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Study of subchronic exposure of ethephon induced cytomorphological and numerical alterations in the blood picture of albino rat.

Mayurakshi Deka and **Uma Dutta**
Department of Zoology, Cotton College, Guwahati, India

Abstract

Ethephon ($C_2H_6ClO_3P$) (ETP) is indiscriminately used as a pre and post-harvest ripening agent in a variety of marketed fruits. The mode of action of ripening by ETP is mediated on liberation of the natural plant ripening hormone-ethylene. ETP also belongs to the class of organophosphorus pesticides. Under this background, the present study was undertaken to evaluate the toxic effect of ETPin the haematological picture of albino rat exposed repeatedly to two different doses of ETP viz. 25 mg, 50 mg/kg body wt./day for a period of 90 days through oral administration. The study was carried out on the basis of cytomorphological and numerical alterations of different blood cells. Cytomorphological and numerical level of different haematological indices such as TLC (total leucocyte count), DLC (differential leucocyte count), TEC (total erythrocyte count), Hb (Haemoglobin) content and PCV (packed cell volume) were performed following standard methods.

Findings of the present study showed a progressive increase in the TLC with an abnormal count of DLC in chemical treated groups compared to their normal counterparts. However, a gradual depletion of Hb content, PCV and TEC were found in all the treated groups. Cytomorphological changes of disc shaped erythrocytes manifested numerous stomatocytes appearance with central pallor, discocyte asymmetry and various forms of membrane deformities, macroscopic hypochromic ring and teardrop-shaped cells. Appearance of Heinz bodies in the erythrocyte was another marked feature of cellular abnormalities. Various immature WBC like ring type eosinophils and blast formation of granulocytic series, fragmented neutrophil along with membrane irregularity in many white blood cells were also evident in the peripheral blood film.

Analysis of the present findings concluded that ETP is a potent agent that can cause alterations in the blood picture at cytomorphological and numerical level. Therefore, it establishes the haematotoxic nature of the chemical.

Keywords: Ethephon, Haematotoxicity, Cytomorphlogy, Albino rat.

1. Introduction

Ripening of fruits can be considered as the final stage of maturity during which it undergoes physiological changes and becomes more edible. In nature, this process is regulated by the natural plant hormone ethylene. However, in the present scenario, many synthetic chemicals are being used to hasten the ripening process by retailers and traders in order to meet the increasing demand of people as well as to earn a quick profit (Rahman *et al.*, 2008 and Asif, 2012). Most of these chemicals have been reported to be health hazardous (Medlicott *et al.*, 1987; Rahman *et al.*, 2008 and Siddiqeui, 2008). Presently, ethanol, ethylene glycol, ethephon, methanol, calcium carbide,

Corresponding author : umadutta@yahoo.com, dekamayurakshi@gmail.com

acetylene gas, potassium sulfate etc. are being indiscriminately used in fruits and vegetables for commercial benefits (Chace, 1934; Nagel, 1989; Goonatilake, 2008; Siddiqui and Dhua, 2009 and Singal *et al.,* 2012). This has raised much concern in many parts of the world especially in developing countries like India, Bangaladesh etc. Among such artificial ripening and maturing agents, ethephon (ETP) is considered to be less toxic and so is preferred and recommended compared to other agents. ETP [2-Chloroethylphosphonic acid ($C_2H_6ClO_3P$)] commonly known as ethereal is used both as a pre and post harvest ripening and maturing agent mainly in the ripening of mango, pineapple, coffee, tomato, watermelon, capsicum, cauliflower cucumber, groundnut and rubber (Dhembare, 2014). It is an organic based inorganic substance and is one of the most commonly used ethylene generating chemicals.Basically, ETP is a plant growth regulator that can regulate different phases of growth and development of plant by application to different growth areas (Kidd and James, 1991). The mode of action of ETP is mediated by liberation of the natural plant ripening hormone, ethylene on chemical conversion (Montogomery, 1993 and Asif, 2012). However, ETP also belongs to the class of organophosphorus pesticides which are known to be environmental carcinogens and mutagens (El-Fiky *et al.*, 1992; Rahman *et al.*, 2002 and Nada, 2006). Organophosphorus pesticides are normally known to react with DNA as alkylating agents and also affect several other biochemical pathways (Waters *et al.*, 1980 and Das *et al.*, 2006). Besides, when indiscriminately used in fruits and vegetables ETP can cause some health related disorders in human such as diarrhea, nausea, vomiting, cramps, slow heart beat, eye and skin irritation, accelerate breathing etc (Reese, 1972 and Dhembare, 2014).

