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CHRONIC EXPOSURE OF CAC_2 INDUCED HAEMATOTOXICITY ON ALBINO RAT

MAYURAKSHI DEKA[a] AND UMA DUTTA[b1]

[ab]Department of Zoology, Cotton College, Guwahati, Assam, India

ABSTRACT

Calcium carbide (CaC_2) is indiscriminately used as a post ripening agent in a variety of marketed fruits which causes toxicity in human and animals as it contains traces of arsenic and phosphorus. On biochemical conversion, it produces free radicals that causes oxidative stress and in turn, damages the tissues. Therefore, the present study was aimed at evaluating the toxic effect of CaC_2 on haematological picture of albino rat exposed repeatedly to two different doses of 25 mg/kg, 50 mg/kg body weight/day for a period of 90 days through oral route. The study was carried out on the basis of cytomorphological alterations of different blood cells following Wright stain. In addition, numerical level of different blood indices such as Total Leucocyte Count (TLC), Total Erythrocyte Count (TEC), Differential Leucocyte count (DLC), Haemoglobin (Hb) concentration and Packed Cell Volume (PCV) were performed following standard haemotological methods. Findings of the study showed an increase in the TLC with abnormal count of DLC whereas a gradual reduction of Hb content and TEC found in all treated groups. Cytomorphological changes of erythrocytes revealed the presence of numerous stomatocytes with central pallor, membrane deformities, macroscopic hypochromic cells, tear drop cells and Heinz bodies along with anisopoikilocytes. Various immature WBC especially ring type eosinophils and blast formation of granulocytes along with fragmented neutrophils and membrane irregularity in many cells were evident in blood film. Analysis of the present findings concluded that CaC_2 is a potent agent that can cause alterations in the blood and thus haematotoxic in nature.

KEYWORDS : Calcium carbide, Haematotoxicity, Cytomorphlogy, Albino rat

In the present scenario, many synthetic chemicals are being used in different food commodities to process and increase their production, so as to meet the needs of the growing population. Among the various food items, fruits and vegetables play an important role in our diet as they are a source of all the essential nutrients including vitamins and minerals and are also known to have antioxidant properties. However, presently many hazardous chemicals are reported to be used in fruits and vegetables for pre and post harvest ripening and maturation process. These chemicals were not only reported to lessen the nutritional content but also harmful to human health (Rahman et al., 2008, Siddiqui and Dhua, 2009). The retailers and traders use different types of artificial ripening and maturing agents to fasten these processes to earn a quick profit. A variety of these synthetic chemicals are being indiscriminately used in the markets today viz. Ethanol, Ethylene glycol, Ethephon, Methanol, Calcium carbide, Acetylene, Potassium sulfate etc (Siddiqui and Dhua, 2009). Among such synthetic ripening and maturing agents, Calcium carbide (CaC_2) is most widely used in different parts of the country even being banned under the 44AA of Prevention of Food adulteration Rules 1955 for its availability and low cost (Rahman et al., 2008). Chemically it reacts with moisture to produce acetylene, which being an analogue of natural ripening hormone- ethylene, can trigger the natural ripening process by mimicking its action. Again, acetylene upon chemical decomposition is known to produce free radicals in the body after ingestion of CaC_2 ripened fruits (Kaczmarski et al., 1999). Being highly reactive, these free radicals leads to oxidative stress in the body which in turn initiate various detrimental effects in the body like premature ageing and even DNA damage (Kaczmarski et al., 1999). In addition to the release of acetylene gas, industrial grade CaC_2 also contains traces of arsenic and phosphorus which further contributes to its toxicity (Rahman et al., 2008).

Blood is one of the specialized body fluids that regulate various vital functions of the body and transports nutrients, gases, minerals, metabolic products and hormones between tissues and organs. The routine haematological parameters provide a crucial information that help in assessing the well being of an organism. Any alterations in the blood picture both at numerical and cytomorphological level are indicative of stress or diseased conditions. As such, haematological parameters can be considered as an important diagnostic tool in health sciences. CaC_2 is known to develop toxicity in the body but its harmful effect at haematological level has not yet been thoroughly studied. Based on it, the present project was aimed at evaluating the toxicological impacts of CaC_2 on haematological pictures of albino rat after repeated

[1]**Corresponding author**

exposure at a dose of 25 mg/kg, 50 mg/kg body weight/day for a period of 90 days through oral route. The study was assessed on the basis of alterations in numerical parameters viz. TLC, DLC, TEC, Hb, PCV and cytomorphology of erythrocytes and leucocytes.

