TOXICOPATHOLOGY OF QUINALPHOS INDUCED TOXICITY IN WISTAR RATS (*Rattusnorvegicus*)

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ABSTRACT

Quinalphos is effective broad-spectrum insecticides. The present study was designed to study clinical signs, hemato-biochemical alterations, oxidative stress and pathomorphological changes induced by quinalphos toxicity in rats. Accordingly, twenty four adult female wistar rats were divided uniformly into four equal groups, Group I, Group II, Group III, and Group IV. Group I (Control) rats received only groundnut oil and served as control. Group II (Low dose), Group III (Medium dose) and Group IV (High dose) rats were given quinalphos @ 0.689 mg/kg, 1.378 mg/kg and 2.756 mg/kg, respectively orally by gavage for 28 days.Quinalphos intoxicated animals showed decrease physical activity, body weight, feed consumption and water intake. Hematological were decrease in mean values of Hb, TLC, MCV, PCV, monocyte and lymphocyte, while increase in mean values of neutrophil. Quinalphos significantly increase in mean values of plasma ALT, AST,creatinine, BUN, LPO and glucose, while decrease in acetyl-cholinesterase, total protein and SOD in treatment as compared to control group. The patho-morphological changes comprised of varying degrees of congestion, hemorrhages, degeneration and necrosis in various visceral organs.

KEY WORDS: Hematology, pathomorphology, quinalphos, rat.

INTRODUCTION

Pesticides have played a pivotal role for the green revolution in India. Since late few decades use of pesticides increase for purpose of getting better production in agriculture, animal husbandry and public health operation to kill the insect, weeds and fungus and to get rid of insect transmitted disease. Quinalphos is one of the best organophosphorus, which is currently gaining more popularity as a potent insecticide and become choice of insecticidal compound for controlling of ectoparacite in farm animals. The present investigation was undertaken to study the possibilities of toxic effect on the biological system of Wistar rats.

Quinalphos is one of the most effective broad-spectrum insecticides. Sometimes insecticides get contact directly or indirectly and cause hazards to both livestock as well as human. The crops protected with the most intense quinalphos use are cotton, corn and almonds. It is used to control ticks on cattle and as a spray to control crop pests. Quinalphos can kill insects by direct contact or ingestion. It kills

insects by disrupting their normal nervous system function.It is assumed that indirect exposure of livestock to quinalphos may occur and may affect the biological systems of livestock. Quinalphos primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning (Anonymous, 2007).

Current high production and the potential for more widespread humans and animal exposure, toxicity studies on quinalphos pesticide poisoning are important and hence, the present study on**"**Toxicopathology of quinalphosinduced toxicity in wistarrats" was carried out with following objectives like clinical symptoms, various hematological and biochemical parameters, oxidative stress and Patho-morphological changes in various target organs associated with quinalphos exposure.The findings of this study will help to understand the mechanism as to how this pesticide alters the hematological, biochemical profile and patho-morphological changes of various organ of rat. It is expected to get some useful knowledge about the extent of adverse effect of continuous exposure of quinalphos to rat, this knowledge could later be used for making a judicious use.

MATERIALS AND METHODS

The present study was carried out in the Department of Pathology, College of Veterinary Science and Animal Husbandry, SardarkrushinagarDantiwada Agricultural University, Sardarkrushinagar. The experiment has been designed to study the toxicopathological effects of quinalphos toxicity at different dose levels in Wistar rats. Quinalphos (70% Technical grade) obtained from Meghmani Organics Limited, Ahmedabad, India.

Experimental Animals

The study was conducted on 24 colony-bred adult female wistar rats. Rats were procured from Zydus Research Centre, Ahmadabad, Gujarat, India. Synopsis of research project was presented before the Institutional Animal Ethical Committee(IAEC) and experimental protocol was approved for conducting the work. The housing and feeding were provided as per the CPCSEA guidelines during all experiments. Before the start of experiment, rats were kept in laboratory conditions for a period of seven days for acclimatization.

Experimental design

All the twenty four adult female wistar rats were divided uniformly into four equal groups, Group I, Group II, Group III, and Group IV. Group I (Control) rats received only groundnut oil and served as control. Group II (Low dose), Group III (Medium dose) and Group IV (High dose) rats were given quinalphos @ 0.689 mg/kg, 1.378 mg/kg and 2.756 mg/kg, respectively orally by gavage for 28 days.