Blood is the most important body fluid that regulates various vital functions of the body (Baynes and Dominiczak, 2006). Blood is easily accessible and is considered to be the first indicator of any pathological conditions of the body. It provides crucial information for assessing the well being of an organism. Therefore, the routine haematological parameters can be considered the most important diagnostic tool in health sciences. As ETP belongs to the category of organophosphorus pesticides and is known to have some neurotoxic, mutagenic and terratogenic effects; but its toxic effects on the haematological parameters has not yet been thoroughly studied. Therefore, in the present investigation, a rodent model, i.e. albino rat was used as an experimental model to assess the toxicity level of ETP on the haematological parameters considering two different doses i.e. 25mg/kg body weight and 50 mg/kg body weight for a period of 90 days through oral administration.

2. Materials and methods

2.1 Experimental animals

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

The approval for conducting experiments on animals was taken from Institutional Ethical Committee (No-902/ac/05/CPCSEA/4/12)before starting the experiment. The experiments on animals were carried out according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on animals (CPCSEA).

2.2 Chemical materials

Ethephon (ETP) was the test substance whose chemical formula is $C_2H_6ClO_3P$ and structure is-

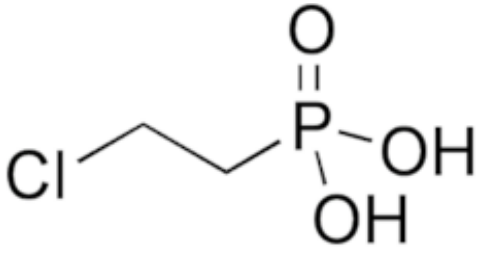

Fig 1 : Structure of ethephon

ETP was obtained from Loba Cheme, Mumbai. Other

reagents and chemicals like Ethanol 95% alcohol were purchased from Hi media, Mumbai, India; Chloroform, formaldehyde, glacial acetic acid and diethyl ether were purchased from Merck, (Germany); Haemotoxylin and Eosin were procured from Sigma, USA and Paraffin (No. 19215, congealing point 58^0C-69^0C, purity 99.5%) were purchased from Qualigens, Mumbai.

2.3 Experimental design

A total of 15 animals were divided into the following three groups in the experimental set up-

Group I (Control Group): 5 male albino rats fed with normal diet and distilled water served as standard or control.

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

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

During the study period, ETP was orally fed to both the treated groups at a dose of 25mg/kg body weight (bw) and 50mg/kgbw daily for a period of 90 days, while the rats fed with similar volume of distilled water served as control. 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 both the treated and control groups for analysis of different haematological parameters without sacrificing them.

2.4 Method of blood sample collection

The animals were anaesthetized with mild etherization for about 3 to 5 minutes for collection of blood samples. With the help of sterilized micro syringe (20 gauze needle) about 1ml of blood was collected from the heart after every 15 days interval of the days of treatment by cardiac puncture method. After collection of blood, the animals were provided with normal salt solution for supplementation after regaining consciousness. The collected blood was immediately transferred into sterilized vials containing an anticoagulant EDTA at a concentration of 1mg:5ml of blood.

2.5 Haematological parameters

2.5.1 Numerical studies

In the present study different haematological parameters were used which include total leucocyte count (TLC), differential leucocyte count (DLC), total erythrocyte count (TEC),haemoglobin concentration (Hb) and packed cell volume (PCV). TEC and TLC were counted by wintrobe method using a Neubaur chamber in haemocytometer (Benjamin,1970 and Jain, 1986), Packed Cell Volume (PCV) was done by Wintrobe Haematocrit method, haemoglobin concentration by Sahli's Haemometer (Kolmer *et al.,* 1969 and Medway, 1973) and differential leucocyte count (DLC) was done by wright stain.