MATERIALS AND METHODS

Experimental Animals

A total of 15 healthy swiss male albino rats (strain, Sprague Dawley) were selected in the study that was procured from the Animal Stock of the Department of Zoology, Gauhati University. The animals (age; 12-14 weeks and weight; 130-140g) were housed in separate propylene cages bedded with paddy husk. They were maintained under uniform husbandry conditions of light (12hr light/dark cycle), temperature (25±2ºC) and relative humidity (52±5%) and were fed with standard rodent food diet and water ad libitum throughout the study period. Prior to the commencement of the experiment, the animals were acclimatized to the laboratory conditions for about 15 days.

Before starting the experiment, approval was taken from Institutional Ethical Committee and experiments on animals were carried out according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on animals (CPCSEA).

Experimental Design

In the experimental set up a total of 15 animals were divided into the following three groups-

Group I Control Group (Olive Oil treated): 5 male albino rats that receive olive oil (dose= 1ml/kg body weight) daily were used as standard or control.

Group II Chemical treated group (CaC_2 + olive oil): 5 male albino rats orally fed with CaC_2 at a dose of 25mg/kg body weight/ml/day for a period of 90 days.

Group III Chemical treated group (CaC_2 + olive oil): 5 male albino rats orally fed with CaC_2 at a dose of 50mg/kg body weight/ml/day for a period of 90 days.

During the study period, olive oil was used as a vehicle for oral administration of CaC_2. CaC_2 was orally fed to both the treated groups at a dose of 25mg/kg body weight and 50mg/kg body weight daily for a period of 90 days while a group of rats fed with similar quantity of olive oil and distilled water served as control. Besides a group was fed with same quantity of distilled water to check impacts of olive oil in the body, if any. As there was no variations found in both the control groups, therefore, vehicle control group have been considered as control group in the experiment. All the treatments were carried out in the morning hours (8.20 am to 9.30 am). At every 15 days interval blood was collected from control and both the treated groups for analysis of different haematological parameters.

Method of Blood Sample Collection

For collection of blood samples animals were anaesthetized with mild etherification for a short period of time. With the help of sterilized micro syringe blood was collected in vials containing EDTA after an interval of 15 days from the heart by cardiac puncture method.

Haematological Parameters

Numerical Studies

The numerical level of different haematological parameters used in the present study include Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Haemoglobin Concentration (Hb) and Packed Cell Volume (PCV). TEC and TLC were studied by Wintrobe method using a Neubaur haemocytometer (Benjamin.1970). Packed Cell Volume (PCV) was studied by Wintrobe Haematocrit method whereas haemoglobin concentration by Sahli's Haemometer (Kolmer et al., 1969) and Differential Leucocyte Count (DLC) by wright stain method.

Cytomorphological Studies

For the analysis of cytomorphological changes in the blood cells, a thin blood film was prepared from freshly collected blood, stained with wright stain and equal volume of distilled water and mounted in DPX by routine techniques. In order to examine the degree of alterations in size and shape as well as detailed cellular structures, the blood film was observed under light microscope using high magnification (X400).

Statistical Analysis

The data obtained from the above experiments were analyzed using the statistical package SPSS for windows (16.0 version). All the data were represented as mean ± S.E of the sample size. The differences were compared for statistical significance by "t- test" and at the

level of $p<0.05$ was considered as statistically significant and superscripts a,b and c were used to denote the significance level. The data were graphically represented using Microsoft Excel 2007.

RESULTS

Numerical Studies of Blood

Total Erythrocyte Count (TEC)

A gradual depletion in the TEC was noted in both the chemical treated groups throughout the exposure paradigm when compared with the control. Maximum reduction was observed in the later days of treatment i.e. 90 days of exposure. The rate of trend of depletion of TEC is higher in Group III (higher dose) than low dosed group. The variation was highly significant ($p<0.05$) in both the treated groups (25mg and 50mg) as compared to the control ones. The details of these changes are shown in table 1 and figure 1.

