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Elucidation of the Determinant Factors Affecting Camels' Health in Some Regions of Saudi Arabia: A Biochemical, Histological, and Toxicological Study

Jalaluddin Aazem Jalal, Khalid Ahmed Albuseeda, Taha A. Kumosani, Khaled Omar Aboulnaja and **Mohamed Farouk Elshal**

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract. Recent reports announced by the Saudi Ministry of Agriculture indicate the death of more than 3,000 camels, cows and goats in different parts of the Kingdom over the past months. Laboratories examinations both inside and outside Saudi Arabia suggested that the samples contained poisonous and toxic materials to camels which may affect the physiological processes that may render animals vulnerable to pathogens such as bacteria and viruses. So, we aimed to investigate blood and plasma chemistry including analysis of oxidants and antioxidants parameters as well as heavy metal determination in blood and tissue samples from diseased animals and compare it to normal animals to elucidate the possible cause(s) of the intoxication. Although we could not obtain any samples from diseased animals, results showed that there were non significant changes in biochemical, heavy metals and blood picture in specimens from the normal animals from Jeddah and Taif areas. However, there was a significant elevation in the level of malondialdhyde (lipid peroxidation marker) in brain, liver and serum associated with a significant decrease in total antioxidant activity in specimens from Jeddah area. This imbalance between oxidants and antioxidants as a marker of abnormal changes may lead to disturbances in some physiological processes in the brain and the liver that could render these animals susceptible to infectious diseases and ultimately to death if not treated.

Abbreviations

ROS**;** Reactive oxygen species, RNS; Reactive nitrogen species, NO; Nitric oxide, MCHC; mean corpuscular hemoglobin concentrations, TBARS; Thiobarbituric acid reactive substances, TAOC; Total antioxidant capacity,

OCP; Organochlorine, pesticides, H&E; hematoxylin and eosin stain, ALP; Alkaline phosphatase, ALT; Alanine aminotransaminase, AST; Aspartate aminotransferase, BABR; Bile acid binding resin, CBC; Complete blood count, CK; Creatine kinase, GGT; Gamma-Glutamyl Transpeptidase, LDH; Lactate dehydrogenase, CK; Creatine kinase, CK-MB; Creatine-Kinase monobasic, LYM; Lymphocytes, MON; Monocytes, GRAN; Granulocytes, RBCs; Red blood cells, HCT; Hematocrit, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular, hemoglobin concentration, RDW; RBC distribution width, PLT; Platelets.

Introduction

Recent reports announced by the Saudi Ministry of Agriculture of the deaths of more than 3,000 camels, cows and goats in different parts of the Kingdom over the past months prompted us to study the possible factors that may cause this kind of mysterious death of animals. This can be done by studying the impact of determinant factors capable of producing an adverse response in a biological system, seriously injuring structure or function or inducing cell death.

Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities, and animals are exposed to them through various pathways[1]. Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals, and an increased metal uptake by food crops grown on such contaminated soils is often observed. In general, wastewater contains substantial amounts of harmful toxic chemicals and toxic heavy metals, which are creating problems for animal and agricultural production, respectively[2,3].

Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal uptake by crops, and thus affect food quality and safety[4]. Heavy metal accumulation in soils and plants is of increasing concern because of the potential human health risks. This food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body. Heavy metal accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to-plant transfer factors of the metals[5].

Most of the evidence that links environmental exposure to adverse health effects comes from studies where the effect of a pollutant is linked to a particular disease phenotype. Such approaches are primarily focused in correlating exposure(s) to disease phenotype(s) while everything else between

exposure and disease is neglected. Because of the limitations of such studies in accurately measuring exposure to many pollutants, scientists have developed molecular epidemiological approaches where they have incorporated molecular and cellular markers for improving disease incidence and thus mortality. In constructing biomarkers descriptive of the events in between exposure and disease progression, one has to investigate the mechanistic basis of how exposure to environmental contaminants induces animal disease. In this context, scientists have confirmed the involvement of oxidative stress in the multistage process of environmentally induced disease development; but the exact mechanism(s) are yet to be confirmed.

In order to elucidate the role of environmental exposure on camel health, a full Plasma chemistry, immunological examinations, oxidative stress were done as well as heavy metal determination in blood, and tissue samples from animals recruited from two different environments, namely Jeddah and Taif areas, were carried out.

Materials and Methods

1) Plasma Chemistry

Blood samples (10 mL) for plasma chemistry and hematology were collected from the jugular vein using vacutainer tubes containing sodium heparin. Plasma concentrations of total protein, albumin, globulins, glucose, uric acid, cholesterol, bilirubin, calcium, and phosphorus and activities of amylase, lipase, lactate dehydrogenase, aspartate aminotransferase, γ-glutamyltransferase, and creatine kinase were determined using standard biochemical techniques.

