Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2014, 5 (3):27-35
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Heat and cadmium stress alter blood electrolytes of wistar rat

Zerfaoui Zehour, Cherif Abdennour, Wafa Siouda and Kamel Khelili

Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Sciences, University Badji Mokhtar-Annaba, Annaba, Algeria

---

ABSTRACT

The fast economic development has polluted the environment and led to a remarkable global warming worldwide characterized by an environmental hyperthermia. In view of these circumstances, the investigation of some biological markers during the combined stress was carried out. Thus, the effect of heat stress (HS), cadmium chloride (Cd) and heat stress-cadmium (HS-Cd) were performed on Wistar rats for a period of 8 consecutive days. The control and the cadmium group (10 mg cadmium chloride daily by gavage) were housed separately (temperature ranges 22.23-25.45 °C). The HS and HS-Cd groups were placed in a thermal chamber where they exposed each day from 09:00h to 13:00h (temperature ranges 34.41-37.23 °C). However, serum total proteins, sodium, chlorine, calcium, potassium and blood components were investigated. The obtained results showed a remarkable increase of serum Na in HS-Cd and serum Cl in HS and HS-Cd were seen. However, in the cadmium treated group, only calcium level was affected by a notable decline after one week. In the combined treatment, the concentrations of serum calcium as well as that of serum total proteins were decreased significantly. Contrary, serum K was within normal ranges in all treated groups. RBC and WBC counts were remarkably lower in the HS-Cd group, while hematocrit was significantly decreased in all treated groups. To conclude, the combined stress has made deep alterations on biological markers of Wistar rats.

Keywords: Heat, cadmium, stress, electrolytes, blood, rats, toxicity.

INTRODUCTION

Uncomfortable weather characterized with high temperature and high humidity make humans and even animals unable to rid out of excess heat in order to regulate body temperature. The **[1]** climate projection is expecting significant changes, as the Southern Europe is becoming warmer, and that will not certainly affect humans only **[2, 1]**, but it may perhaps disturbs the ecological systems including many plants and animal species. Accordingly, environmental hyperthermia is making today deep alterations concerning the unbalance of ecosystems 'biodiversity **[3, 4, 5].** Therefore, the rise in temperature for longer periods would create drought, and then many species will disappear to be replaced with other new species able to resist the changing climate **[6].**

Due to variation in the structure and function of ecosystems **[7]**, some natural physiological adaptations could occur to cope against heat stress **[8, 9].** For this reason animal physiological mechanism concerning heat stress need to be understood deeply in order to apply preventive and control measures **[10]**, as for example animals having more sweat glands are able to regulate their body temperature better than those getting fewer glands. Furthermore, the

thermal regulation can also be different even within the same species due to acclimation, as in humans who permanently live either in cold or in hot climate **[11].**

Therefore, the objective of this study is to investigate some biological markers of male rats exposed to thermal stress and cadmium pollution.

MATERIALS AND METHODS

Male Wistar rats were placed in a controlled house (temperature, relative humidity, and photoperiod) with a standard pellet diet and water *ad libitum.* Animals were divided into four groups of 10 individuals each. Group 1 was served as a control; group 3 was exposed each day to heat stress (HS) from 09:00h to 13:00h, group 2 was treated with 10 mg cadmium chloride daily by gavage and group 4 was exposed to a combined treatment of heat stress-cadmium (HS-Cd). However, the control and the Cd group were housed at the same room (temperature ranges 22.23-25.45 °C), while those of HS and HS-Cd groups were housed in a thermal chamber (temperature ranges 34.41-37.23 °C). The experiment was lasted 8 consecutive days for all groups. The temperature was continuously monitored by a hygrometer throughout the experiment (Tchilo model). Animals were decapitated on the eighth day of the experiment and blood samples were collected from each animal into dry tubes and were obtained by blood centrifugation at 3000 rpm/min for 10 mn. Serum was used for the determination of total proteins, calcium, sodium, chlorine and potassium using the biochemical multiparameters (Dialab Autoanalyser). A portion of blood was also collected in EDTA tubes to be analyzed by "Abacus 4 Hematology Analyzer, Hungary". Results are expressed as mean ± standard deviation. Student's *t*-test was used to compare the mean of each treated group against the control. However, the significant test was used at $p<0.05$ level.