Total Leucocyte Count (TLC)

Chronic administration of CaC_2 at a dose of 25 mg/kg body weight and 50 mg/kg body weight showed a gradual increase in TLC which was significant at $p<0.05$ level when compared to the control group of animals. Moreover, the observed elevation in the TLC of both the treated groups occurred in a time dependent and dose dependent manner as shown in table 1 and figure 2.

Haemoglobin Concentration (Hb)

A significant ($p<0.05$) reduction in the level of haemoglobin concentration was recorded in the 25mg and 50 mg CaC_2 treated groups as compared to the control counterparts. However, maximum reduction was noted in the group of animals exposed to higher dose (50 mg group) in the later part of exposure paradigm. Moreover, the reduced trend was found to be dose and time dependent, the details of which is given in table 1 and figure 3.

Packed Cell Volume (PCV)

The detail changes in PCV levels of control and chemical treated groups are depicted in table 1 and figure 4. In the present study, a significant decrease ($p<0.05$) in the Packed Cell Volume was observed in both the treated groups exposed to both 25mg/kg body weight and 50mg/kg body weight of CaC_2. The decrease was more pronounced in the higher dosed group as compared to low dosed group

Table 1 : Showing TEC (million/µl), TLC (thousand/ µl), Hb (gm/dl) and PCV (%) in Control and CaC_2 Treated Groups of Albino Rat (Values are Mean±S.E of 5 Animals)

Sl. No	Blood Parameters	Groups	Period of Exposure						
			0	15	30	45	60	75	90
1	TEC (million/µl)	I	6.96^{a}±0.11	6.61^{a}±0.13	6.45^{a}±0.17	5.93^{a}±0.15	6.89^{a}±0.31	6.63^{a}±0.13	6.87^{a}±0.07
		II	6.55^{a}±0.08	5.51^{b}±0.21	5.47^{b}±0.11	5.13^{a}±0.12	4.26^{c}±0.29	4.91^{b}±0.18	3.21^{c}±0.10
		III	6.83^{a}±0.21	5.12^{b}±0.29	5.92^{a}±0.19	4.90^{b}±0.23	3.42^{c}±0.27	4.46^{b}±0.14	2.92^{c}±0.31
2	TLC (Thousand/µl)	I	6.88^{a}±0.10	6.78^{a}±0.27	7.14^{a}±0.29	6.32^{a}±0.23	5.38^{a}±0.11	6.80^{a}±0.31	6.12^{a}±0.07
		II	6.58^{a}±0.05	7.33^{a}±0.17	7.56^{a}±0.31	9.19^{b}±0.06	10.38^{c}±0.15	10.82^{c}±0.18	10.94^{c}±0.27
		III	6.40^{a}±0.11	9.32^{b}±0.13	8.34^{b}±0.21	10.98^{c}±0.17	11.91^{c}±0.12	11.87^{c}±0.23	12.52^{c}±0.31
3	Hb (gm/dl)	I	17.43^{a}±0.16	17.01^{a}±0.13	16.87^{a}±0.11	16.29^{a}±0.24	17.27^{a}±0.17	16.76^{a}±0.06	17.37^{a}±0.18
		II	17.52^{a}±0.05	15.02^{b}±0.09	14.17^{b}±0.18	13.73^{b}±0.25	12.40^{b}±0.03	12.14^{b}±0.02	11.22^{c}±0.17
		III	17.34^{a}±0.12	13.73^{b}±0.32	12.40^{b}±0.10	11.90^{c}±0.21	12.85^{b}±0.07	10.11^{c}±0.09	9.80^{c}±0.14
4	PCV (%)	I	36.24^{a}±0.11	36.22^{a}±0.03	35.18^{a}±0.21	36.02^{a}±0.22	35.08^{a}±0.32	35.90^{a}±0.06	36.35^{a}±0.25
		II	36.45^{a}±0.12	35.18^{a}±0.09	33.64^{b}±0.08	30.12^{b}±0.14	31.96^{b}±0.49	30.71^{b}±0.13	31.07^{b}±0.02
		III	35.96^{a}±0.21	32.56^{b}±0.51	30.76^{c}±0.12	29.78^{c}±0.18	29.17^{c}±0.33	28.67^{c}±0.52	28.60^{c}±0.13

