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Review Article

Research performance evaluation and citation in the Arabic countries

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Abstract

There are a vast number of journals in different disciplines issued in the Arabic language, however the majority of these journals are not currently indexed internationally or provide abstracts. This short communication presents information about the ranking of the Arabic countries in regard to published documents, citations and H-index to the period from 1996-2011 according SJR SCIMAGO LAB powered by Scopus, in addition to presenting the numbers of veterinary sciences documents that were published from 1996-2011 in each country, and the name of journals that included in the Scopus index, and originated from different Arabic countries.

Key words: Algeria, Egypt, Iraq, research performance, H-Index

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Introduction

Research is defined as the establishment of new knowledge and/or the use of current and existing knowledge in a new and innovative way in order to generate new concepts, methodologies and understandings. This could include synthesis and analysis of previous research to the extent that it leads to new and innovative outcomes.

Recently the numbers of governmental and private universities have heavily increased in different Arabic countries, which have led to a significant increase in scientific research activities. It is important to evaluate the academic performances and research activities in these universities. The predominant reason for evaluating academic research is the quality assurance of the research, as well as to improve research and research policy, by giving feedback to the academic community. This is because research is a central function and the university must evaluate its performance. Research activity in universities and research institutes is evaluated both internally and externally. This short communication is designed to

introduce a short review about the ranking of Arabic countries according to published documents, citations and H-index from 1996-2011 presented by SJR SCIMAGO LAB powered by Scopus for all countries in the world. In addition, the number of veterinary sciences documents that were published from 1996-2011 in each country, are presented, as well as the name of journals that included in the Scopus index, and originated from different Arabic countries.

Research evaluation

Research evaluations are based on expert evaluation. Recently, the publishing and citation data in the form of bibliometric indicators is becoming increasingly common to use. The fact that bibliometric indicators have become more common in evaluation can be explained by manageability and ease of access. Even though indicators are often seen as objective measures, and many of them are well known and widely used, one should interpret them with caution. All indicator calculation methods and databases that form the foundation for referencing information have limitations, and these limitations can be misleading. Hence, quantitative information produced by bibliometric indicators should only be used as a supplement to research activity evaluation by experts.

Currently, there are three major factors that are used to evaluate academic performances: the number of publications, the impact factor of those journals (Al-Salihi, 2012; Eric and Vincent, 2009), and the H factor (Hirsch, 2005). Leo Egghe, 2006, has suggested what is called the g-index. This index is used to quantify scientific productivity based on the publication record and is calculated based on the distribution of citations received by a given researcher's publications.

A list of over 25,000 journals is maintained by the Institute of Science Index (ISI). The list includes over 1200 arts and humanities journals as well as scientific journals. Listing is based on published selection criteria and is an important indicator of journal quality and impact (http://www.isi-thonsomreuters.org/).

There are a vast numbers of journals in different discipline issued in the Arabic language, however the majority of these journals are not currently indexed internationally or provide abstracts. Mapping the Arabic regional journals is the best way, which will increase the exposure of these journals at national, regional and at the global levels. In order to map the Arabic journals a building of a new model system index is required. Two simple indices to classify journals, published in the Arabic language, and different researchers had been suggested by Abdel-Aty, 2011. These indices depend upon the known impact factor and h-index. The new indices give an easy way to judge the rank of any journal (output of any researcher) without looking for other journals (output of other researchers) (Abdel-Aty, 2011). However, actual applications of these new indices are not yet used in the evaluation of research output and publications.

Journal and Country Ranking was developed by SCImago according to the information contained in the Scopus® database (Elsevier B.V.). These indicators can be used to assess and analyze scientific domains. SJR SCIMAGO LAB powered by Scopus was presented the ranking of all countries in the world. The data showed that the Arabic countries situated in different locations according to published documents, citations and H-index from 1996-2011(Table 1). The number of veterinary sciences documents that published from 1996-2011 in each country is also presented (Table 2). Although Iraq is situated at the place 93 and 11 globally and regionally respectively, but it is the seventh Arab country in respect to research published in Veterinary Science (Table 3 and Fig 1). There are 74 Arabic journals from different countries that are included in the Scopus index (Table 4).

