.96	14.25 ± 0.37	15.40 ± 2.16	16.20 ± 2.32	16.43 ± 0.76
Fraction (A)	16.62 ± 1.89	15.13 ± 1,11	15.38 ± 3.79	12.83 ± 1.15	12.17 ± 1.88	13.80 ± 0.86	* 13.00 ± 0.52	14.40 ± 0.45
Fraction (B)	12.90 ± 1.06	14.16 ± 1.90	14.16 ± 1.44	12.50 ± 2.39	** 11,04 ± 1.65	** 9.26 ± 0.54	** 9.27 ± 1.94	** 11,06 ± 0.93
Fraction (C)	14.25 ± 0.87	14.13 ± 1.93	13.75 ± 0.29	** 12.20 ± 0.76	** 11.70 ± 2.20	** 11.87 ± 1.05	** 9,07 ± 1.45	** 11.51 ± 0.73
Control	15.56 ± 1.52	15.85 ± 1.35	15.00 ± 1.88	15.75 ± 1.40	15.59 ± 1.55	16.02 ± 1.7	15.60 ± 1.59	15.56 ± 1.52

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. : Week

Table (4): Mean value ±S.E. of MCV picture (μ3) of albino mice post-intubation by daily 1/10 LD50 of Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	** 72.72 ± 1.63	** 80.18 ± 1.01	** 84.84 ± 1.02	** 89.02 ± 1.20	** 71.42 ± 0.58	** 69.74 ± 0.57	** 66.66 ± 1.07	** 66.10 ± 1.06
Fraction (A)	** 72.26 ± 1.49	** 79.43 ± 0.47	** 92.43 ± 0.47	** 86.90 ± 1.18	** 73.96 ± 1.55	** 75.25 ± 0.52	** 80.60 ± 0.84	** 108.99 ± 0.63
Fraction (B)	* 67.62 ± 3.26	** 71.25 ± 0.70	** 79.54 ± 1.11	** 83.67 ± 0.40	** 76.80 ± 1.45	** 76.21 ± 0.46	* 74.29 ± 0.99	** 78.65 ± 0.09
Fraction (C)	** 70.14 ± 1.01	** 75.39 ± 0.64	** 72.24 ± 0.64	** 90.04 ± 0.68	** 86.25 ± 0.89	** 79.85 ± 0.83	** 105.30 ± 0.80	** 76.66 ± 1.68
Control	60.7 ± 1.63	60.84 ± 1.60	62.41 ± 1.85	53.67 ± 1.56	60.88 ± 1.47	60.27 ± 1.56	63.6 ± 1.53	63.49 ± 1.67

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. : Week

Table (5): Mean value ±S.E. of M.CHC picture (%) of albino mice post-intubation by daily 1/10 LD50 OF Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	** 41.89 ± 2.43	39.80 ± 2.28	35.62 ± 0.98	35.75 ± 1.93	38.00 ± 0.64	** 40.00 ± 1.43	** 42.75 ± 1.97	** 43.20 ± 1.19
Fraction (A)	38.65 ± 2.26	36.79 ± 0.84	** 46.60 ± 2.30	33.78 ± 1.61	32.30 ± 2.38	37.80 ± 1.62	** 41.80 ± 0.96	** 46.87 ± 0.72
Fraction (B)	30.58 ± 3.39	31.79 ± 1.63	33.59 ± 1.63	** 30.69 ± 1.44	** 29.63 ± 2.14	** 25.99 ± 0.62	** 24.49 ± 1.73	* 31.61 ± 0.46
Fraction (C)	36.53 ± 0.94	37.18 ± 1.17	35.71 ± 0.54	** 28.71 ± 0.70	** 30.00 ± 1.60	** 29.81 ± 1.10	** 24.96 ± 1.30	* 30.37 ± 1.70
Control	35.2 ± 1.87	35.20 ± 1.80	32.92 ± 2.03	35.79 ± 1.75	35.27 ± 1.75	36.40 ± 1.87	35.05 ± 1.85	34.15 ± 1.86

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. : Week

Table (6): Mean value ±S.E. of leucocytic count (thousand) of albino mice post-intubation by daily 1/10 LD50 of Allium sativum L. extracts

Type of extracts	Time post-intubation							
	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	8th Wk.
Crude extract	** 12.65 ± 2.12	8.42 ± 1.07	7.54 ± 0.94	8.52 ± 0.80	8.27 ± 0.49	10.41 ± 2.57	7,07 ± 0.97	8.62 ± 0.62
Fraction (A)	** 15.16 ± 0.76	** 13.65 ± 2.80	** 12.00 ± 1.03	** 11.66 ± 1.15	** 12.32 ± 2.09	9.13 ± 3.43	8.94 ± 1.99	6.35 ± 0.88
Fraction (B)	** 13,05 ± 1.10	** 13.75 ± 3.19	** 16.66 ± 1.89	10,07 ± 3.37	7.34 ± 6.29	7.00 ± 0.36	8.22 ± 3.72	8.92 ± 3.66
Fraction (C)	** 14.64 ± 3.40	** 10.54 ± 1.39	** 16.00 0.56	** 10.76 ± 1.20	** 11.75 ± 1.70	9.18 ± 3.96	9.98 ± 2.57	7.33 ± 2.66
Control	6.62 ± 1.40	6.85 ± 1.35	6.40 ± 1.45	6.50 ± 1.50	6.85 ± 1.60	7.00 ± 1.85	6.95 ± 1.50	6.60 ± 1.41