Values having different superscripts (a,b,c) differ significantly ($p<0.05$)

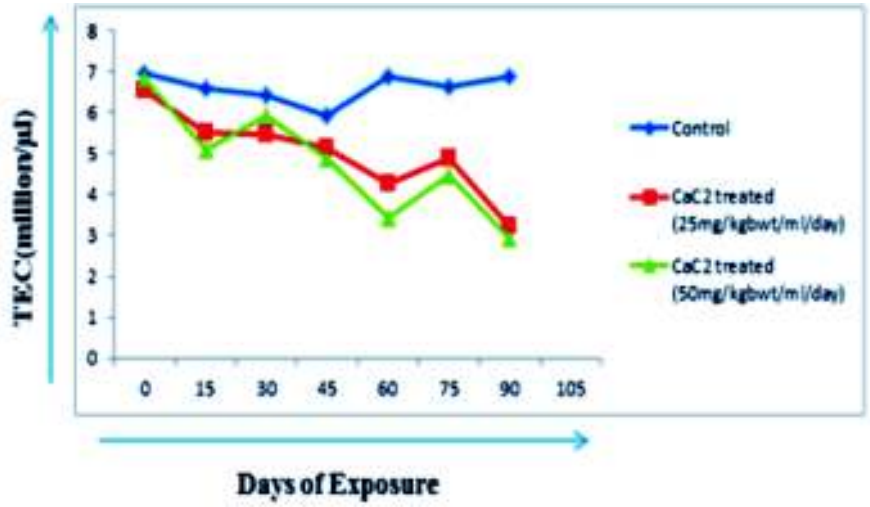

Figure 1 : Total Erythrocyte Count (TEC) (million/µl) of Control and CaC_2 Groups of Albino Rat

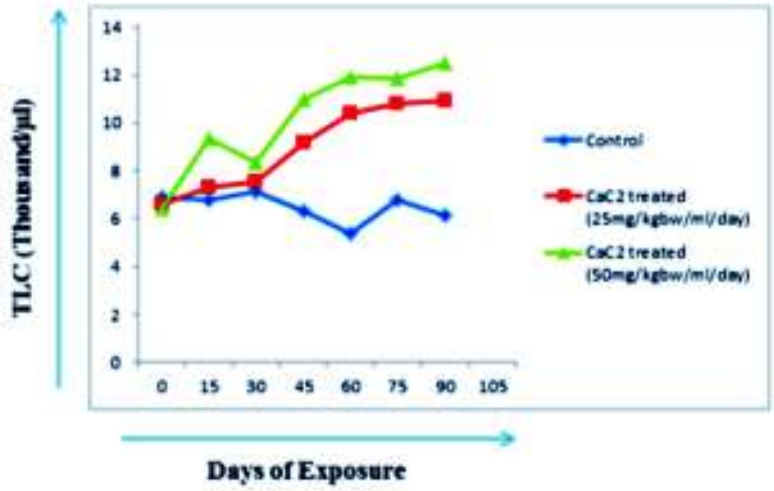

Figure 2 : Total Leucocyte Count (TLC) (Thousand/µl) in Control and CaC_2 Treated Groups of Albino Rat

and later days of exposure indicating that the variation was dependent both on time and dose.

Differential Leucocyte Count

In the present study the number of neutrophils were found to be significantly increased ($p<0.05$) in both the exposed groups as compared to the control ones (Table 2). Also, an increased appearance of immature and abnormal cells of different types of leucocytes were noted in both the treated groups which was significant ($p<0.05$) when compared to control group of rats. However, the number of lymphocytes, eosinophils and monocytes showed a decreasing trend that was significant ($p<0.05$) with the normal counterparts.