2.5.2 Cytomorphological studies

A blood film was prepared from freshly collected blood, stained with wright stain and mounted in DPX for cytomorphological analysis. After that the slide was observed under light microscope using high magnification (X400) in order to examine the degree of variation in size and shape as well as detailed cellular structures and morphological deformities.

2.6 Statistical analysis

The statistical package SPSS (16.0 version) was used for analyzing the collected data. All the data were represented as mean ± S.E of the sample size. The significant differences between groups were tested by "t- test" and the probability level $p<0.05$ was considered as statistically significant. Appropriate superscripts were incorporated in the table to denote significant differences. Also the data were graphically represented using Microsoft Excel 2007.

3. Results

3.1 Numerical studies of blood

3.1.1 Total erythrocyte count

A gradual reduction in TEC was noted throughout the experimental period. However, the reductional pace was more pronounced in the groups exposed to higher dose and more depletion was also noted in the later days of treatment especially 90 days of exposure. The variation was 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 fig 2A.

3.1.2 Total leucocyte count (TLC)

Table 1 : Showing total erytrocyte count (million/µl) in control and ETP treated groups of albino rat (values are mean±S.E of 5 animals)

Time (days)	Control	ETP treated (25mg/kg BW)	ETP treated (50mg/kgBw)
0	6.71^{a}±0.17	6.68^{a}±0.13	6.43^{a}±0.27
15	6.29^{a}±0.12	6.03^{a}±0.07	5.09^{b}±0.31
30	6.67^{a}±0.09	5.86^{a}±0.15	4.78^{b}±0.14
45	6.41^{a}±0.19	5.49^{a}±0.11	4.90^{b}±0.32
60	5.96^{a}±0.21	5.14^{b}±0.32	3.89^{c}±0.27
75	6.53^{a}±0.31	4.95^{b}±0.23	3.64^{c}±0.13
90	6.84^{a}±0.08	3.98^{c}±0.21	2.08^{c}±0.37

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

A significant (p<0.05) gradual increase in TLC were observed on chronic administration of ETPat a dose of 25 mg/kg body weight (ranges 6.51±0.07 to 10.81±0.31 thousand/µl) and 50 mg/kg body (ranges 6.73±0.17 to 12.42±0.32 thousand/µl) when compared to the control group (ranges 5.80±0.25 to 7.34±0.21thousand/µl). Besides, the rate of elevation observed in the TLC of both the treated groups occurred in a time dependent and dose dependent manner as shown in table 2 and fig 2B.

3.1.3 Haemoglobin concentration:

Table 2 : Showing total Leucocyte Count (Thousand/µl) in control and ETP treated groups of albino rat (values are mean ± S.E of 5 animals)

Time (days)	Control	ETP treated (25mg/kg BW)	ETP treated (50mg/kgBw)
0	6.69^{a}±0.16	6.51^{a}±0.07	6.73^{a}±0.17
15	6.73^{a}±0.24	7.53^{a}±0.27	9.42^{b}±0.19
30	7.34^{a}±0.21	7.69^{a}±0.33	8.85^{b}±0.29
45	6.46^{a}±0.29	9.04^{b}±0.19	10.71^{c}±0.11
60	6.38^{a}±0.09	9.98^{b}±0.28	11.97^{c}±0.31
75	5.80^{a}±0.25	10.36^{c}±0.11	11.57^{c}±0.23
90	6.60^{a}±0.12	10.81^{c}±0.31	12.42^{c}±0.32

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

Chronic administration of ETP at a dose of 25 mg/kg body weight and 50 mg/kg body weight showed a significant ($p<0.05$) reduction in the haemoglobin concentration as compared to the control counterparts. Maximum reduction was noted in the 50 mg group in the later days of exposure period i.e. 90 days which showed that the reduction was dose and time dependent. The details of the changes in the numerical value is given in table 3 and fig 2C.