2) The Determination of Heavy Metals

Determination of heavy metals in tissue samples were performed on a hydride attachment to the AAS-30 atomic absorption spectrophotometer using heavy metals-hydride attachment to the Buck AAS-30 atomic absorption spectrophotometer. This method is an integral component of chemical toxicological analysis in expert evaluation of poisonings[6].

3) Hematology

Hematocrit was determined with a Micro-Capillary Reader after a 5-min centrifugation[6]. Hemoglobin was measured as cyanomethemoglobin with a Hemoglobinometer[7] after the red blood cells were lysed. In addition, mean corpuscular hemoglobin concentrations (MCHC) was calculated as MCHC = hemoglobin/hematocrit.

Differential white blood cell counts was performed using blood smears stained with modified Wright's stain using the Hematek Stain Pak. One hundred white blood cells was examined per animal using a Nikon microscope set at 400×magnification, and heterophils, lymphocytes, monocytes, eosinophils, and basophils were identified. In addition, total counts of heterophils and eosinophils were made with a Neubauer hemacytometer and a Nikon microscope set at 100×magnification. Numbers of lymphocytes, monocytes, and basophils were determined by calculation.

4) Oxidative Stress

Lipid peroxide level in the liver homogenate was estimated by the production of Thiobarbituric acid reactive substances (TBARS) according to the method described by (7). Plasma total antioxidant capacity (TAOC) was measured after a 1:1 dilution with a spectrophotometer (Shimadzu UV-1601), on which absorbance values were recorded over 3 min, according to the method of Armstrong and Browne[7]. The Folin reagent was used to measure protein concentration at 540 nm[8].

5) Detection of Pesticide

The presence of Organochlorine pesticides (OCPs) (mainly-HCH, -HCH, -HCH, DDT, DDD, DDE, and isomers) in samples from diseased animals must be emphasized. Identification and quantification of pesticide residues in samples is generally carried out by gas chromatography[9].

6) Histopathology

Tissues and rumen contents from all animals necropsied were submitted either fresh or frozen for laboratory testing. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4mm, stained with hematoxylin and eosin (HE), and examined by light microscopy. Selected sections of affected skeletal and cardiac muscles were stained with Masson's trichrome[10].

Statistical Analysis

SPSS computer program was used to analyze the data. A P value < 0.05 was *considered* statistically significant, and a P *value* < 0.01 was *considered* statistically highly significant.

Results and Discussion

Some of the most dangerous emerging diseases facing society today are directly related to exposures to deadly environmental toxins. Of particular concern are oxidative (damaging) toxins from pesticides and heavy metals. Both national and international public health agencies aim to prevent health problems

induced by toxic chemicals and/or their heavy metals found in the environment. Such exposures have been linked primarily with environmental and occupational sources. In fact, nutrition makes up 40% of what we know as environmental sources of animal exposure. Moreover, there are influences induced by demographic factors that further contribute to the etiology of environmentally induced animal disease. Therefore, it becomes of outmost importance not only to identify these potential harmful sources but also to be able to understand the mechanistic basis of their progression to disease.

The presence of Organochlorine pesticides (OCPs) (mainly-HCH, -HCH, -HCH, DDT, DDD, DDE, and isomers) in fodders as well as in samples from diseased animals must be emphasized, because OCPs have been restricted or banned for agriculture use since 1978 in the USA and Europe, due to their persistence and bioaccumulation in the environment. However, these pesticides are still frequently found in soils, from which they continue to cycle through the environment. Pesticide standards must be protected from light and the solvent used to prepare them must be carefully chosen based on literature or experimentally checked in order to avoid standards degradation. Identification and quantification of pesticide residues in samples is generally carried out by gas chromatography[(9)]. Non of the pesticides or toxins were detected in blood, liver and muscles of normal camels from Jeddah and Taif.

Heavy metals accumulate in various tissues and are associated with increases in today's biggest killers: Cardiovascular disease and cancer. Reducing these heavy metals from the body has been a challenge to modern day medicine. Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis[11]. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno and/or propano adducts)[12]. Whilst iron (Fe), copper (Cu), chromium (Cr), vanadium (V) and cobalt (Co) undergo redox-cycling reactions, for a second group of metals, mercury (Hg), cadmium (Cd) and nickel (Ni), the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins[13-15]. Arsenic (As) is thought to bind directly to critical thiols, however, other mechanisms, involving formation of hydrogen peroxide under physiological conditions, have been proposed[16,17]. The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of reactive oxygen and nitrogen species[18]. Common mechanisms involving the Fenton reaction, generation of the superoxide radical and the hydroxyl radical appear to be involved for iron, copper, chromium, vanadium and cobalt primarily associated with mitochondria, microsomes and peroxisomes[19].