RESULTS AND DISCUSSION

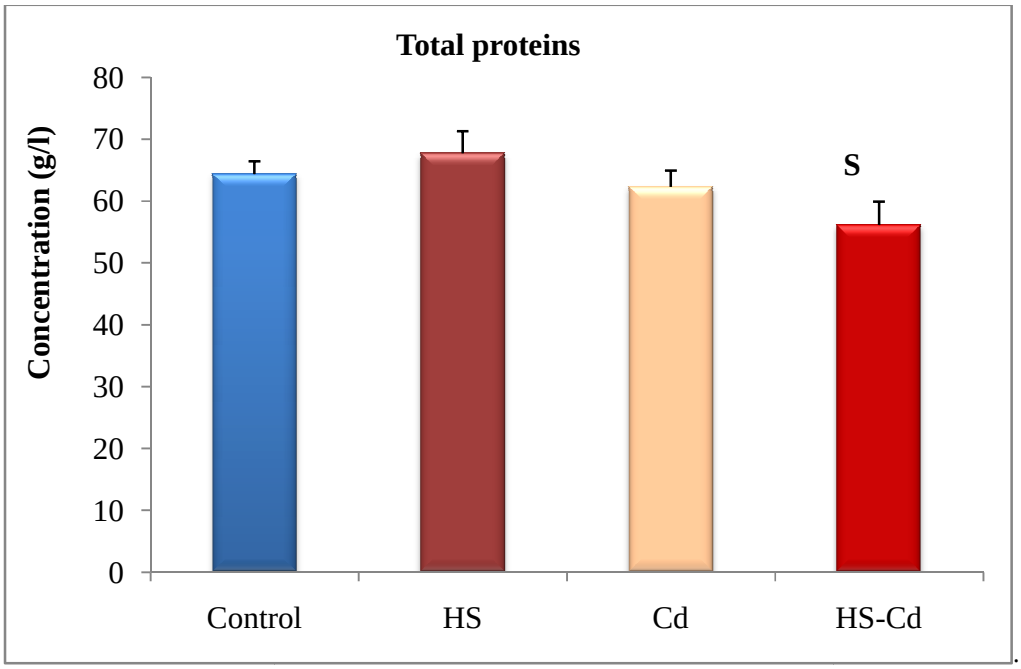

Fig 01: Variation of serum total proteins concentration (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control

In this experiment, the level of serum total proteins was slightly increased in rats experienced HS. Such finding is nearly similar to that of **Kataria et al., [12]** who reported only a negligible rise in plasma protein concentrations of Marwari goats during summer, which was linked to the capability of animals to maintain normal blood circulatory volumes even at hot season. The elevation in serum protein levels is likely related to hemo-concentration, which resulted from body fluid losses through excessive sweating. On the other hand, the observed increase of serum

proteins might indicate a loss of extracellular fluid due to high temperature. These results were similar to the data cited by **Rasooli et al., [13]** in Holstein heifers during summer time. Moreover, lactating cows have produced fewer proteins in their milk under hyperthermic situation **[14]**. Furthermore, the fall of milk production by 33%) was accompanied with a fall in milk proteins and an elevation in plasma urea of lactating cows after 21 days cyclic heat stress (daily temperatures ranging from 29.4 to 37.8°C and 20% humidity) **[15]**. At this circumstance, milk protein reductions were likely affected by the milk production process in the mammary glands, or/and they were affected by the serum protein deficiency.

In the present work, cadmium has made no noticeable variation in the concentration of serum total proteins after a one week chronic exposure. Some authors **[16, 17]** observed a decrease in serum total protein levels in rats after four weeks cadmium exposure. Moreover, the decrease in serum total protein levels of rats exposed to cadmium for a period of two weeks **[18]**, might be resulted from the increase in the urinary excretion of high molecular weight proteins **[19]**. Cadmium accumulation in the kidney may cause renal dysfunction, and in turn it induces proteinuria. Contrary, the actual result is different than those of **Ajilore et al., [20]** who found an increase of serum total proteins, accompanied by a decrease in liver total proteins of rats exposed to cadmium for four consecutive weeks.

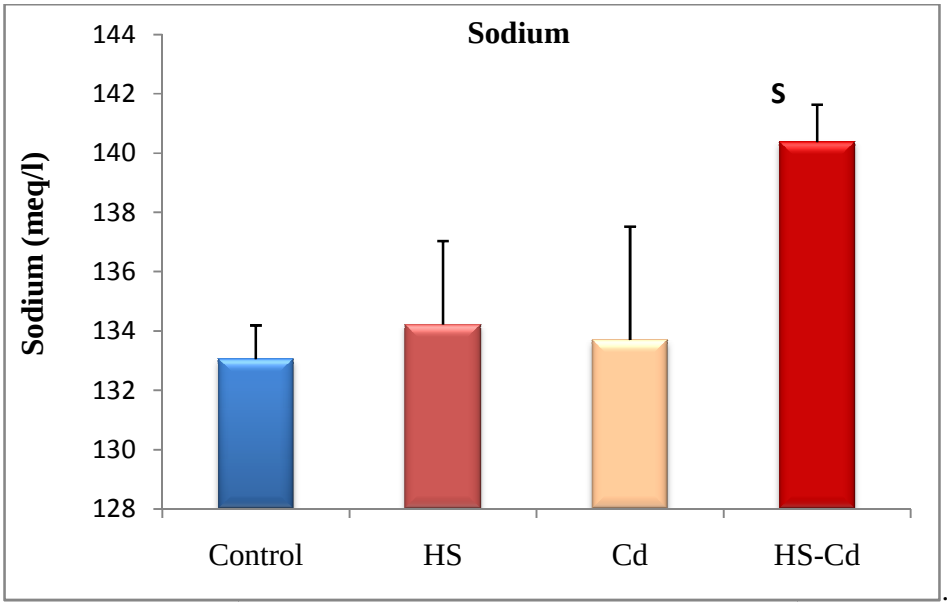

Fig 2: Variation of serum sodium concentration (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control.