Collection of material

Rats were anesthetized by using diethyl ether and blood was collected on 0 and 28th days of experiment from retro-orbital plexus with the help of capillary tube. Blood was collected in sterilized vials containing 4% EDTA as an anticoagulant for hematological parameters and in heparinised vial (10 IU) to separate plasma for biochemical estimates and RBC pellets was used for estimations of oxidative stress related parameter. Blood smears were prepared for differential leukocyte count. Prior to each collection, rats were fasted for 12 hours.Tissues from various organs of the rats sacrificed on

29th day of post-treatment were collected in 10 per cent neutral buffered formalin. Tissue samples viz. kidney, brain, liver, lung, heart, stomach, spleen, intestine, and ovary were collected for histopathological study.

Parameters studied

Symptomatology: All the experimental rats were closely observed for any behavioural change or mortality during the entire period of experiment. Feed consumption and daily water intake was recorded. The body weight was recorded weekly.

***Hematology*:** Hematological parameters were performed with auto blood analyzer. The following hematological parameters were studied:Hemoglobin estimation (Hb), Packed Cell Volume (PCV),Total Erythrocyte Count (TEC),Mean Corpuscular Volume (MCV),Mean Corpuscular Hemoglobin (MCH),Mean Corpuscular Hemoglobin Concentration (MCHC),Total Leucocyte Count (TLC),and Differential Leucocyte Count (DLC).

***Biochemicalstudy*:** All the biochemical parameters except lipid peroxidase were analyzed by using Mercks Kits by Clinical Analyzer. The following biochemical estimates were analyzed:Plasma Aspartate Aminotransferase (AST),Plasma Alanine Aminotransferase (ALT),Plasma Creatinine, Blood Urea Nitrogen (BUN), Cholinesterase Activity, Total Plasma Protein, Plasma Glucoseand Super Oxide Dismutase (SOD).

***Pathomorphology*:** Necropsy was conducted on the rats, which sacrificed at the end of experiment. Gross and histo-pathological changes in different organs were studied.After recording the gross lesions the tissues from affected kidney, brain, liver, lung, heart, stomach, spleen, intestine and ovary were collected from sacrificed animals and subsequently preserved in 10 per cent neutral buffered formalin for at least 24-48 hours. Further, these tissues were processed by routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Sections of 5-6 µ thickness were prepared with the use of Microtome Machine and processed by routine Hematoxyline and Eosin method to study the general histopathological alterations (Luna, 1968).

Statistical analysis

The statistical analysis of data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD) (Snedecor and Cochran, 1980) and using CD values compared the treatment means (Steel and Torrie, 1984).

RESULT AND DISCUSSION

Symptomatology

Quinalphos intoxicated animals was observed decrease physical activity in the dose rate of 2.756 mg/kg post treatment through gavage. The same effects ofquinalphos were reported in adult male ratsby Sarkar *et al.* (2000).

***Bodyweight*:** Significant ($P<0.05$) decreases in body weight was observed in rats of Group II, Group III and Group IV from 14th day onwards when compared with the control group showed in Table 1. Same results were reported bySurana*et al.* (2008).

***Organweight*:** The mean weight of liver, brain, and lung in female rats of all the treatment groups revealed significant ($P<0.05$) decrease. It may be due to retarded growth and development of rats fed on pesticide.

***Feed consumption and Water intake*:** In female rats, there was dose dependant significant ($P<0.05$)

reduction in mean value of average weekly feed consumption and water intake throughout the experiment in all treatment groups as compared to control.Reduction in feed consumption and water intake may be due to toxic effect of insecticide on gastrointestinal tract resulting in decreased appetite or decreased feed intake may be either due to anorectic properties of the insecticide (Mohapatra and Mallick, 1998).

***Haematology*:**

Haemoglobin (g/dl), Packed Cell Volume (%), Mean Corpuscular Volume (fl) and Total Leucocyte count (thousands/cumm):There was dose dependant significant ($P < 0.05$) decrease in mean values of haemoglobin and MCV in Group III and Group IV, whereas PCV and TLC in Group II, Group III and Group IV rats treated with quinalphos as compared to control. The lowest values of haemoglobin, PCV, MCV and TEC were observed in group IV rats receiving higher level of quinalphos.Decreased in the values of TLC, PCV, Hb and TEC werealso reported byGarg*etal.*, 1992, Kumar *et al.*, 1996b, Kumar *et al.*, 1996a and Kumar and Uppal (1996). The results observed in the present study revealed anemia (decrease in hemoglobin concentration, PCV %) and leucopenia because of decrease value of TLC.

Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH)andTotal Erythrocyte Count (millions/cumm):There was non-significant difference in values of MCH, MCHC and TEC in rats belonging to different groups at 28th day of post treatment as compared to Group I control rats. The findings of the present study go well with these reported earlier by Garg*et al.* (2004) and Sayim*et al.* (2005).

Differential leucocyte count (%): The mean values of neutrophil count showed the significant ($P<0.05$) increase in the Group II, Group III and Group IV rats as compared to Group I rats after 28thdays post treatment, whereas the mean values of lymphocyte and monocyte showed the dose dependent significant ($P<0.05$) decrease after 28 days post treatment. However, the mean values basophil and eosinophil does not reveled any significant changes in any of the quinalphos treated group.Same finding was observed by Benson *et al.* (1989) in rats.

***Biochemical alterations*:**

Alanine aminotransferase and Aspartate Aminotransferase (IU/L):Alanine aminotransferase and Aspartate Aminotransferase values of all the treatment groups revealed dose dependent significant ($P < 0.05$) increase on 28th day post treatment as compared to control Group I rats.Studied Quinalphos in male wistar rats shown elevation levels of SGPT and SGOT. In the present study, degenerative changes of varied degree were found in liver (Surana*et al.*, 2008).

***Plasma Creatinine*:**The mean plasma creatinine (mg/dl) values revealed dose dependant significant ($P <0.05$) increase in Group IIIand Group IV rats when compared with mean value of Group I and Group II rats. Same results were reported byChandane (2009).

***Blood urea nitrogen*:**The mean plasma blood urea nitrogen (mg/dl) values revealed dose dependant significant ($P <0.05$) increase in Group II, Group III and Group IV rats when compared with mean value of Group I control rats. Same results were reported byChoudhary*et al.* (2003).

***Plasma Glucose*:**The mean value of plasma glucose (g/dl) revealed dose

dependent significant (P<0.05) increase on 28th day post treatment in all groups compared to control.Similar finding were also observed earlier by with quinalphos (Chandane, 2009).

***Total Plasma Protein*:** The mean value of total protein (g/dl) revealed dose dependent significant (P<0.05) decrease on 28th day post treatment in all groups compared to control.Similar finding were observed earlier in broiler birds with quinalphos (Chandane, 2009).

***Acetyl-Cholinesterase (AChE)*:** The mean value of acetyl-cholinesterase (IU/L) revealed dose dependent significant (P<0.05) decrease on 28th day post treatment in all groups compared to control.Similar finding were also observed earlier by studied effect of quinalphos in chicken found the maximum inhibition of blood, plasma and erythrocyte cholinesterase (Vairamuthu and Sundararaj, 2005).

***Lipid Peroxidation (nmol/ml of RBC)*:** Lipid peroxide activity increased significantly (P<0.05) in Group II and Group VI of the experimental groups as compared to Group III and Group I control. Group IV rats showed highest plasma lipid peroxidase activity as compared with other treatment groups. Literature scanned revealed support to our findings of significant increase in LPO level in male wistar rats treated quinalphos reported bySurana*et al.* (2008).

***Superoxide Dismutase (U)*:** SOD values decreased significantly (P<0.05) in Group II and Group IV of the experimental groups as compared to Group III and Group I control. Group IV rats showed lowest plasma SOD activity as compared with other treatment groups.The present findings of significant decrease in SOD activity are in agreement with the findings ofSurana et al. (2008) with Quinalphos toxicity in male wistar rats.

***Pathomorphology*:**

Thepatho-morphological changes in the rats treated with quinalphos resulted into varying level of degenerative, vascular and necrotic lesions. The lesions were dose depend in nature in lung, brain, liver, kidney, heart and spleen were found to be most severely affected organs followed by ovary, intestine and stomach.

***Liver*:** Grossly, In Group IV rats, liver was found moderately congestion with mild infraction patches (Fig.1). The histo-pathological changes of liver of Group III rats showed venous hemorrhages with sinusoidal dilatation. Group IV rats receiving the highest dose of quinalphos showed severe fatty changes with sinusoidal congestion and also shown focal congestion in central veins (Fig.2), Besides these infiltrations of the mono nuclear inflammatory cells around the portal triad, congestion and haemorrhages were also evident. The present findings were in conformity with the finding observed in rats byChoudhary*et al.* (2003).

