ORIGINAL ARTICLE

LIVER FUNCTION TESTS, RED CELL INDICES AND OXIDATIVE STRESS IN HEALTHY MALE SPRAGUE DAWLEY RATS

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Background: Sprague Dawley rats are commonly used in laboratory research. The present study compared the levels of serum malondialdehyde (MDA), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and red cell indices of Sprague Dawley rats used in laboratory research at National Institute of Health, Islamabad, with that of published data. **Methods:** Thirty five young healthy male Sprague Dawley rats (age, 92±3.4 days; weight 228±5.6 gm) were obtained from National Institute of Health, Islamabad. Rats were anaesthetized and 4 ml of blood was drawn from each rat for estimation of serum MDA, ALT, AST levels and red cell indices. **Results:** Serum MDA levels in the study group were 3.2±0.39 µmol/l, Serum ALT levels were 44.1±3.26 IU/l, and serum AST levels were 156.2±4.97 IU/l. Haematological parameters revealed RBC count 7.63±0.33 (10^6/µl), haemoglobin 13.16±0.57 gm/dl, haematocrit 41.93±2.06%, MCV 55.69±0.93 fl, MCH 17.33±0.35 pg, and MCHC 31.45±0.53 g/dl. **Conclusion:** Levels of serum MDA, ALT, AST and red cell indices in the Sprague Dawley rats at National Institute of Health, Islamabad are comparable with published work on this strain. These parameters can be used in assessing the oxidative stress, hepatotoxicity and haematological derangements produced by various agents.

Keywords: Red cell indices, malondialdehyde, alanine aminotransferase, aspartate aminotransferase, Sprague Dawley rats, Liver Function Tests, Oxidative stress

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INTRODUCTION

Sprague Dawley rats are among the most commonly used laboratory animals, and have comparable haematological and biochemical parameters with human. Effects of various environmental pollutants, toxins, drugs and dietary supplements have been evaluated in Sprague Dawley rats. Many of these agents cause disturbance between pro-oxidants and antioxidants in the body, damage to hepatocytes and haematological derangements.[1,2]

Oxidative stress is produced by excessive production of reactive oxygen species (ROS) and depletion of the antioxidant system by various agents. ROS cause lipid peroxidation of the cell membranes which begins as a chain reaction mediated by the presence of free radicals and leads to the production of lipid hydroperoxides and their metabolites. Lipid peroxidation of polyunsaturated fatty acids releases malondialdehyde (MDA) as a byproduct which is used as an indicator and a marker of oxidative stress.[3,4]

Hepatotoxicity is produced by various drugs and chemicals either by direct damage to the hepatocytes or by the oxidative stress produced by these chemicals. Hepatocytes are very active cells which perform multiple metabolic functions and normally generate ROS. In presence of various chemicals and drugs, both the excessive generation of ROS as well as the depletion of the antioxidant reserves, cause accumulation of ROS in hepatocytes. These ROS initiate lipid peroxidation in cell membranes, inactivate sulfhydryl antioxidants, damage nucleic acids and inhibit DNA repair in hepatocytes. Damage to hepatocytes can be quantitatively assessed by estimation of enzymes released by the damaged hepatocytes in to the blood when these are ruptured. Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) levels are used as markers of hepatotoxicity.[5,6]

Erythrocytes are the most vulnerable cells for damage by various toxins and drugs which produce oxidative stress. RBCs lack normal cytoplasmic organelles especially rough endoplasmic reticulum which synthesizes antioxidant enzymes. Haematological derangements produced by various agents are best evaluated by red cell indices including levels of haemoglobin, RBC count, haematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV). Determination of red cell indices enables the quantitative estimation of integrity of haematopoietic system, severity of anaemia and clues about the probable cause of anemia.[7]

The present study was designed to determine the basal levels of parameters of oxidative stress, liver functions and red cell indices in Sprague Dawley rats available at National Institute of Health (NIH) Islamabad. The results were compared with the published data on the same strain of rats to see any statistically significant difference between the results of this study and the already published work.