The combined stress by HS-Cd has remarkably decreased serum total proteins in rats after one week exposure. Previously, it has been hypothesized that heat stressed ruminants could motivate proteins' catabolism in order to produce energy through the gluconeogenesis **[21]**. Besides, creatinine phosphokinase, urea and even lactate high levels were found to be sensitive indicators for heat stroke in rabbits exposed to temperature above 37°C **[22]**. Therefore, it is possible that the remarkable decrease of total body weights of HSH rats was promoted by proteins' breakdown to produce sufficient energy; especially rats were unable to eat much food under the hard hyperthermic conditions. Furtheremore, plasma proteins were decreased in lambs exposed to 9h/d for 17 days at a temperature of 35 °C **[23]**.

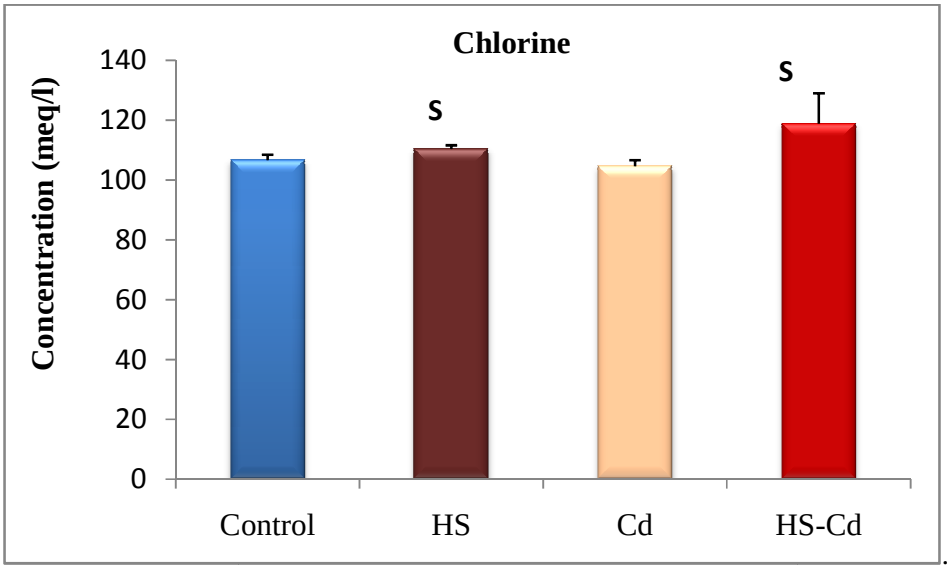

Fig 3: Variation of serum chlorine concentration (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control.

According to the obtained results, serum sodium and chlorine have been disturbed by heat stress, whereas those of potassium and calcium were not changed significantly. In view of that, **Mahiye and Orhan [24]** found a decrease in calcium and an increase in sodium of Japanese quails during heat exposition 6th week. Likewise, lower values of serum calcium were seen in heat stressed rabbits after four weeks exposure **[25]** and in lambs exposed to 9h/d for 17 days at 35 °C **[23]**. The rise in serum chlorine may be resulted from sweating, despite the normal range of serum potassium. Besides, serum sodium, potassium and chlorine concentrations increased in crossbreed cattle under heat stress of up to 42 °C during summer time **[26]**. Elsewhere, serum sodium augmentation was accompanied with potassium diminution, causing however, muscle injury and dysfunction **[27]**. Thus, aldosterone, which rises in the event of fluid diminution, is known to increase sodium and decline potassium in the blood. Furthermore, milk potassium depletion was seen in cows lived in high environmental temperature **[14]**. In the actual study, tiredness is one of the main observed sign in all heat stressed rats. Though, fluid depletion, hypoxia, glycogen metabolism and membrane potential **[27]** could participate in muscles fatigue and the restricted movements of heat stressed rats. Therefore, acidosis and muscle cell damage are amongst many factors that participate in hyperkalemia, while the stimulation of sodium-potassium pump by catecholamines and insulin leads to hypokalemia **[28]**. Hence, metabolic acidosis appears when there is no enough oxygen delivery to the tissues **[29]**. However, as it was recently reported, human skeletal muscles contain the largest single pool of potassium in the body **[28]**. Besides, hypokalemia was only seen in 42 % of men and women, with an average age of 55 years, who suffered from environmental heat stroke, despite the recorded metabolic acidosis **[30].** Moreover, calcium is known to be an essential element for muscle contraction and nervous system function. Though, hypocalcaemia is a common feature in heat stroked persons **[31]**. Sodium chloride represents the biggest portion of sweat, followed by smaller amounts of potassium, calcium, and magnesium **[32]**.