Table 3 : Showing haemoglobin concentration (gm/dl) in control and ETP treated groups of albino rats (values are mean±S.E of 5 animals)

Time (days)	Control	ETP treated (25mg/kg bw)	ETP treated (50mg/kgbw)
0	$17.13^{a}\pm0.11$	$17.39^{a}\pm0.06$	$17.54^{a}\pm0.13$
15	$17.51^{a}\pm0.07$	$15.62^{a}\pm0.08$	$13.63^{b}\pm0.36$
30	$16.97^{a}\pm0.09$	$14.43^{b}\pm0.17$	$11.81^{c}\pm0.16$
45	$16.79^{a}\pm0.21$	$13.83^{b}\pm0.28$	$11.95^{c}\pm0.28$
60	$17.47^{a}\pm0.27$	$12.78^{b}\pm0.13$	$12.61^{b}\pm0.17$
75	$16.76^{a}\pm0.16$	$12.04^{c}\pm0.32$	$10.41^{c}\pm0.25$
90	$17.31^{a}\pm0.13$	$11.22^{c}\pm0.16$	$9.89^{c}\pm0.21$

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

3.1.4 Packed cell volume (PCV)

The detailed changes in the PCV are depicted in table 4 and fig 2D. In this study, the packed cell volume showed a significant decrease ($p<0.05$) in both the treated groups exposed to 25mg/kgbw and 50mg/kgbw of ETP. Although, the reduction was more pronounced in the higher dosed group and later days of exposure paradigm indicating the dose and time dependent nature of the variation.

Table 4 : Showing packed cell volume (%) in control and ETP treated groups of albino rats (values are mean ± S.E of 5 animals)

Time (days)	Control	ETP treated (25mg/kg bw)	ETP treated (50mg/kgbw)
0	$36.46^{a}\pm0.02$	$36.13^{a}\pm0.13$	$36.09^{a}\pm0.03$
15	$36.72^{a}\pm0.09$	$35.48^{a}\pm0.29$	$33.56^{b}\pm0.52$
30	$36.18^{a}\pm0.01$	$34.84^{a}\pm0.18$	$31.46^{c}\pm0.39$
45	$35.72^{a}\pm0.12$	$32.72^{b}\pm0.35$	$30.48^{c}\pm0.23$
60	$36.08^{a}\pm0.22$	$32.96^{b}\pm0.49$	$29.47^{c}\pm0.31$
75	$35.96^{a}\pm0.05$	$31.89^{c}\pm0.37$	$28.87^{c}\pm0.21$
90	$36.05^{a}\pm0.15$	$31.43^{c}\pm0.27$	$28.75^{c}\pm0.51$