MATERIAL AND METHODS

Male Sprague Dawley healthy rats of age 92±3.4 days and weight 228±5.6 gm were taken from National Institute of Health (NIH) Islamabad. At one time, 5 rats were placed in a closed chamber containing ether soaked cotton to anaesthetise them. Four ml of blood was drawn into disposable syringes, by intracardiac sampling from each animal. One ml blood of each sample was transferred to an EDTA tube, while 3 ml of blood was transferred into a plain tube and allowed to clot. Blood-containing plain tubes were centrifuged for 15 min for separation of serum in cold centrifuge machine (Model 5810R; Eppendorf, Germany). Temperature of centrifuge was adjusted at 4 ºC and speed at 4,000 rpm. After cold centrifugation, serum was pipetted out. Approximately 1.5 ml of serum was obtained from each blood sample, transferred to serum tubes (Eppendorf, Germany) which were labelled and stored at -80 °C in deep freezer (Model DFU-446 CE, Operon, Korea) till the assay for MDA, ALT and AST levels by using commercial kits on spectrophotometer and Microlab 200®.

Estimation of serum malondialdehyde (MDA) levels was done by thiobarbituric acid reactive substances (TBARS) assay kit, Cayman, USA by using spectrophotometer[8]. Estimation of serum alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) levels was done by using commercial kits on Merck Microlab 200®.[5] Blood samples in the EDTA tubes were used to determine haemoglobin concentration, erythrocyte count and red cell indices by haematology analyser Sysmex KX-21. Haematology analyser determined cell type, cell count and haemoglobin directly were as red cell indices, namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration, were determined by the built in software of the analyzer.[7]

Data was entered into SPSS-18. Mean and standard deviation of all the continuous parameters were determined. The results were compared with the internationally published data by applying ANOVA and Post Hoc Test which reflected the statistical significance between the differences of means of various parameters.

RESULTS

Serum MDA levels in Sprague Dawley rats in this study were 3.2±0.39 µmol/l, Serum ALT levels 44.1±3.26 IU/l, and serum AST levels 156.2±4.97 IU/l. Haematological parameters revealed RBC count $7.63\pm33\times10^6$/µl, haemoglobin levels 13.16±0.57 gm/dl, haematocrit 41.93±2.06%, MCV 55.69±0.93 fl, MCH 17.33±0.35 pg, and MCHC 31.45±0.53 g/dl. Results of this study are compared with published data in Table-1 and 2.

Table-1: Comparison of serum MDA, ALT and AST levels of male Sprague Dawley rats with the published data of various studies

Study	Sample size (n)	MDA (µmol/l)	ALT (IU/l)	AST (IU/l)
Present study	35	3.2±0.39	44.1±3.26	156.2±4.97
Ruifen *et al*[9]	12	3.9±0.4	-	-
Nevin and Seckin[10]	9	3.4±0.8	47.5±8.9	136±13
Feng-Xia *et al*[11]	15	3.0±0.8	-	-
Young *et al*[12]	24	-	47±3.9	131±20
Ismail and Suzek[13]	12	-	29.5±7.5	137.3±11.8

Table-2: Comparison of haematological parameters in the study group with the results of various studies on Sprague Dawley rats

Authors	Sample (n)	Hb (g/dl)	RBC count (10^6/mm^3)	Hct (%)	MCV (fl)	MCH (pg/RBC)	MCHC (g/dl)
Present study	35	13.16±57	7.63±0.33	41.93±0.06	55.69±0.93	17.33±0.35	31.45±0.53
Ismail and Suzek[13]	12	14.6±1	8.7±0.4	42.4±0.8	50.2±1.5	17.9±0.5	34.5±0.3
Mugahi *et al*[15]	15	14.5±3	7.6±0.7	41±1	50.3±2.1	17.9±0.7	-
Suzuki and Yoshida[16]	27	13.1±0.27	-	-	-	-	-
Rahiem *et al*[17]	16	13.8±0.4	6.5±0.2	-	-	-	-

DISCUSSION

Serum MDA levels in Sprague Dawley rats (3.2±0.39 µmol/l) of this study is comparable with the published data of different studies.[9–11] In 2004, Ruifen Zhang *et al*[9] measured the serum MDA levels in healthy Sprague Dawley rats as 3.9±0.44 µmol/l. Nevin and Seckin[10] documented the level of MDA in healthy control as 3.4±0.8 µmol/l. Feng-Xia *et al*[11] evaluated the serum MDA levels in healthy Sprague Dawley rats and found as 3.0±0.8 µmol/l. When the MDA levels of present study were compared with the three above mentioned studies, there was no statistically significant difference between the serum MDA levels of the studies. This reflects that the serum MDA levels in this strain of rats are in a narrow range and the data of research work at various places is consistent.