However, a recent discovery that the upperlimit of "free pools" of copper is far less than a single atom per cell casts serious doubt on the in vivo role of copper in Fenton-like generation of free radicals. Nitric oxide (NO) seems to be involved in arsenite-induced DNA damage and pyrimidine excision inhibition[20].

In the present study, there are no statistical significant difference was found in serum heavy metals (ppm) between normal camels of Jeddah and Taif in animals.

Oxidative stress, defined as the imbalance between oxidant and antioxidants in favor of the oxidants, potentially leading to damage, has been associated with a number of diseases[21,22]. Moreover, it was found that oxidative stress is associated with abnormal changes that may lead to disturbances in some physiological processes in the brain and the liver that could render these animals susceptible to infectious diseases and ultimately to death if not treated either in human or animals[23-25].

With the term “oxidative stress” we refer to a cellular state emerging from an excessive generation of oxidants overcoming our antioxidant capability in metabolizing them. These oxidants are often referred to as “free radical species” implying the presence of one or more unpaired electrons. This characteristic confers them with an “unstable” chemical nature capable of highly reacting with major cellular macromolecules like DNA, RNA, proteins and lipids[26-28]. Free radicals include both Reactive Oxygen (ROS) and Nitrogen (RNS) Species and their production can be from endogenous as well as exogenous sources. For instance, endogenous sources include those of mitochondrial oxidative metabolism, P450 metabolizing system, inflammatory cell activation, *etc*., whereas exogenous sources include those of radiation, ozone, various industrial chemicals and xenobiotics[29]. It is obvious therefore that most environmental and occupational settings are capable of generating free radicals that can mediate progression to human disease[28]. Such mediation has been proposed to occur primarily by the ability to metabolize these free radical species and thus generate secondary molecules that are even more toxic and harmful. For instance, only the last few years scientists have established that free radicals are important molecules involved in the pathophysiology of many diseases including those of cancer, lung, heart disease, *etc*[30-32].

Antioxidants (both enzymatic and non-enzymatic) provide protection against deleterious metal-mediated free radical attacks. Vitamin E and melatonin can prevent the majority of metal-mediated (iron, copper, cadmium) damage both in vitro systems and in metal-loaded animals. Toxicity studies involving chromium have shown that the protective effect of vitamin E against lipid peroxidation may be associated rather with the level of non-enzymatic

antioxidants than the activity of enzymatic antioxidants. However, a very recent epidemiological study has shown that a daily intake of vitamin E of more than 400 IU increases the risk of death and should be avoided[33]. While previous studies have proposed a deleterious pro-oxidant effect of vitamin C (ascorbate) in the presence of iron (or copper), recent results have shown that even in the presence of redox-active iron (or copper) and hydrogen peroxide, ascorbate acts as an antioxidant that prevents lipid peroxidation and does not promote protein oxidation in humans *in vitro*[34]. The impact of zinc (Zn) on the immune system, and the ability of zinc to act as an antioxidant in order to reduce oxidative stress was also reported[35].

A major defense mechanism involves the antioxidant enzymes including SOD and glutothoine peroxidase which convert active oxygen molecules into non–toxic compounds. As in other mammals, camel tissues contain a powerful antioxidant system. The liver has the highest contents of antioxidants and antioxidant enzymes indicating that it plays an important role in pro-oxidants detoxification[36].

Table 1. Statistical analysis of Serum Heavy Metals (ppm) in normal camels of Jeddah and Taif, (mean ± S.D.).

Parameter	Normal Jeddah Group	Normal Taif Group	t-test	p- value
Lead	3.73±1.21	3.49±1.06	0.4208	N.S.
Arsenic	0.36±0.12	0.34±0.23	0.1898	N.S.
Mercury	0.27±0.04	0.27±0.05	0.1520	N.S.
Cadmium	0.08±0.06	0.07±0.03	0.2444	N.S.
Antimony	0.00±0.00	0.00±0.00	----	N.S.

In the present study there was a significant elevation of the level of malonaldhyde and a significant decrease in total antioxidant activity in camels of Jeddah than that of Taif (Table 2).

Table 2. Statistical analysis of Serum and Tissue Malondialdhyde (MDA) and total antioxidant activity in normal camels of Jeddah and Taif, (mean ± S.D).