The administration of cadmium chloride for one week showed a noticeable decline of rat's serum calcium, which is in line with the finding of **Abd-El-Baset [33]** who observed a significant decline of this element during cadmium intoxication. Such variation was related to the decreased calcium absorption seen in rats during cadmium exposure **[34]** since cadmium competes with calcium absorption. Furthermore, cadmium may replace calcium in the bones and cause hypercalcuria **[35].**

Concerning the electrolytes, cadmium has not in this experiment changed the levels of serum sodium, chlorine and potassium. Though, blood sodium and chlorine concentrations have been elevated after five weeks of exposure to cadmium **[33]**. At longer period of 6 weeks to cadmium exposure, the electrolyte levels were still higher in rats' serum **[36]**. Also, cadmium fed rats for a low or moderate chronic exposure has had accumulated higher amount of dietary radio-sodium **[37]**. Even after a long exposure to low level cadmium in drinking water for nearly eleven months, water retention was high in both sexes of growing rats **[38]**. However, the high blood pressure was

positively related to water retention only during the first week of cadmium injected male rats **[39]**. Moreover, the latter authors have observed a remarkable decrease in urinary sodium and potassium only during the first three days of cadmium exposure. However, in the present study these two electrolytes were unchanged after one week of exposure, a result which could be in line with **[39]**.

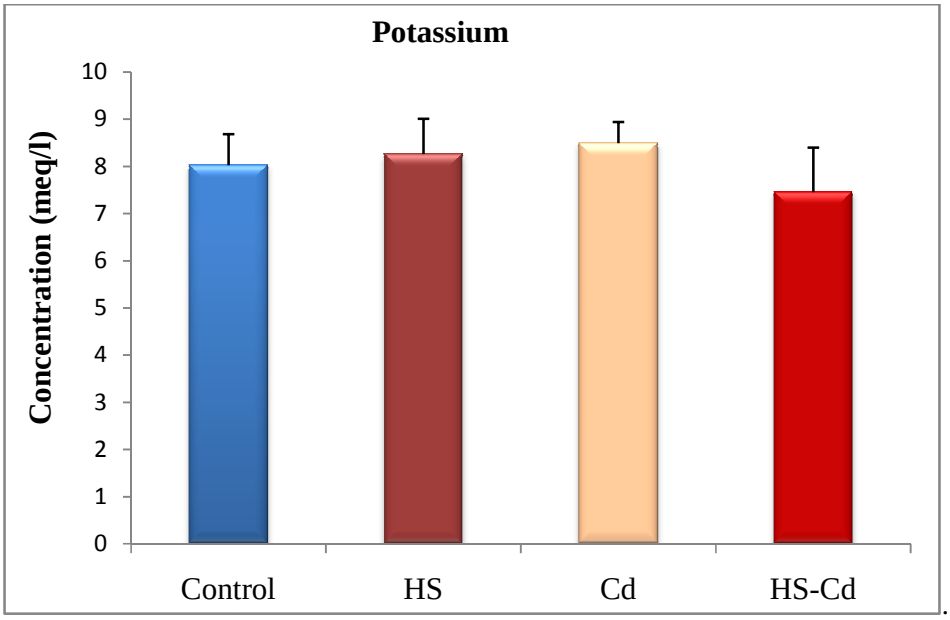

Fig 4: Variation of serum potassium concentration (X±SD) of rats exposed to HS and Cd for 1 week

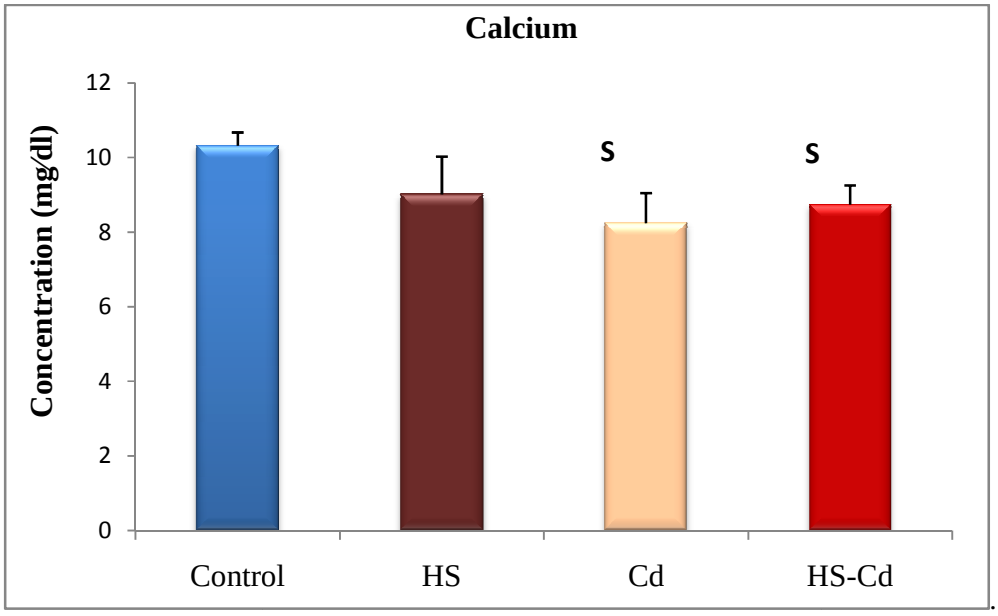

Fig 05: Variation of serum calcium concentration (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control.