MDA is the bi-product of lipid peroxidation mediated by reactive oxygen species in the membranes of cell and subcellular organelle. It has been documented that serum MDA levels is an indirect measure and a reliable tool for the assessment of oxidative stress. During health, the reactive oxygen species (ROS) are neutralised by the defence system of the cell which prevents abnormal lipid peroxidation in the body, hence normal MDA levels are found in the narrow range in healthy subjects.[3,4]

The levels of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) recorded in this study are compared with the levels in healthy Sprague Dawley rats as documented in other international studies. Nevin and Seckin[10] measured serum ALT and AST levels in the Sprague Dawley rats. In their study levels of serum ALT and AST in healthy rats were 47.5±8.9 IU/l and 136.4±13.8 IU/l respectively. Young *et al*[12] evaluated the levels of markers of hepatotoxicity in Sprague Dawley rats and documented the levels of serum ALT (47±3.9 IU/l) and serum AST (131±20.5 IU/l) in healthy control group. In 2008, Ismail and Suzek[13] measured the serum ALT and AST levels in healthy control as 29.5±7.5 IU/l and 137.3±11.8 IU/l respectively.

Serum AST levels of present study are not statistically significantly different from the data of both the above quoted international studies whereas serum ALT levels of present study are statistically significantly different from the data of Ismail and Suzek[13]. They used rats with the weight between 150–200 gm which is less than the mean weigh of rats of present study (228±5.6 gm) which may be factor for the difference. In addition, the study design of Ismail and Suzek contained only 6 rats and probably less number of rats may lead to a mean which is statistically significantly less than the present study. Serum ALT levels of present study are not statistically significantly different from the data of Young *et al*.[12]

Serum ALT and AST levels have long been used by many researchers to assess the integrity of hepatocytes and response to various toxins and environmental pollutants. Although normal range of these enzymes is different in Sprague Dawley rats compared to humans but, rise in levels above normal limits indicates damage to hepatocytes. When hepatocytes are damaged, it causes release of these enzymes (ALT and AST) into the plasma that are used as markers of hepatotoxicy.[14]

Haematological parameters measured by various workers have been found consistent as regards the values of haemoglobin and RBC indices in this species of rat. Mugahi *et al*[15] evaluated haemoglobin and RBC indices in the Sprague Dawley rats: haemoglobin 14.5±3 g/dl, MCV 50.3±0.1 fl, and MCH 17.7±0.7 pg/RBC. Ismail and Suzek[13] evaluated the haematological parameters of Sprague Dawley rats as: RBC Count 8.7±0.4×10^6/mm^3, haemoglobin 14.6±1 g/dl, haematocrit 42.4±2.8%, MCV 50.2±1.5 (μm^3), MCH 17.9±0.5 pg, and MCHC 34.5±0.3%. Suzuki and Yoshida[16] measured the haemoglobin in Sprague Dawley rats as 13.1±0.27 g/dl. Rahiem *et al*[17] measured haemoglobin and RBC count as 6.5±0.2×10^6/mm^3 and 13.8±0.4 g/dl respectively.

Haemoglobin levels of present study are not significantly different from results of Suzuki and Yoshida[16] and Rahiem *et al*[17] whereas they are significantly different from the results of Ismail and Suzuik[13] and Mugahi *et al*[15]. The variation in haematological parameters between various studies could probably be due to different balanced chow used as food of rats containing different quantities of iron and vitamins. The composition of the balanced chow is of critical importance for determining the haematological indices as the availability of iron and vitamins is dependent on it and they are required for the haemopoiesis.

CONCLUSION

Levels of serum MDA, ALT, AST and red cell indices in the Sprague Dawley rats, available in this region, are comparable with the international reference values. These parameters can be used in assessing the oxidative stress, hepatotoxicity and haematological derangements produced by various agents.

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