Cytomorphological Studies Of Blood

The present study revealed an abnormal variation in the cytomorphology of erythrocytes and leucocytes in the peripheral blood throughout the study period as a result of chemical exposure when compared to control group (figure 5A). Presence of large number of enlarged ringed shaped RBC as well as central slits like pallor in the cells were

observed in the peripheral blood. Again numerous deformed RBC showing different grades of membrane

Table 2 : Differential Leucocyte Count (DLC) (%) in Control and Calcium Carbide Treated Groups of Albino Rat (Values are Mean±S.E of 5 Animals)

Group	Days	Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte	Immature cells	Abnormal cells
Control		23.89^{a} ±1.16	3.20^{a} ±0.76	0.87^{a} ± 0.06	68.49^{a} ±0.76	2.95^{a} ±0.21	0.10^{a} ±0.6	0.00^{a}±0.00
CaC_2 treated (25mg/kgbw)	**0**	22.96^{a} ±.38	3.31^{a} ±0.12	0.91^{a} ±.19	69.34^{a} ±0.42	2.83^{a} ±0.03	0.65^{a} ±0.09	0.00^{a} ±0.00
	30	27.37^{b}±0.13	3.01^{a} ±0.17	1.01^{a} ±.78	60.43^{a} ±0.43	2.02^{b} ±.04	3.31^{b} ±0.24	2.85^{c} ±0.22
	60	29.06^{c} ±0.16	2.35^{b} ±0.21	0.73^{b} ±0.17	55.09^{b} ±0.11	2.01^{b} ±0.54	7.81^{c} ±0.31	2.5^{c} ±0.03
	90	31.27^{c} ±0.07	2.13^{b} ±0.14	0.61^{c} ±0.02	54.13^{b} ±0.23	1.57^{c}±0.13	6.37^{c} ±0.19	3.74^{c} ±0.11
CaC_2 treated (50mg/kgbw)	**0**	23.21^{a} ±0.56	3.45^{a} ±0.65	1.09^{a} ±0.15	68.43^{a} ±0.06	2.12^{a} ±0.26	1.7^{a} ±0.59	0.00^{a}±0.00
	30	23.01^{a} ±0.02	3.27^{a} ±0.13	1.13^{a} ±0.34	59.62^{b} ±0.24	1.98^{a} ±1.11	7.25^{c} ±0.17	3.74^{c} ±0.19
	60	26.31^{b} ±0.21	2.98^{a} ±0.05	0.89^{b} ±0.22	55.27^{b} ±0.31	1.59^{b} ±0.18	10.11^{c} ±0.04	2.85^{c} ±0.27
	90	28.78^{c}±0.39	1.23^{b} ±0.16	0.76^{c} ±0.03	48.91^{c} ±0.28	1.04^{c} ±0.14	16.72^{c} ±0.39	2.56^{c} ±0.07

Values having different superscripts (a,b,c) differ significantly ($p<0.05$)

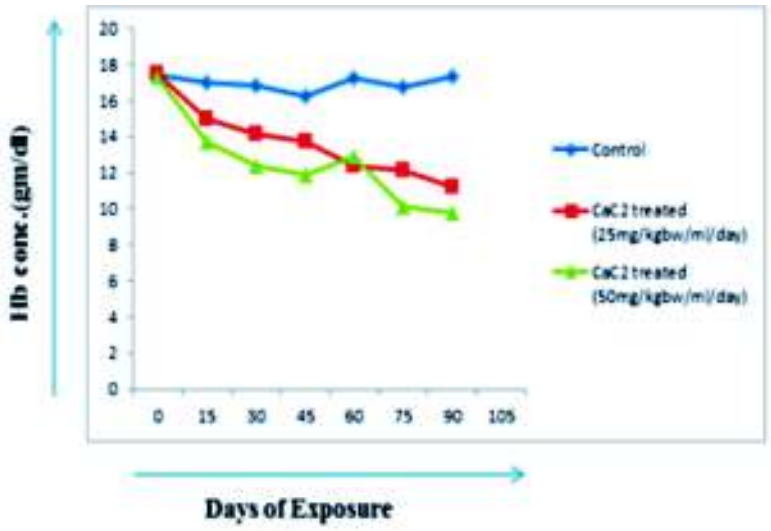

Figure 3 : Haemoglobin Concentration (gm/dl) in Control and CaC_2 Treated Groups of Albino Rat