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

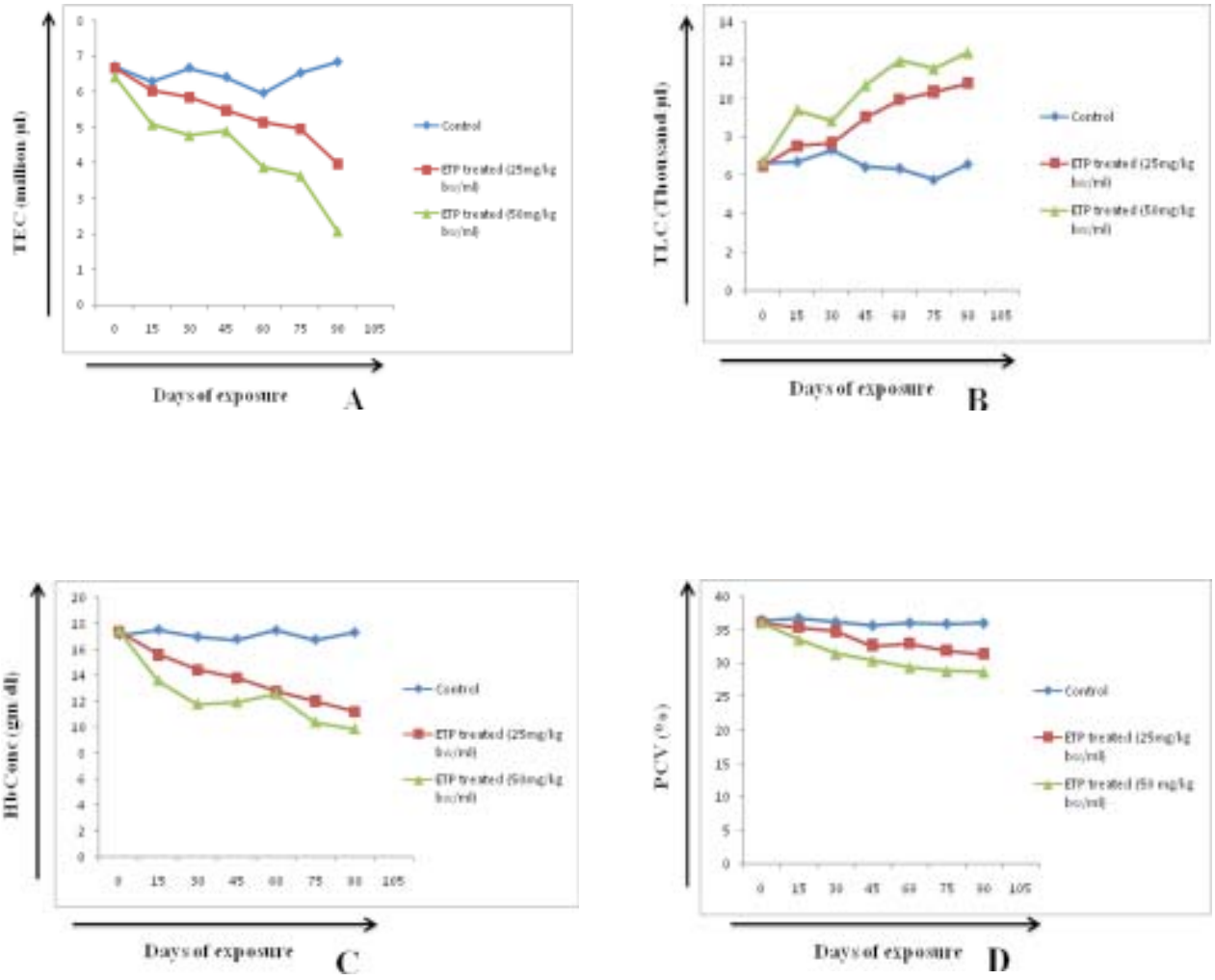

Fig 2 (A-D) : Showing different haematological parameters in control and ETP treated animal groups

A- Total Erythrocyte Count (TEC)

B- Total Leucocyte Count (TLC)

C-Haemoglobin concentration

D- Packed Cell Volume

3.1.5 Differential leucocyte count

A significant ($p<0.05$) increase in neutrophils count of both the ETP exposed group was well marked in the present findings as compared to the control ones. Besides, increased number of abnormal and immature cells of melocyte and metamyelocyte series were another marked feature in both the treated groups, although these changes in number were more pronounced in the groups exposed to high dose than those exposed to low dose or control group. On the other hand, the number of lymphocytes, eosinophils and monocytes showed a significant ($p<0.05$) decreasing trend without showing any significant variation in basophil number. The details of these variations are shown in table 5.

Table 5 : Showing differential leucocyte count (DLC) (%) in control and ETP exposed groups of albino rats (values are mean ± S.E of 5 animals)