Regarding the combined stress (HS-Cd), calcium level has recorded a significant drop, where it nearly followed the same trend as that of HSH group. Thought, water evaporation due to heat stress, and the hypercalcuria provoked by

cadmium intoxication, might contribute in such diminution. However, hypocalcaemia might affect muscle contraction and participates more in muscle weakness and rat fatigue.

Furthermore, in this study, the combined stress has made a noticeable elevation in serum sodium and chlorine concentrations. Accordingly, sodium retention is one of the known effects of cadmium on kidney functions, causing however high blood pressure **[37]**. Hyperthermia could reduce urine volume, simply because most water is being lost via the sweat glands of the skin, which might led to more serum sodium retention.

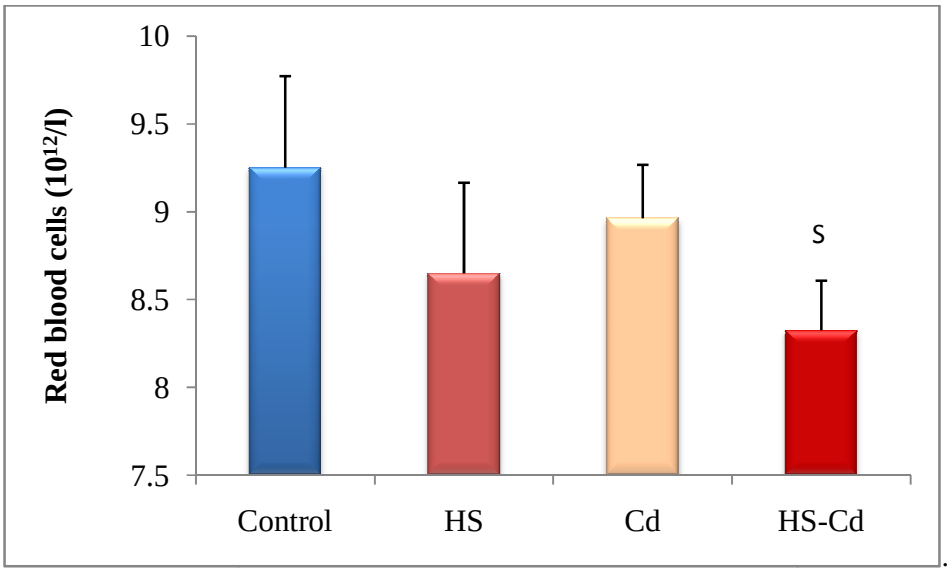

Fig 06: Variation of red blood cell counts (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control.

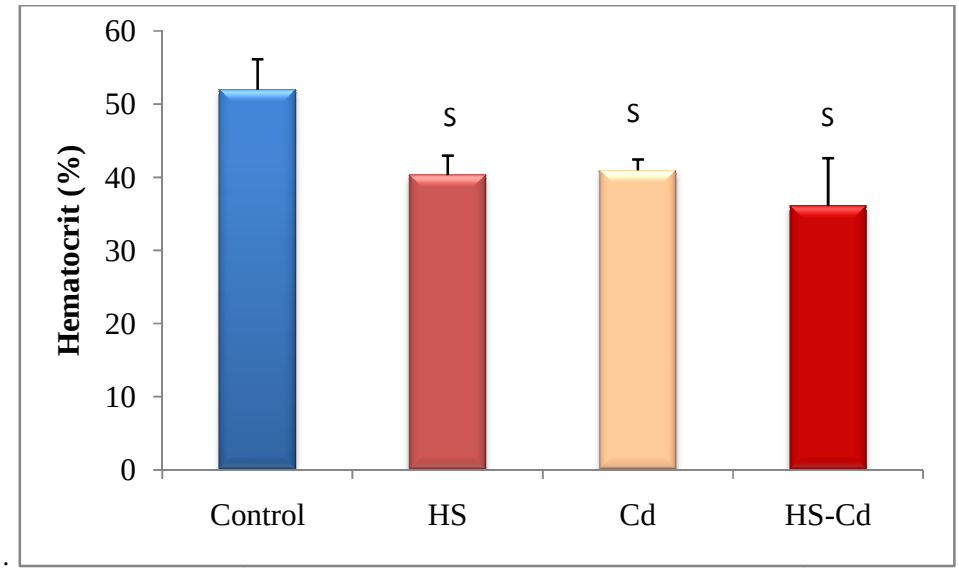

Fig 07: Variation of hematocrit (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at p<0.05 level when compared to the control.

Concerning blood components, the HS-Cd has reduced red blood cell counts significantly, but hemoglobin concentration has not been affected by the three treated groups after one week exposure. The decrease in RBC leads to a shortage in oxygen needs, and that probably why animals were seen to have increased respiratory rate.