No.	Rank according SJR SCIMAGO LAB powered by Scopus	Country	Documents	Citable documents	Citations	Self-Citations	Citations per Document	H- index
1	41	Egypt	75.610	73.968	438.912	91.957	7,23	122
2	48	Saudi Arabia	46.167	44.089	241.843	35.926	6,82	114
3	52	Tunisia	32.250	30.884	141.848	32.694	6,65	80
4	55	Morocco	23.446	22.480	135.411	25.033	6,82	90
5	59	Algeria	21.059	20.770	88.422	17.264	6,34	74
6	61	Jordan	17.126	16.807	90.151	13.333	6,83	72
7	65	United Arab Emirates	15.698	15.039	83.109	9.530	7,36	81
8	68	Kuwait	12.254	11.943	80.980	11.653	7,43	77
9	69	Lebanon	11.672	10.852	82.250	8.564	9,39	91
10	80	Oman	6.875	6.542	36.901	4.770	7,02	58
11	93	Iraq	4.420	4.170	11.812	1.378	4,53	37
12	95	Qatar	4.398	4.196	18.382	1.923	5,55	44
13	100	Sudan	3.384	3.273	21.343	3.214	8,99	48
14	101	Syrian Arab Republic	3.379	3.288	24.751	3.341	9,53	53
15	109	Bahrain	2.817	2.624	11.059	1.225	4,98	36
16	110	Libyan Arab Jamahiriya	2.304	2.236	7.428	465	4,79	32
17	112	Palestine	2.273	2.202	11.764	1.852	7,68	41
18	122	Yemen	1.395	1.350	7.259	841	7,46	34
19	169	Mauritania	292	283	2.188	114	8,56	22
20	191	Djibouti	109	99	567	30	6,47	12
21	209	Comoros	50	47	377	24	7,67	8

Table 1. Ranking of Arabic countries according to published documents, citations and H-index to period from 1996-2011. (According SJR SCIMAGO LAB powered by Scopus) (http://www.scimagojr.com/countryrank.php)

Conclusion

Research performance evaluation and citation in the Arabic countries requires the building of a new model system index. The research activity evaluation is based on several evaluation methods, which are chosen to be relevant to the evaluation situations. The Arabic countries appeared to be situated in different places within the SJR SCIMAGO ranking list. Iraq located at the seventh place within the Arabic countries in regard to veterinary publications. Seventy four Arabic journals are included in the Scopus index in respect to their country of origins.

Country	Year from 1996-2012																
	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	Total
Egypt	31	34	32	32	16	29	24	20	25	31	37	74	90	97	105	157	834
Saudia Arabia	20	16	17	19	16	24	20	18	18	15	11	13	22	28	45	45	347
Tunisia	7	4	7	12	11	5	9	11	17	17	18	14	20	24	24	29	229
Morocco	10	11	7	9	6	5	14	11	10	11	7	10	6	7	5	15	144
Algeria	2	-	3	1	-	-	1	4	-	3	4	10	9	14	8	16	75
Jordan	6	12	15	13	10	8	11	9	15	8	20	23	35	26	14	20	245
United Arab Emirates	20	9	16	8	11	14	2	13	15	16	23	17	18	11	12	12	217
Kuwait	-	1	6	7	4	-	4	2	3	6	3	2	4	5	6	5	58
Lebanon	3	3	1	1	-	5	1	1	2	1	1	1	-	1	9	8	38
Oman	3	1	3	6	5	2	3	5	3	7	2	3	5	2	8	2	60
Iraq	9	4	3	4	1	3	2	2	2	5	5	2	13	29	42	46	172
Qatar	-	-	-	-	-	-	2	-	1	1	3	4	1	1	4	4	21
Sudan	4	8	11	9	5	5	9	14	11	14	17	14	26	31	34	22	234
Syrian Arab Republic	5	2	-	5	1	1	-	5	4	2	3	6	4	8	20	18	84
Bahrain	1	-	1	1	-	-	-	-	1	-	-	-	-	-	-	-	3
Libyan Arab Jamahiriya	1	-	-	3	-	1	1	3	1	-	2	2	-	2	3	7	26
Palestine	-	-	-	-	1	1	-	-	-	-	-	3	2	3	1	3	14
Yemen	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	2
Mauritania	1	1	-	2	2	1	-	-	-	2	1	-	1	-	1	-	12
Djibouti	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Comoros	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1

Table.2: shows the published documents in veterinary sciences from 1996-2011 according to SJR SCIMAGO LAB powered by Scopus