Group	Days	Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte	Immature cells	Abnormal cells
Control		23.70^{a}±1.09	3.89^{a}±0.56	0.49^{a}± 0.09	68. 87^{a}±1.13	2.75^{a}±0.27	0.30^{a}±0.16	0.00^{a}±0.00
ETPtreated (25mg/kgbw)	0	23.31^{a}±0.18	3.51^{a}±0.17	0.71^{a}±.09	68.74^{a}±0.22	2.98^{a}±0.13	0.75^{a}±0.02	0.00^{a}±0.00
	30	27.07^{b}±0.33	2.95^{a}±0.10	1.01^{a}±.48	58.43^{c}±0.53	2.32^{a}±0.31	4.37^{c}±0.21	3.85^{c}±0.12
	60	28.11^{c}±0.19	2.42^{b}±0.03	0.78^{a}±0.11	54.35^{c}±0.17	2.04^{b}±0.34	7.80^{c}±0.41	4.50^{c}±0.13
	90	30.21^{c}±0.27	1.87^{c}±0.19	0.61^{a}±0.12	51.19^{c}±0.33	1.47^{b}±0.10	8.71^{c}±0.39	5.94^{c}±0.31
ETPtreated (50mg/kgbw)	0	23.33^{a} ±0.16	3.34^{a}±0.25	1.07^{a}±0.19	68.48^{a} ±0.02	2.87 ^{a}±0.21	0.91 ^{a}±0.39	0.00^{a} ±0.00
	30	26.11^{b} ±0.09	2.47^{b} ±0.03	1.05^{a} ±0.23	56.48^{c}±0.44	1.88 ^{b}±1.01	7.42 ^{c}±0.57	4.59^{c} ±0.15
	60	29.37^{c} ±0.27	2.08^{b} ±0.13	0.83^{a} ±0.02	52.20^{c} ±0.41	1.46^{b}±0.28	9.21^{c}±0.41	4.85^{c} ±0.37
	90	31.08 ^{c}±0.39	1.51^{c}±0.16	0.71^{a} ±0.03	49.58^{c}±0.28	1.14^{c} ±0.14	10.62 ^{c}±0.39	5.36^{c}±0.07

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

3.2 Cytomorphological studies of blood

The present study revealed an abnormal alteration in the cytomorphology of the erythrocytes and leucocytes throughout the period of exposure paradigm due to chemical induced toxic insultation. Many abnormal variations in the morphology of RBC and WBC were observed in the peripheral blood picture when compared to the control counterparts showing uniformity (Fig. 3A). Numerous RBCs showing different types of deformities such as irregular shaped or discocyte asymmetry, some cells with central pallor like slits, Maxican Hat shaped, Triangular shaped RBC as well as presence of some fragmented RBC were found in the peripheral blood film in both the ETP treated groups (Fig. 3B, D and E). Appearance of an innumerable number of ring shaped RBC along with number of tear drop cells, membrane infoldings and appearance of Heinz bodies were another well marked alterations observed in the RBC of treated groups (Fig. 3B and C). These changes however varied in a time and dose dependent manner. In the later part of exposure paradigm, number of reticulocytes and nucleated RBCs were also observed in the peripheral blood film (Fig. 3D). Besides, the appearance of numerous abnormal and immature myelocytes and metamyelocytes and ring shaped eosinophils were noted in the blood film of chemical exposed rats. Membrane deformities of various grades in different leucocytes were also observed in the treated groups. In the later days of treatment in both chemical treated groups, the leucocytes were basket shaped in appearance called as smudge due to presence of foamy cytoplasm and irregular membrane with cytoplasmic extensions (Fig. 3F). These changes were very much pronounced in the groups exposed to high dose than those groups exposed to lose dose. Again, appearance of hyperchromic and fragmented neutrophils associated with different grades of degenerative changes were observed in the blood film of the chemical exposed animals.Further, appearance of abnormally shaped large sized platelets were found to be aggregated in the blood smear. Platelets also showed changes in their shapes from normal disc shaped to swollen sphere with the appearance of irregular cytoplasmic processes protruding out from the cells. The projections appeared as fine threads that shoot out radially in all directions or may appear as long filial pseudopodia like structure from one end of the cell.