Parameter	**Animals from Jeddah area**	**Animals from Taif area**	**t-test**	***p*- value**
Brain MDA	29±3.1	21±1.1	0.4208	**p< 0.01 .**
Liver MDA	46±4.3	32±2.9	0.1898	**p< 0.05**
Serum MDA	38±2.9	21±2.0	0.1520	**p< 0.05**
Total antioxidant activity	17±1.5	25±1.3	0.2444	**p< 0.01**

In the present study, liver functions, kidney functions, lipid profile did not show any difference between serum camel of Jeddah and Taif (Table 3). A

significant increase in plasma urea nitrogen (PUN) was found Jeddah animals in comparison with Taif animal. Plasma urea nitrogen was reported significantly higher in the one-humped camel (Camelus dromedarius) feed with foodstuff contaminated by aflatoxin[37]. Therefore, these results suggest that animals from Jeddah area affected with the increase in lipid peroxidation and reduced anti-oxidant activity.

Table 3. Statistical analysis of liver function and kidney function tests, Serum Lipid Profile and other Biochemical activity in normal camels of Jeddah and Taif, (mean ± S.D.).

Parameters		Normal Jeddah Group	Normal Taif Group	t-test	p- value
Liver Function tests	ALP (u / l)	106.25±27.59	110.15±23.23	0.4418	N.S.
	ALT (u / l)	54.07±25.52	60.26±16.60	0.6164	N.S.
	AST (u / l)	31.19±8.70	35.45±12.12	0.781341	N.S.
	GGT (u / l)	44.54±14.99	49.56±14.38	0.874940	N.S
	Total Bilirubin (mg / dl)	0.65±0.13	0.78±0.21	0.3267	N.S.
	Direct Bilirubin (mg /dl)	0.26±0.09	0.25±0.13	0.0964	N.S.
	Total Protein (g / dl)	7.58±0.84	8.44±0.58	0.3645	N.S.
	Urea nitrogen (mmol / l)	7.13±1.05	4.74±0.97	3.6375	p< 0.001
	Albumin (g / dl)	4.38±0.65	5.08±0.40	1.0754	N.S.
Kidney Function tests	Uric Acid (mg / dl)	5.89±0.97	7.32±1.24	1.0032	N.S.
	Creatinine (umol / l)	86.02±22.86	95.69±12.76	0.0619	N.S.
	sodium (mmol / l)	141.29±2.52	149.78±3.40	1.812	N.S.
	potassium (mmol / l)	4.37±0.6121	5.31±0.40	0.4385	N.S.
	Calcium(mg / dl)	9.25±0.440	10.32±0.63	0.290	N.S.
	Chloride (mmol / l)	101.46±1.64	112.57±2.62	1.679	N.S.
	Phosphorous (mmol / l)	----	2.10±0.13	----	----
Lipid Profile	Total Cholesterol (mmol/ l)	4.96±0.86	5.67±0.66	1.3250	N.S.
	HDL-C (mg /dl)	44.29±7.62	45.60±5.92	1.4393	N.S.
	LDL-C (mg / dl)	127.37±34.022	126.70±16.72	2.8368	N.S
	Triglyceride (mg / dl)	125.33±61.25	175.33±61.25	0.8053	N.S.
Others	Glucose (mmol / l)	5.64±1.72	7.78±1.73	0.2789	N.S.
	Cortisol (nmol / l)	380.70±114.06	430.7± 136.28	0.4892	N.S.
	CK (u / l)	183.54±93.73	143.95±33.47	3.36134	p< 0.05
	LDH (u / l)	241.82±124.40	165.17±21.63	3.31891	p< 0.05

LDH and CK LDH is a cytoplasmic enzyme with a high activity in heart, skeletal muscle, liver, kidney, and red blood cells. These enzymes are indicators

of a higher level of cellular damage and their increased activity is a consequence of their increased release from the damaged cells and a reflection of metabolic changes in the inflamed tissues especially in the heart[38,39]. The higher activities of CK and LDH in animal samples from Jeddah may indicate such problems.

Seasonal variations have been suggested to affect the hematological and serum biochemical parameters in camels. Leukocyte count, erythrocyte count and haemoglobin levels did not differ significantly during different seasons, but hematocrit values were significantly higher ($P<0.01$) during summer due to increased mean corpuscular volume of erythrocytes. Total proteins, albumin, creatine kinase and creatine values were similar ($P<0.01$) during summer and winter. However, statistically significant ($P<0.01$) seasonal variations were observed in serum levels of glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and iron. GOT, BUN and iron levels were higher in winter while LDH was higher in summer[40].