***Kidney*:** Gross lesions comprised of mild hyperemia in Group III rats, whereas in Group IV rats receiving quinalphos high dose; kidneys showed moderate congestion with mild swelling. Microscopic Lesions in Group II rats comprised of scattered hemorrhages along with mild tubular degeneration. In Group III rats hemorrhages in glomeruli, tubules and inter-tubular space. Rats of group IV showed severe tubular congestion and hemorrhages were evident. The findings of the present study correlate with previous observations made bySurana*et al.* (2008).

***Brain*:** Grossly, Group IV rats brain was found cerebrum mild congestion. Microscopic changes in brain of rats

belonging to Group II showed mild vacuolation in cerebellum, neuronal degeneration and congestion in meningeal vessels. Group III comprised of cerebellum congestion, neuronal degeneration and vacuolation. In rats of Group IV, moderate cerebral congestion, moderate neuronal degeneration and vacuolation in cerebellum was observed. The lesions observed in the present study were in conformity with the findings of Sharma *et al.* (2005) in rats.

***Heart*:** Group III rats, mild engorgement of ventricular veins. Group IV rats were observed engorgement of ventricular blood vessels with rounded borders. Microscopic changes in heart of Group III rats comprised of mild to moderate fragmentation of myocardial fibers along with congestion. Heart of Group IV rats showed severe myocardial congestion with separation of myofibrils and hemorrhages. The lesions observed in the present study were in conformity with the findings in broiler birdsChandane (2009).

***Lung*:** Gross examination of rats belonging to different groups revealed mild to moderate congestion in lung parenchyma especially in Group IV rats (Fig.3). Microscopic changes in lungs of Group II rats revealed emphysema. In rats belonging to group III, thickening of interalveolar septa with mononuclear cells infiltration. In rats of group IV besides emphysema, moderate to severe hemorrhages along with thickening of alveolar septa and severe oedema was also evident (Fig. 4). The findings of the present study correlate with previous observations made by Erdogan *et al.* (2006) in rat and Chandane (2009) in broiler birds.

***Spleen*:** Gross examination of spleen of different groups revealed mild to moderate vascular changes characterized by severe congestion with sharp border (Fig.5). Histo-pathological examination in Group IV receiving the highest dose of quinalphos mild to moderate depletion of lymphocytes in the germinal centers of spleenic corpuscles, rarefaction of lymphocytes, thickening ofintertrabacularsepta and scanty of lymphoblast in germinal centre was observed (Fig.6). The lesions observed in the present study were in conformity with the findings in mice (Garg*et al.*, 1992).

***Ovary*:** Grossly, ovary of rats belonging to Group IV showed mild congestion, whereas no appreciable gross lesions were observed in ovary of Group II and Group III rats. Histo-pathological lesions in ovary of Group IV receiving the highest dose of quinalphos, moderate congestion with scattered hemorrhages, desquamation with peripheral congestion.

***Stomach*:** The stomach of Group IV rats was found mild sloughing and congestion. The microscopic changes in Group IV rats receiving the highest dose of quinalphos revealed congestion as evident by submucosal congestion, serosal hemorrhages with sloughing, glandular degeneration with intra-glandular congestion.

***Intestine*:**No appreciable gross lesions were observed in Group II, Group III and Group IV rats. The microscopic changes in Group IV rats receiving the highest dose of quinalphosrevealed shortening of villi with mild goblet cell proliferation, desquamation with congestion, Blunting and sloughing of villi with hemorrhages at tips was also evident.The lesions observed in the present study were in conformity with the findings in broiler birds (Chandane, 2009).

CONCLUSION

Quinalphos at high dose rats showed decreased physical activity, feed consumption and body weight.

Patho-morphological lesions induced by quinalphos toxicity were of varying degrees of congestion, hemorrhages, degeneration and necrosis in visceral organs in nature and dose dependant. Kidney, Liver, Lung, Brain, Heart, Spleen, Intestine and Ovary were found as the most affected target organs of quinalphos intoxication in rats. Further work on quinalphos toxicity with special reference to genotoxicity and teratogenecity may be carried out to explore the nature of compound.