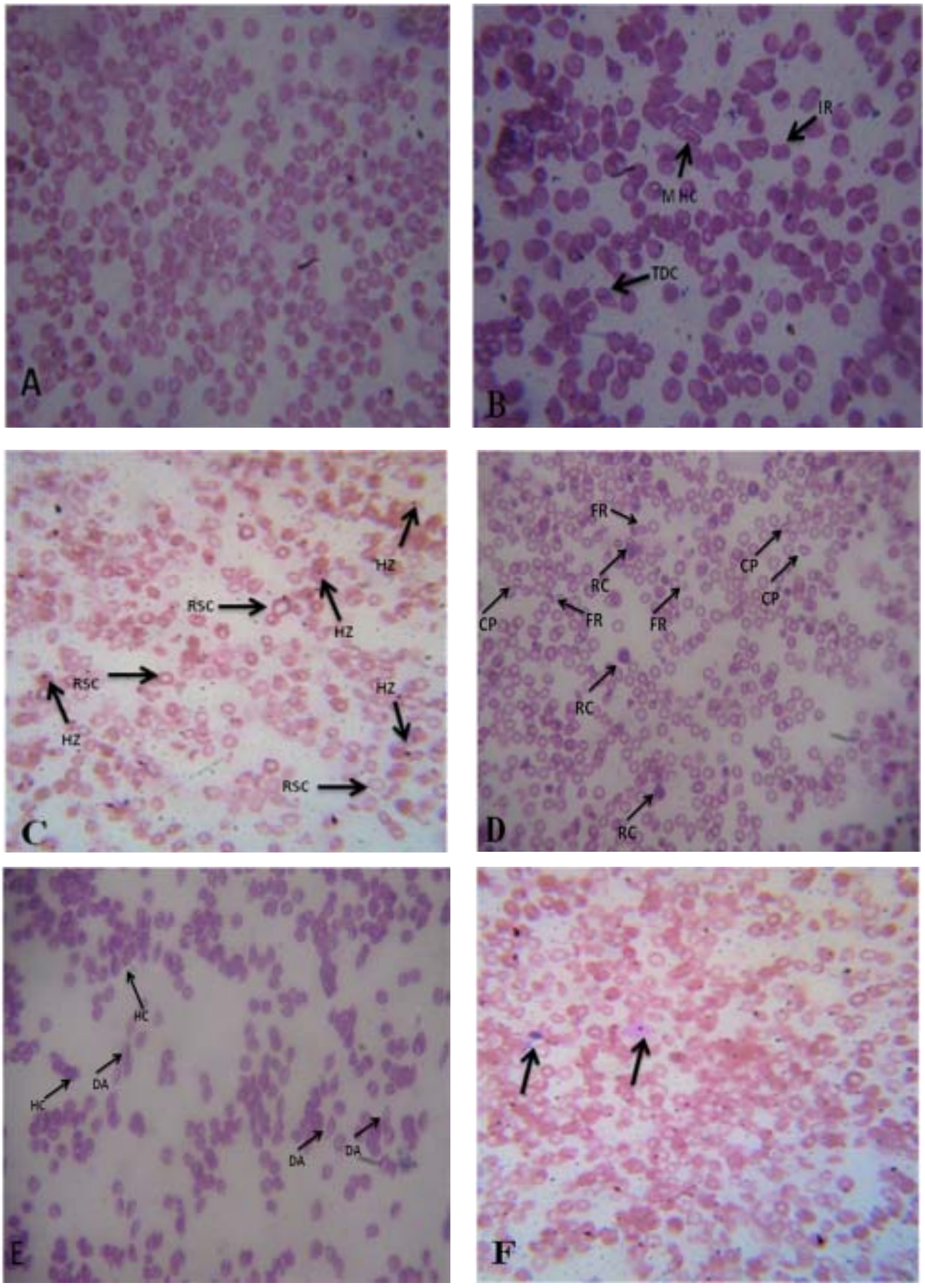

Fig 3 : A: Peripheral blood smear showing the uniform blood picture of control groups.

B-F: Peripheral blood smear of ETP exposed groups (50mg/kgbw group) (90days) showing numerous deformities in RBC and WBC.

B : showing presence of Maxican Hat shaped cells (MHC), tear drop cells (TDC) and irregular shaped cells (IR) (X400).

C : showing numerous ring shaped cells (RSC) and presence of Heinz bodies (HZ) (X400).

D : showing reticulocytes (RC), cells with central slit like pallor (CP) and fragmented cells (FR) (X400).

E : showing discocyte asymmetry (DA) and hyperchromic cells (HC) (X400).

F : showing leucocytes with degenerating membrane and smudge formation (X400).

4. Discussion

Blood is the most important body fluid that plays an important role in various functions of the body. However, the haematological parameters are found to be affected by various factors such as environmental, physiological, stress, chemical intoxication, bacterial infection etc (Uboh *et al.*, 2009 and Savithri *et al.*, 2010). As such, haematological indices are an important indicator of the pathophysiological status of the whole body and also helps to confirm the toxic nature of the administered chemical (Promise *et al.*, 2014). Therefore, in the present investigation different haematological indices were studied to test the toxicity level of ETP on albino rat.