Hematocrit on the other hand was altered in all treated groups. Accordingly, the decrease in hematocrit of the combined group is in line with that of RBC, which confirms the increasing needs of the body to oxygen demands. Respiratory rate was mentioned to increase in cattle during elevated temperature of up to 39 °C **[40]**. Moreover, previous studies have observed a remarkable decrease in hematocrit of broiler birds put under 38 °C for three hours **[41]**, for 21 days at 35-41 °C **[42]** and in lambs exposed to 9h/d for 17 days at 35 °C **[23]**. Even though, dehydrated blood was found not to be responsible on the elevation of some serum indicators of humans exposed to heat stroke, at above 40 °C in Sahara desert, as it demonstrated by normal hematocrit level **[30]**. Also blood hemoconcentration of cows was only observed after one day heat stress, but it was normal after a period of one week **[43]**. Beyond that, RBC number, hemoglobin level and hematocrit have been increased in crossbreed cattle under heat stress of up to 42 °C during summer time **[26]**.

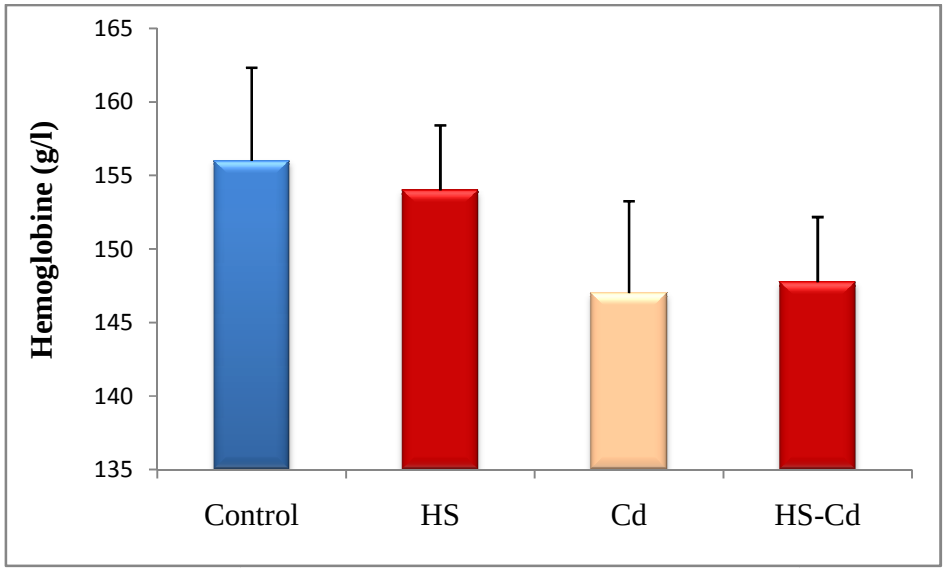

Fig 08: Variation of hemoglobin concentration (X±SD) of rats exposed to HS and Cd for 1 week

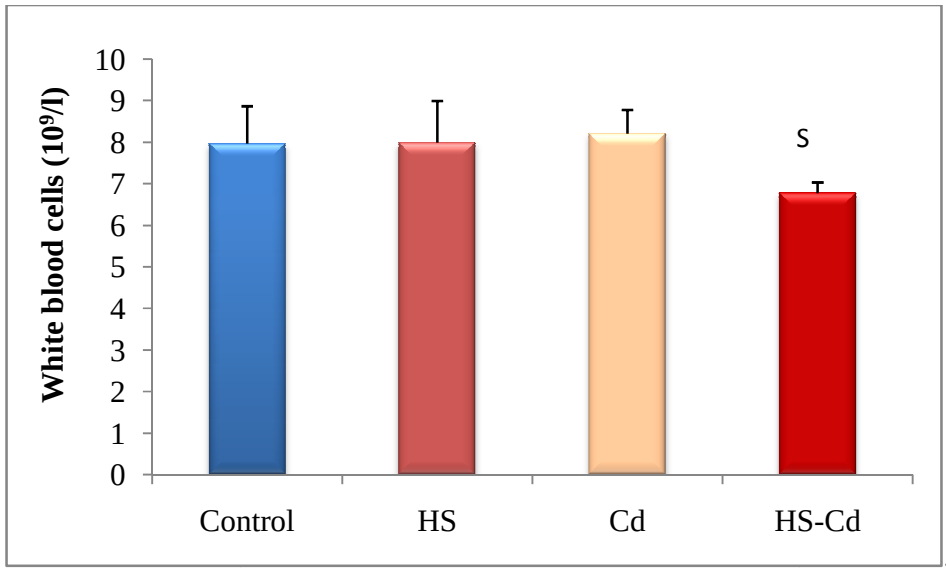

Fig 9: Variation of white blood cell counts (X±SD) of rats exposed to HS and Cd for 1 week
S: significant at $p<0.05$ level when compared to the control.