In the present study, all samples were obtained in the same period of the year. No statistical significant difference was found in hematological parameters studied in blood specimens from animals from Jeddah and Taif areas (Table 4). This finding indicate that, at least there is no abnormalities in hematological parameters during the time of the present study.

Table 4. Statistical analysis of complete blood cell count in normal camels of Jeddah and Taif.

Parameter	Normal Jeddah Group	Normal Taif Group	t-test	P- value
WBC (k / ul)	7.33±1.72	7.91±1.93	1.486	N.S.
LYM	3.04±1.05	3.59±0.72	2.166	N.S.
LYM (%L)	33.78±12.66	34.60±9.12	1.548	N.S.
GRAN	4.64±1.46	4.03±1.54	0.665	N.S.
GRAN (%G)	54.07±9.42	56.11±10.50	0.517	N.S.
RBC (m / ul)	6.46±0.51	6.42±0.41	0.288	N.S.
HCT (%)	49.90±3.79	46.18±3.02	1.332	N.S.
MCV (fl)	80.55±7.12	83.34±5.06	1.618	N.S.
MCH (pg)	30.55±2.92	31.07±2.09	2.131	N.S
MCHC (g / dl)	32.55±0.86	34.21±0.73	2.912	N.S
RDW (%)	15.86±1.43	14.56±0.91	3.858	N.S
PLT (k / ul)	275.00±71.80	277.56±53.89	1.267	N.S.

Histopathological examinations revealed normal arrangement of the cell of different tissues (liver, heart, brain, spleen, muscle) with clear nuclei and Pathological conditions of lungs, particularly pneumonia in farm animals may

result in severe reduction in production and even terminate in death causing economic losses to the farmers. Literature revealed only sparse reports about pathological changes and associated bacterial isolation from the camel lungs (36,40). Examination of the histological slides from animals either from Jeddah or Taif showed no evidence of pathological changes.

Conclusions

Although we could not obtain any sample from the diseased camels, the measurements of oxidants and antioxidant molecules in normal animals revealed significant differences between the two groups of animals from Jeddah and Taif areas. These differences may emphasize the impact of environment as reflected by the imbalance occurred in the oxidants-antioxidants system that considered a marker of abnormal changes and could lead to disturbances in some physiological processes in the brain and the liver rendering these animals susceptible to infectious diseases and ultimately to death if not treated. Finally, we recommend instead of focusing on crisis management and a reducing of determinant factors affecting animal health, there is a need to concentrate on prevention.

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استدلال الأسباب التي تؤدى إلى نفوق الإبل باستخدام طرق كيموحيوية، ميكروبية سمية ومناعية

جلال الدين أعظم جلال، وخالد أحمد البوسعدة، وطه عبدالله قمصانى وخالد عمر أبو النجا، ومحمد فاروق الشال

قسم الكيمياء الحيوية، كلية العلوم، جامعة الملك عبدالعزيز جدة – المملكة العربية السعودية

المستخلص. التقارير الأخيرة التي أعلنتها وزارة الزراعة السعودية تشير إلى نفوق أكثر من ٣٠٠٠ رأس من الإبل والبقر والماعز في مختلف أنحاء المملكة خلال الأشهر الماضية. و قد أشارت فحوص المختبرات داخل وخارج السعودية أن العينات المأخوذة من الحيوانات تحتوي على مواد سامة إلى الإبل التي قد تؤثر على العمليات الحيوية بجسم الحيوانات مما تجعلها عرضة للعوامل المعدية مثل البكتيريا والفيروسات. ولذلك كان هدف البحث هو توضيح سبب التسمم بإجراء دراسة كاملة تشمل تحليل المعادن الثقيلة في الدم، والاختبارات الكيموحيوية للبلازما، ودراسة الأنسجة من عينات الحيوانات السليمة، ومقارنتها بعينات من الحيوانات المصابة إن وجدت. بالرغم من تعذر الحصول على عينات من الحيوانات المصابة إلا أن النتائج قد أظهرت عدم اختلاف في أي من التحاليل الكيموحيوية، والمعادن الثقيلة، وصورة الدم في العينات المأخوذة من الحيوانات السليمة بمنطقتي جدة والطائف. كما أظهرت النتائج وجود ارتفاع ملحوظ في مستوى المالونالدهيد في المخ، والكبد، والدم ونقص في مستوى مضادات الأكسدة في مصل الدم لهذه الحيوانات، مما يدل على عدم وجود اتزان بين عمليات الأكسدة ومضادات الأكسدة بجسم هذه

الحيوانات، مما قد يؤدي إلى ظهور خلل في بعض الوظائف الحيوية، وبالتالي تكون عرضة للإصابات الميكروبية، وبعض الأمراض التي قد تسبب نفوق الإبل.