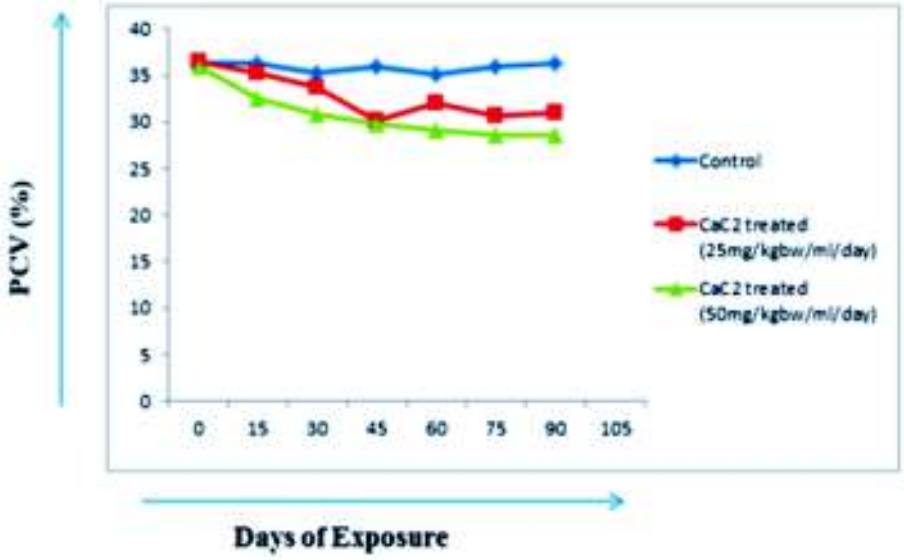

Figure 4 : Showing PCV (%) in Control and CaC_2 Treated Groups of Albino Rats

content observed in the study (Dhembare, 2014). Moreover, reduction in RBC count can also be the result of microcytic or macrocytic or dimorphic anaemia as depicted by the presence of number of ring shaped hypochromic microcytes and hyperchromic macrocytes along with presence of immature reticulocytes. The presence of reticulocytes or nucleated RBC in peripheral circulation further indicated anaemia as well as shift of marrow reticulocytes to circulating blood. The observations made in the present study are in agreement with the reports of Dhembare et al., (2011). Again, treatment with CaC_2 showed a significant reduction in haemoglobin content of the exposed rats. This CaC_2 induced effect on haemoglobin content was in consortium with earlier reports (Dhembare et al, 2011). These decreased haemoglobin concentration may be due to decreased number of RBC's, release of immature