* Significant at P < 0.05
** Significant at P < 0.01
S.E. : Standard error
Wk. :Week

Fig (1) Erythrocytic count (Millions/ml)

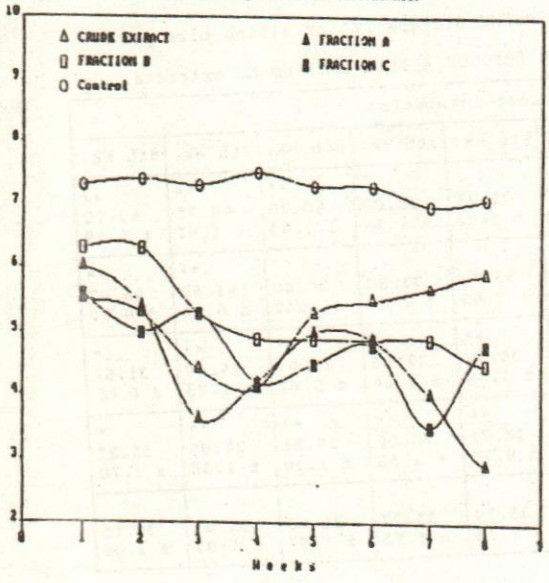


Fig (2) Packed cell volume (%)

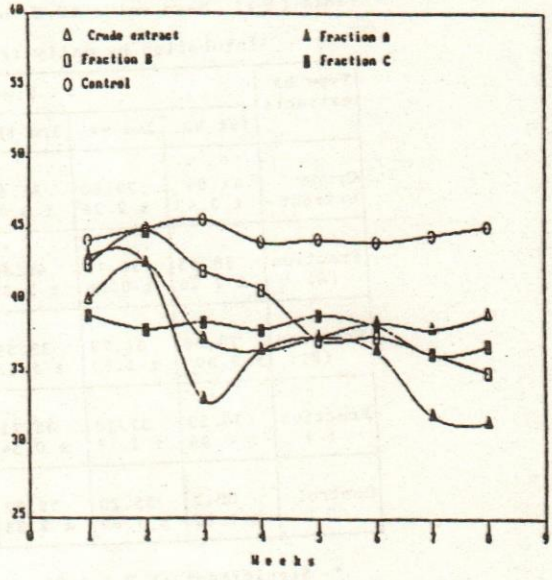


Fig (3) Haemoglobin concentration (gm %)

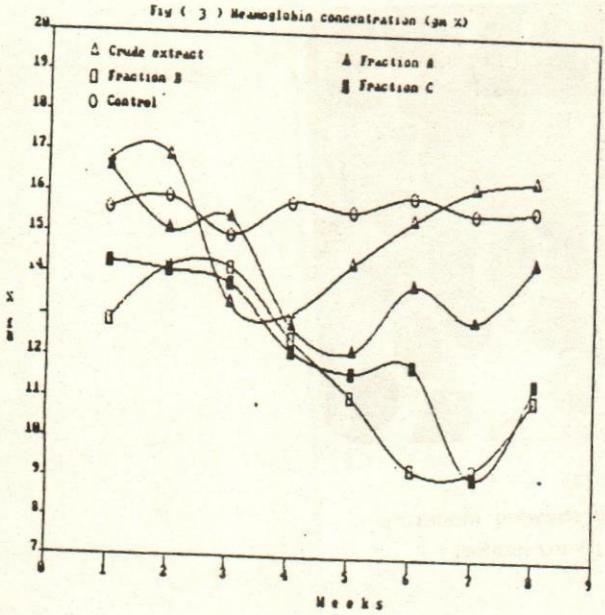


Fig (4) H C V picture (%)