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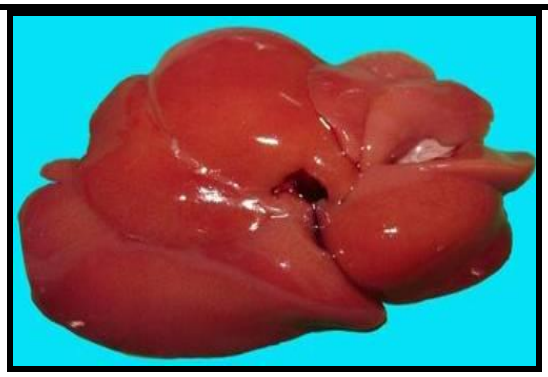

Figure 1*: Liver of group IV showing moderately congestion with mild infraction patches*

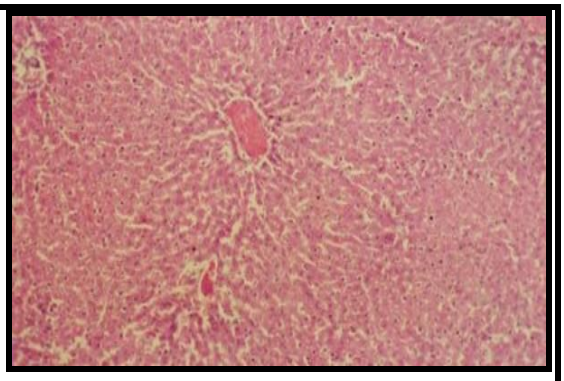

Figure 2*: Liver of group III and IV showing severe fatty changes with sinusoidal congestion and also shown focal congestion in central veins*

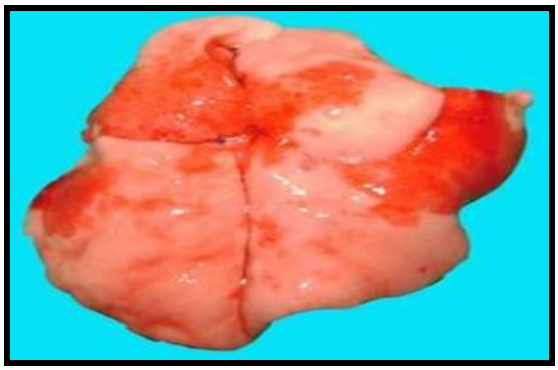

Figure 3: *Lung of group IV showing moderate congestion in lung parenchyma*

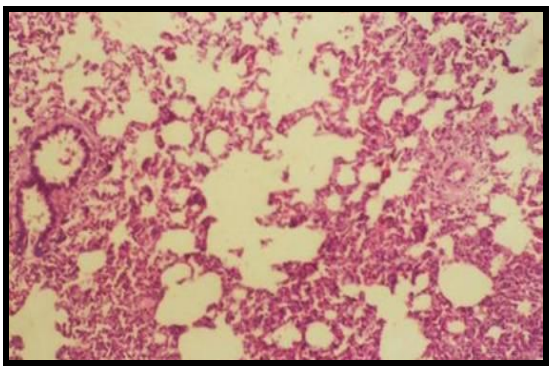

Figure 4*: Lung of group IV showing emphysema, moderate to severe hemorrhages along with thickening of alveolar septa and severe oedema*

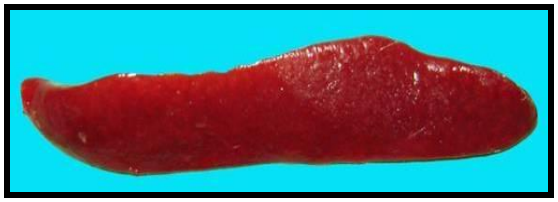

Figure 5*: Spleen of group IV showing severe congestion with sharp border*

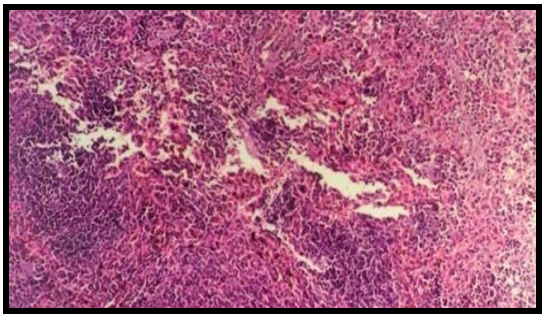

Figure 6*: Spleen of group IV showing moderate depletion of lymphocytes in the germinal centers and scanty of lymphoblast in germinal centre*

Table 1: Comparison of weekly body weight (Mean ± S.E., g) in female rats of different experimental groups (Values are mean ± SE,n=6).

Group	Days				
	0	**7th day**	**14th day**	**21st day**	**28th day**
I (Control)	203[a]±1.000	215[a]±6.943	223[a]±6.112	233[a]±5.169	241[a]±5.063
II	201[a]±7.813	207[a]±7.765	211[b]±6.965	217[b]±7.609	222[b]±7.470
III	202[a]±8.010	207[a]±8.383	213[b]±6.726	219[b]±7.225	224[b]±15.983
IV	205[a]±3.028	210[a]±3.028	211[b]±3.155	214[b]±2.781	218[b]±3.197

Table 2: Effect of Quinalphos on Haemogram in rats after daily Oral administration for 28 days.