A significant reduction in the number of RBCs in both the groups of ETP exposed rats were reported in the present study which may indicate the toxic effect of the chemical in the bone marrow resulting in the reduced rate of erythropoeisis. Similar kinds of changes were reported earlier in animals exposed to organophosphorus pesticides (Dutta *et al.*, 1992).The reduced number of RBC count may also be on account of microcytic or dimorphic anaemia which was confirmed in the study by the appearance of number of ring shaped hypochromic microcytes and hyperchromic macrocytes along with presence of immature reticulocytes in the peripheral blood film of treated rats. These present findings were in consortium with the earlier reports (Eisler *et al.*, 1966 and Dhembare, 2014). Further, a gradual depletion in the haemoglobin content of blood was noted throughout the experimental paradigm. This was associated with the decreased count of RBC that indicated anaemic condition. The depleted haemoglobin concentration may be due to disturbed haemoglobin synthesis, decreased number of RBC's, release of immature form of reticulocytes, or inhibition of erythropoietin production coupled with enhanced rate of erythrocyte deformities (Kumar and Reddy, 1997 and Srivastava and Srivastava, 2002). A dose and time dependent reduction was observed in the PCV values during the present investigation which might have occurred due to hypoxia produced in the body on chemical conversion after exposure to ETP or may be attributed to the depletion in the total number of cells in the blood stream caused by disturbances in steady state mechanisms of the haematopoietic tissues (Dhembare, 2014). Besides, chemical induced stress causing erythropenia or lower haematocytic count, loss of electrolyte, impaired osmoregulation and interference of the chemical with the haematopoietic tissues may be the other factors related to depletion in PCV levels(Grizzle, 1977; Wedemeyer and Leay, 1981; and Srivastava and Srivastava, 2002).

On the other hand, WBC showed an increasing trend in their number in the ETP treated groups. As WBC is known to play an important role in the defense mechanism of the body,its increase can be considered as an indication of the immediate activation of the body's immune system (Chandra *et al.*, 2005). Moreover, the rate of increase in WBC count also depends on the severity of the causative stress of the chemical that finally resulted in increased rate of leucocyte mobilizations (Kar *et al.*, 2001). Again, a change in the DLC count was observed in the treated rats as compared to the normal counterparts. The increased number of neutrophils found in the present study may be because of the fact that neutrophils are phagocytic in nature that act in acute infections and carry many digesting enzymes for destroying invading foreign pathogens or body's own inactive damaged cells. An increased number of abnormal and immature white cells were reported in the peripheral blood which may be due to the direct

interference of ETP on the haematopoietic process. The changes in DLC count as compared to the control ones can be considered as a mode of defense against the invading toxicant.

An increase in morphological deformities in the circulating erythrocytes indicated destruction of mature cells. The appearance of irregularity in the membranes 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. This in turn leads to membrane disintegrity or disturbance of uniformity of membrane resulting in stomatocytosis (Smith *et al.*,1982). Also the different grades of deformities observed in RBCs may be due to increased sensitivity to hypoxia and impaired osmoregulation. Further, the presence of numerous ring shaped RBCs and Heinz bodies indicated anaemic condition and haemoglobin depletion (Dhembare, 2014). Presence of abundance of abnormally large platelets with filial pseudopodia like outgrowths or projections and their aggregation in the later days of chemical treatment indicated macrocytosis with an alteration in their functional status. Prentice *et al.*, (1972) reported these type of morphological alterations in the blood platelets in some pathophysiological disorders. The release of 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 ETP induced toxicological stress at the level of bone marrow. Similar kind of changes in the haematological picture were reported in benzene toxicity (Aksoy *et al.*, 1976, Dutta *et al.*, 1992).

5. Conclusion

The present study on the detailed haematological picture of ETP exposed rats clearly revealed the toxic effect of ETP at the haematopoietic level which was manifested by the presence of immature blood cells, degenerated cells with various grades of membrane deformities of RBC, WBC and platelets in the peripheral blood picture and alterations of numerical values of the haematological indices. Based on the present findings it can be concluded that ETP has the potentiality to cause haematotoxic effect.

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