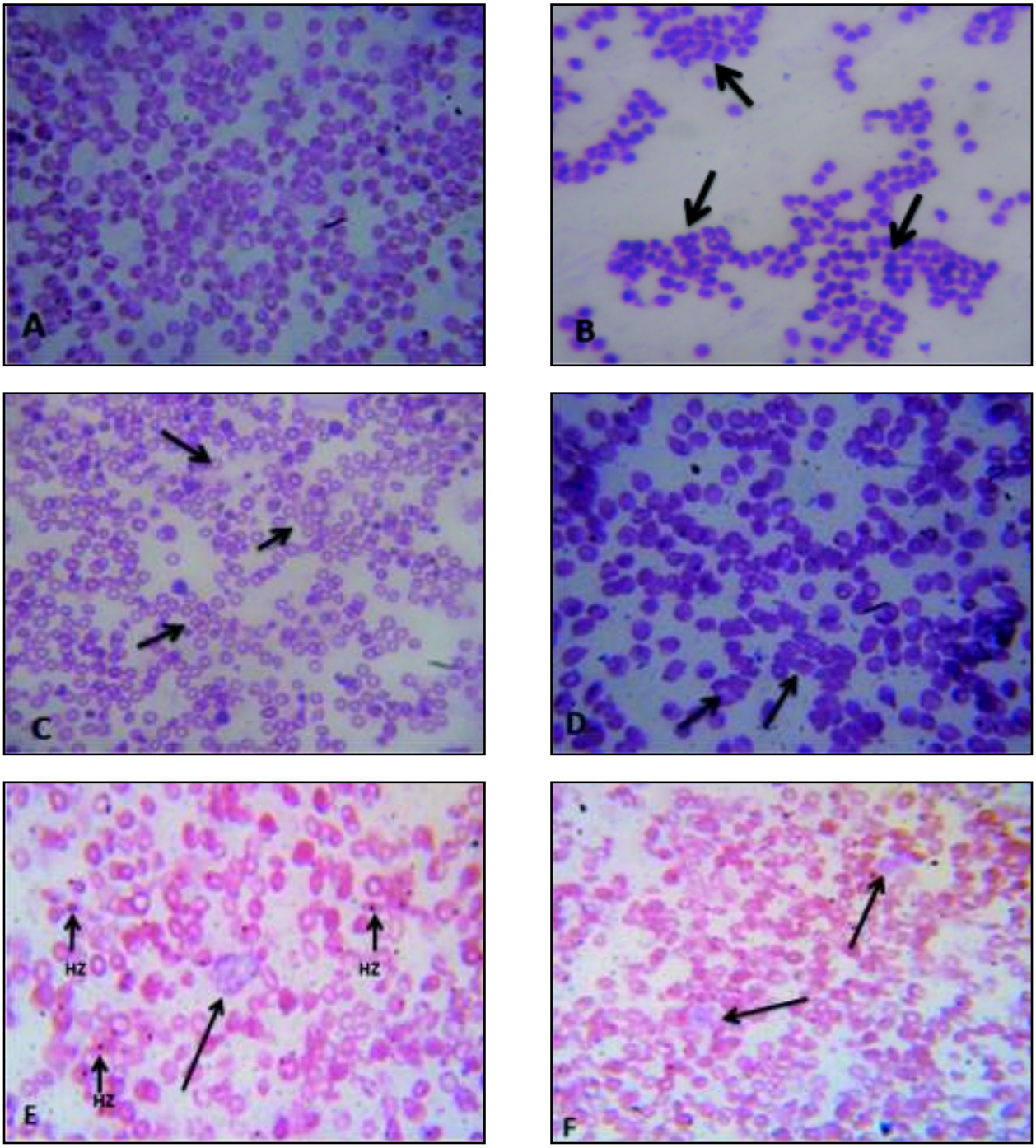

Figure 5(A-F); Peripheral blood smear of control and treated rats (50mg/kg body weight).

A; Peripheral blood smear showing the uniform blood picture from control group (X400).

B; Blood film showing the presence of hyperchromic nucleated and membrane deformed RBCs (X400).

C; Blood film showing numerous ring shaped RBCs with loss of Haemoglobin as well as presence of reticulocytes (X400).

D; Blood film showing membrane infoldings, stomatocytes formation and discocyte asymmetry after 60 days of treatment (X400).

E & F; Blood film showing leucocytes with degenerating membrane and smudge formation and also presence of Heinz bodies in RBCs (X400).

form of reticulocytes, disturbed haemoglobin synthesis or inhibition of erythropoietin production coupled with enhanced rate of erythrocyte deformities. An increase in morphological abnormalities in the circulating erythrocytes indicated destruction of mature cells which in turn may adversely affect O_2 carrying capacity of cells. This may be accounted for the release of immature forms of RBC and reticulocytes in the circulating blood to meet the demand of O_2 in the tissues.