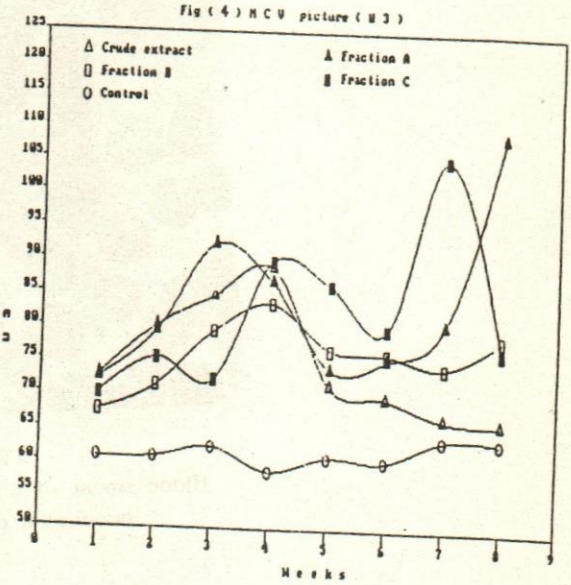
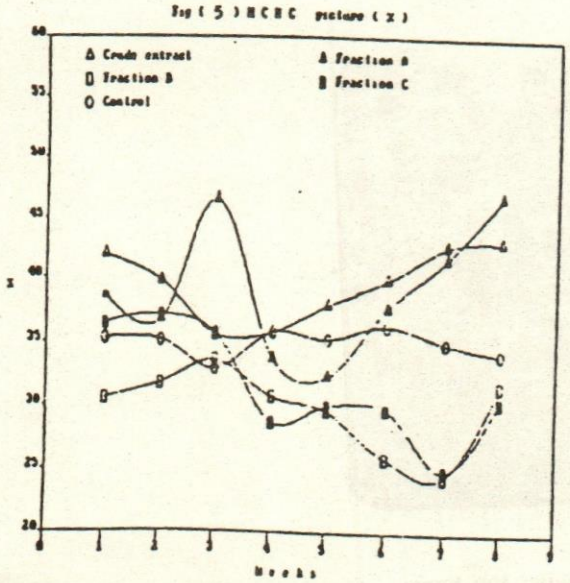
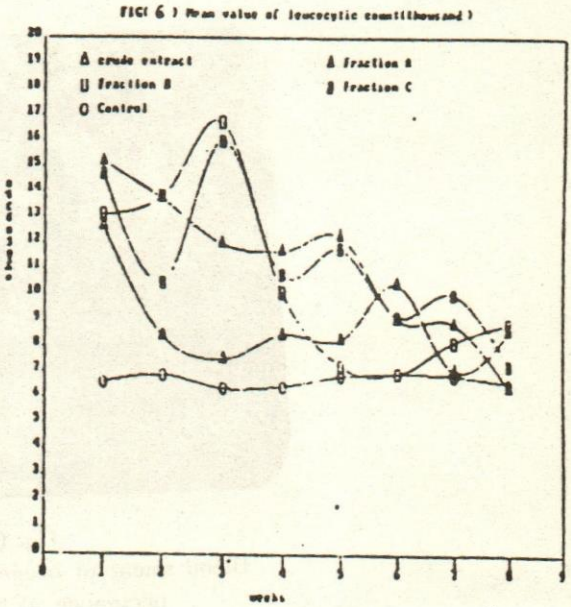


Fig (5) M C H C picture (%)



FIG(6) Mean value of leucocytic count(thousand)



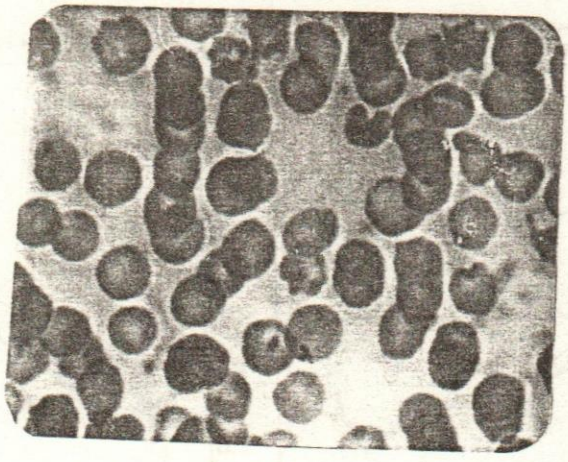


Fig. (7)
Blood smear of mice showing moderate occurance of Heinz-bodies

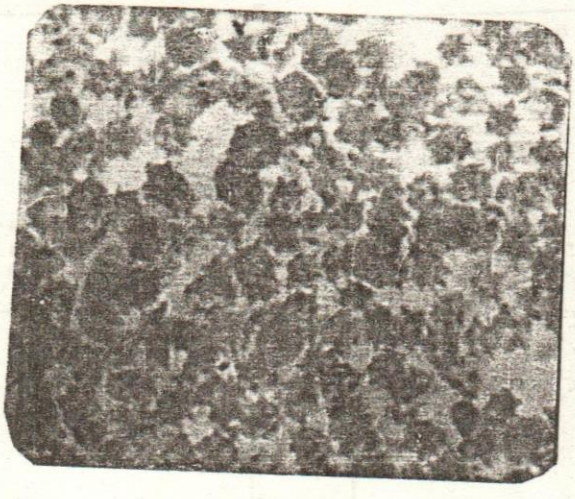


Fig. (8)
Blood smear of mice showing the heavy occurance of hienz-bodies