In this study animals in all groups were possibly hydrated during the exposure, and the observed elevated water consumption throughout the experiment may confirm that. As a result, the decreased hematocrit is likely to come from blood hemodilution. Furthermore, the observed decrease of hematocrit is likely to come from the disturbances of red blood cell turnover. Cadmium was reported to decrease the hematocrit level and provoke splenomegaly 5 days after injecting 1mg cadmium to male rats **[44]**, to cause anemia **[45]** or to have no effect on hemoglobin and hematocrit levels after eight weeks exposure **[46]**.

White blood cell counts were significantly decreased only in the combined stress, which is an indication of the immune system impairment. In view of that, crossbred cattle exposed to temperature of 32 °C for 21 days had lower white blood cell numbers **[47].** Accordingly, leucocytes were remarkably reduced in growing females of New Zealand rabbits at 36 °C **[25]**. In rabbits, leukocyte counts were suppressed by Cd **[48]**, but they were increased by Pb during two week treatment **[49]**. Physiological impairments were reported in rats exposed to cadmium **[50]** and in farmers working in greenhouses in which they have been exposed to continuous combined stress of environmental temperature and pesticides' pollution **[51]**. In reality, biota is under exposure to many stressors, including global warming and industrial pollution, and human activities are the main causes of these changes **[52]**. Such change might affect environmental health deeply and in some cases without return.

CONCLUSION

In conclusion, biological markers of rats have been altered by environmental stressors, especially when exposed to the combined treatment of heat and cadmium.

Acknowledgements
This work was supported by the MATE (ONEDD), Algiers; project code 10/DG/ONEDD/2011.

REFERENCES

[1] J Lelieveld; P Hadjinicolaou; E Kostopoulou; J Chenoweth; M El Maayar; C Giannakopoulos; C Hannides; M Lange; M Tanarhte; E Tyrlis and E Xoplaki. *URL http://dx.doi.org/10.1007/s10584-012-0418-4*, **2012**, 114(3), 667–687.
[2] C Schär and G Jendritzky. *Nature*, **2004**, 432, 559-560.
[3] D Tilman; RM May, CL Lehman and MA Nowak. *Nature*, **1994**, 371, 65–66.
[4] OL Petchey; TP McPhearson; TM Casey and PJ Morin. *Nature*, **1999**, 402, 69-72.
[5] RH Waring; NC Coops and SW Running. *Remote Sens Environ*, **2011**, 115(12), 3554–3566.
[6] TL Root; JT Price; KR Hall; SH Schneider; C Rosenzweig and JA Pounds. *Nature*, **2003**, 421, 57-60.
[7] KE Francl; K Hayhoe; M Saunders and EP Maurer. *J Great Lakes Res*, **2010**, 36, 86-93.
[8] GN Somero . *J Exp Biol*. **2010**, 15, 213(6), 912-20.
[9] GJ Tattersall; BJ Sinclair; PC Withers; PA Fields; F Seebacher; CE Cooper; SK Maloney. *Comp BiochemPhysiol Part A: Molec Integr Physiol*,. **2012**, 129(2-3), 519-525.
[10] JO Daramola; MO Abioja and OM Onagbesan. Heat Stress Impact on Livestock Production *In:* Environmental Stress and Amelioration in Livestock Production. 2012, pp 53-73.
[11] U.S. EPA/*OAP* -U.S. Environmental Protection Agency, Office *of* Atmospheric Programs. Excessive Heat Events. Guidebook.EPA 2006a, 430-B-06-005.
[12] AK Kataria; N Kataria; JS Bhatia and AK Ghosal. *Ind. Vet. J*, **1993**, 70, 761-762.
[13] A Rasooli; M Nouri; GH Khadjeh and A Rasekh. influences of seasonal variations on thyroid activity. *Iranian J Veter Res*, **2004**, 5(2), Ser No 10 1383.
[14] CR Staples and WW Thatcher. Stress in Dairy Animals | Heat Stress: Effects on Milk Production and Composition. Encyclopedia of dairy sciences, Second edition, University of Florida, USA, **2011**; pp. 561–566. http://dx.doi.org/10.1016/B978-0-12-374407-4.00467-2.
[15] G Shwartz; ML Rhoads; MJ VanBaale; RP Rhoads and LH Baumgard. *J. Dairy Sci*, **2009**, 92, 935-942.
[16] N Layachi and Z Kechrid. *African Journal of Biotechnology*, **2012,** 11(93), 16013-16020.
[17] AA Moshtaghie; A Raisi and H Goodarzi. *J Islamic Acad Sci*, **1991,** 4(3), 192-195.
[18] A Samir; BSA Jalila; N Kais; BY Ridha; MH Mona; AE Ahmed; AW Mosaad and O Ridha. *Intern Immunopharmacol*, **2007**, 7, 750-760.
[19] T Christopher. *J Occup Med*, **1991**, 33, 1175–9.
[20] BS Ajilore; TG Atere; WA Oluogun; VA Aderemi. *Intern J Phototherapy res*, **2012**, 2(3), 42-5.