Packed Cell Volume (PCV) is an indication of the proportion of blood volume that is occupied by Red Blood Cells (RBC). In the present study a dose and time dependent depression was observed in the PCV values. This can be attributed to total cell depletion in the blood stream caused by disturbances in steady state mechanisms of the haematopoietic tissues as well as due to hypoxic condition produced in the body after CaC_2 exposure. CaC_2 after ingestion produces free radicals which in turn produces hypoxia. Further, reduction in PCV values may be associated with chemical induced stress that causes erythropenia or lower haematocytic count associated with interference in the haematopoiesis process. Unlike RBC and Haemoglobin, WBC showed an increasing trend in CaC_2 treated groups. It is a well known fact that WBC plays a chief role in the defence system of the body. Therefore, the increase in leucocyte concentration is an indication of the immediate activation of the immune system. The increased WBC count may be due to the direct stimulating effect of the chemical on the lymphoid tissues that induces tissue damage and disturbance of the non specific immune system causing increased production of leucocytes (Promise et al, 2014). Moreover, in the present investigation a change in the DLC count of the treated rats were also reported. The number of neutrophils was also found to be increased. Neutrophils are phagocytic cells that act in acute infections. Neutrophils carry many digesting enzymes for destroying invading cells. This may be one of the causes for the rise in neutrophil count. Also, the number of abnormal and immature cells was reported to be elevated in the peripheral blood. This may be due to the direct effect of CaC_2 on the haematopoiesis process. The DLC count showed changes as compared to the control ones which can be considered as a mode of defence against the invading toxicant.

Presence of irregular membrane of erythrocytes may be due to the asymmetrical distribution of protein and lipids in the two halves of the bilayer that act as a bilayer couple and thereby respond differentially during the interaction of the chemicals that in turn leads to membrane disintegrity or disturbance of uniformity of membrane resulting in stomatocytosis. Membrane folding observed in the study may be related to the higher degree of haemolysis, splenic disorder and anaemia which was suggested by earlier scientist (Barnhart et al, 1978). Presence of abundance of abnormally large platelets with filial pseudopodia like outgrowths or projections and their aggregation in the later days of chemical treastment indicated macrocytosis with an alteration in their functional status. Release of the precursors of the granulocytes alongwith large immature leucocytes in the blood of peripheral circulation may be suggestive of serious bone marrow depletion which may be the result of CaC_2 induced toxicological stress at the bone marrow level.

CONCLUSION

Studies of detailed haematological picture clearly pronounced the toxicological effect of CaC_2 at the haematopoietic level that was manifested by the presence of immature blood cells, degeneration of cells coupled with membrane deformities in the peripheral blood picture. The present investigation clearly suggested or concluded haematotoxic nature of CaC_2.

REFERENCES

Barnhart M.I., Wallace A.M. and Lusher, J.M., 1978. Erythrocyte membrane dynamics in hereditary spherocytosis. Scan. Electron Microsc., **2**:497-510.

Benjamin MM., 1970. Outline of Veterinary Clinical Pathology. 3rd edn Kalyani publishers, New Delhi.

Dhembare A. J., Gholap A. B., and Mohite V. B., 2011. Haematological modulation in European Rabbit,

Oryctolagus cuniculus (Linn) induced by Calcium carbide, **14** (1): 153-56.

Dhembare A. J., 2014. Haematological alterations in European rabbit, Oryctolagus cuniculus (Linn.) exposed to ethereal. Adv. in Appl. Sci. Research, **5** (3) : 59-63.

Kaczmarski M. J., Wojicicki L., Samochowiee T., Dutkiewicz and Sych Z., 1999. The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. Pharmazie, **54**: 303-06.

Kolmer J.A., Spaulding E.H. and Robinson H.W., 1969. Approved Laboratory Techniques 5th edn. Scientific Book Gency, Calcutta : 50-60.

Promise N., Agomuo E.N., Uloneme G. C., Egwurugwu J.N., Omeh Y.N., and Nwakwuo G. C., 2014. Effect of *Phyllanthus amarus* leaf extract on alterations of haematological parameters infested wister albino rats, **9** (1): 7-12.

Rahman A., Chowdhury F.R. and Alam M.B., 2008. Artificial ripening : What we are eating. J. Med., **9** : 42-44.

Siddique M. W. and Dhua R. S., 2009. Standardization of ethrel treatment for inducing ripening of mngo var 'Himsagar', in proceedings of International Conference on Horticulture (ICH-2009), Bangalore, **16** : 41-48.

Uboh F. E., Akpanabiatu M. I., Alozie Y., Edet E. E., Ndem J. I. and Ebong P. E., 2009. Comparative effects of vitamin A and E on gasoline vapours- induced haematotoxicity and weight loss in male rats. International Journal of Pharmacology, **5** (3): 215-221.