Parameters	Groups	Days	
		0 Days	28 Days
Hb (g/dl)	I	16.83 ± 0.215[a]	16.24 ± 0.286[a]
	II	16.63 ± 0.298[a]	15.41 ± 0.291[b]
	III	16.72 ± 0.269[a]	14.53 ± 0.375[b]
	IV	16.46 ± 0.260[a]	14.46 ± 0.678[b]
PCV (%)	I	46.43 ± 0.599[a]	46.32 ± 0.626[a]
	II	45.94 ± 0.708[a]	45.12 ± 0.483[a]
	III	47.61 ±0.560[a]	43.91 ± 0.985[b]
	IV	46.28 ± 0.810[a]	43.06 ± 0.80 [b]
TEC(millions/cumm)	I	8.87± 0.133[a]	8.77 ± 0.303[a]
	II	8068 ± 0.255[a]	7.68± 0.234[a]
	III	8.30 ± 0.263[a]	7.47 ± 0.350[a]
	IV	8.41 ± 0.303[a]	7.12 ± 0.418[a]
MCV(fl)	I	57.75 ± 0.498[a]	58.25 ± 0.797[a]
	II	56.75± 0.409[a]	56.09 ± 1.169[b]
	III	56.67 ± 0.563[a]	55.15 ± 0.573[b]
	IV	57.68 ± 0.541[a]	53.63 ± 0.300[c]
MCH(pg)	I	18.30 ± 0.293[a]	18.94 ± 0.261[a]
	II	18.27 ± 0.304[a]	18.83 ± 0.195[a]
	III	18.60 ± 0.428[a]	18.57 ± 0.166[a]
	IV	18.63 ± 0.379[a]	17.90 ± 0.292[a]
MCHC(%)	I	35.88 ± 0.747[a]	35.62 ± 0.295[a]
	II	35.84 ± 0.276[a]	35.28 ± 0.195[a]
	III	35.00 ± 0.539[a]	35.05 ± 0.243[a]
	IV	35.85± 0.668[a]	34.56± 0.253[a]
TLC (thousand/cumm)	I	10.07 ± 0.266[a]	10.05 ± 0.132[a]
	II	9.01 ± 0.341[a]	8.81 ± 0.523[a]
	III	9.88 ± 0.503[a]	8.75 ± 0.630[b]
	IV	10.24 ± 0.328[a]	7.88 ± 0.776[b]

Table 2 Continue…..			
DLC			
Neutrophil (%)	I	21.63±0.28[a]	20.50 ± 0.437[a]
	II	21.99±0.378[a]	22.11 ± 0.320[b]
	III	22.34±0.345[a]	25.53 ± 0.697[c]
	IV	22.72±0.316[a]	28.53 ± 0.809[d]
Eosinoiphil (%)	I	2.36±0.076[a]	2.38 ± 0.073[a]
	II	2.25 ± 0.077[a]	2.46 ± 0.068[a]
	III	2.20 ± 0.093[a]	2.36 ± 0.119[a]
	IV	2.13 ± 0.088[a]	2.33 ± 0.076[a]
Basophil (%)	I	0.63 ± 0.051[a]	0.60± 0.034[a]
	II	0.51 ± 0.544[a]	0.61 ± 0.023[a]
	III	0.58 ± 0.046[a]	0.63 ± 0.043[a]
	IV	0.65± 0.047[a]	0.65 ± 0.059[a]
Lymphocyte (%)	I	70.85± 0.528[a]	70.86 ± 0.336[a]
	II	71.45 ± 0.416[a]	67.50 ± 0.773[b]
	III	70.66 ± 0.645[a]	66.18± 0.755[b]
	IV	70.96 ± 0.570[a]	64.78 ± 0.960[c]
Monocyte (%)	I	4.58 ± 0.194[a]	4.98 ± 0.263[a]
	II	4.73 ± 0.348[a]	3.81 ± 0.186[b]
	III	4.90 ± 0.095[a]	3.55 ± 0.265[b]
	IV	4.833 ± 0.232[a]	3.16 ± 0.246[b]

- ***Superscripts are to be read column wise for mean comparison.***
- ***Mean with similar superscripts in column do not differ significantly (P < 0.05).***

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