[21] RP Brockman. Pancreatic *and* adrenal hormonal regulation *of* metabolism. *In:* Milligan LP, Grovum WL, Dobson *A editor*. Control *of* digestion *and* metabolism *in* ruminants. Englewood Cliffs, NJ*:* Prentice Hall*;* **1986***;p.* 405.
[22] AM Abdelatif and SM Modawi. *J Therm Biol*, **1994**, 9(6), 357–363.
[23] NE Odongo; O Al-Zahal; MI Lindinger; TF Duffield; EV Valdes ; SP Terrell and BW McBride. *J. Anim. Sci*, **2006**, 84(2), 447-455.
[24] O Mahiye and O Orhan. *Arch. Tierz. Dummerstorf*, **2004**, 47(1), 93-98.
[25] L Ondruska; J Rafay; AB Okab; MA Ayoub; AA Al-Haidary; EM Samara; V Parkanyi; L Chrastinova; R Jurcik; P Massanyi; N Lukac and P Supuka. *Veterinarni Medic*, **2011**, 56(4), 180–186.
[26] F Ferreira; WE Campos and AU Carvalho. *Arq. Bras. Med. Vet. Zootec*, **2009**, 61(4), 769-776.
[27] JP Knochel. Rhabdomyolysis and effects of potassium deficiency on muscle structure and function. Cardiovasc Med. **1978**, 3, 247-*261*.
[28] T Clausen. *Fundament. Clin. Pharmacol*, **2010**, 24, 595-605.
[29] SH Buck and AL Zaritsky. *Pediatrics*, **1989**, 83, 782-784.
[30] AH Alzeer; Mohsen; AF El-Hazmi; AS Warsy; ZA Ansari and MS Yrkendi. *Clinic Chem*, **1997**, 43(7), 1182-1187.
[31] AM Costrini; HA Pitt; AB Gustafson and DE Uddin. *Am J Med*, **1979**, 66, 296.
[32] MN Sawka and SJ Montain. *Amer Soc Clinic Nutr*. **2000**, 72(2), 564s-572s.
[33] Abd-EL-Baset MA Abd EL-Reheem. *Austral J Basic Appl Sci*, **2008**, 2(4), 1438-1453.
[34] S Feldman and R Cousins. *Nutr. Rep. Int*. **1974**, 8, 251-259.
[35] MM Brzóska and J Moniuszko-Jakoniuk. *Bone*, **2004**, 35(5), 1180-91.
[36] UA Ibiam; EI Ugwuja; C Ejeogo; PM Aja; C Afiukwa; OU Oji and AJ Uraku. *J Pharm Biol Sci*, **2012**, 4(1), 48-53.
[37] HM Perry and MW Erlanger. *Sci Total Environ*, **1981**, 22(1), 31–38.
[38] JJ Doyle; RA Bernhoft and HH Sandstead. *J Lab Clin Med*, **1975**, 86(1), 57-63.
[39] **S** Nishiyama; K Nakamura and Y Konishi. *Environ Res*. **1986**, 40(2), 357-64.
[40] GL Hahn and TL Mader. Heat waves in relation to thermoregulation, feeding behavior and mortality of feedlot cattle. *In*: Proc. 5th Intl. Livest. Environ. Symp. (Vol I**).** ASAE SP-01-97, American Society Agricultural Engineers, St. Joseph, MI. **1997**; pp 563-571.
[41] O Altan; A Pabuçcuoğlu; A Altan; S Konyalioğlu and H Bayraktar. *Br Poult Sci*, **2003**, 44(4), 545-50.
[42] R Muchacka; I Skomorucha; E Sosnówka-Czajka; G Formicki1; A Greń and Z Goc1. *J Microbiol, Biotech Food Sci*, **2012**, 2(1), 378-388.
[43] M Koubkova; I Kniwkova; P Kunc; H Hartlova; J Flusser and O Doleszal. *Czech J. Anim. Sci*. **2002**, 47(8), 309–318**.**
[44] M Kunimoto and T Miura. *Environ Res*, **1986**, 39(1), 86-95.
[45] IO Branka ; PZ Slađan ; ŽV Radoslav ; ŠS Andraš ; MD Snežana ; SS Zorica and PM Vojislav. *Kragujevac J Sci*. **2000**, 22, 93-99.
[46] KN Chetty;_ L Drummond and D Desaiah . *J Environ Sci Health B*, **1980**, 15(4), 379-93.
[47] J Morrow-Tesch; N Woolen and L Hahn. *J Therm Biol*, **1996**, 21: l0l-108.
[48] I Abdelaziz; MI Elhabiby and AA Ashour. *Hum Exp Toxicol*, **2013**, 32(4),; *Annals Biol Res*, 2 (3): 389-393.
[49] D Gaurav; S Preet and KK Dua. *Arch of App Sci Res*, **2010**, 2 (1), 390-397.
[50] S Slimani; MS Boulakoud and C Abdennour. *Annals biol res*, **2011**, *2(2)*, 290-297.
[51] J Masih. *Arch of App Sci Res*, **2010**, 2 (2), 100-108.