Country	Total number of publication in veterinary sciences from 1996-2011	Country	Total number of publication in veterinary sciences from 1996-2011
1. Egypt	834	2. Saudia Arabia	347
3. Jordan	245	4. Sudan	234
5. Tunisia	229	6. United Arab Emirates	217
7. Iraq	172	8. Morocco	144
9. Syrian Arab Republic	84	10. Algeria	75
11. Oman	60	12. Kuwait	58
13. Lebanon	38	14. Libyan Arab Jamahiriya	26
15. Qatar	21	16. Palestine	14
17. Mauritania	12	18. Bahrain	3
19. Yemen	2	20. Djibouti	2
21. Comoros	1		

Table 3: shows the ranking of Arabic countries according to published documents in veterinary sciences from 1996-2011 depending on the information presented in SJR SCIMAGO LAB powered by Scopus

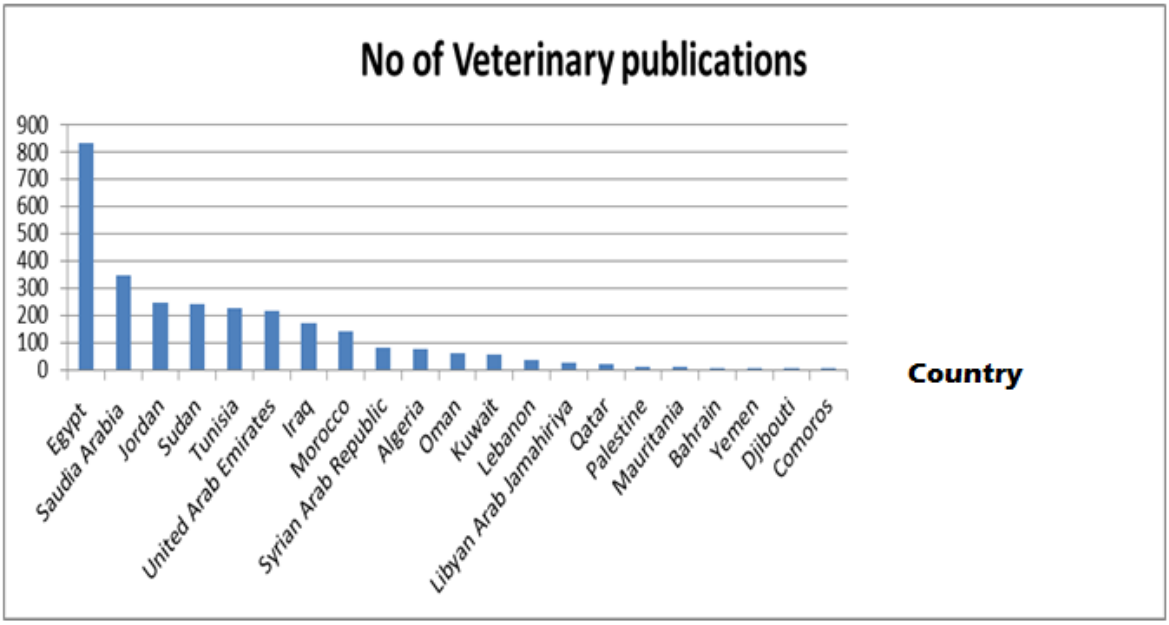

Fig1: Shows the descending distributions of Arabic countries in regards to number of published documents in veterinary sciences from 1996-2011.

Country	Name of journal	Country	Name of journal
1. Egypt	1.Advances in Human-Computer Interaction 2. Interdisciplinary Perspectives on Infectious Diseases. 3. Advances in Hematology 4. Advances in Tribology 5. Journal of Tropical Medicine 6. Egyptian Journal of Biological Pest Control 7. Journal of Environmental and Public Health 8.Journal of the Egyptian Society of Parasitology 9. Advances in Bioinformatics 10.International Journal of Telemedicine and Applications 11. Journal of Toxicology 12. International Journal of Antennas and Propagation 13.AEJ - Alexandria Engineering Journal 14. Anesthesiology Research and Practice 15.Journal of Engineering and Applied Science 16.Journal of the Egyptian Public Health Association, The 17. Egyptian journal of immunology / Egyptian Association of Immunologists, The 18. Middle East Fertility Society Journal 19. Egyptian Journal of Neurology, Psychiatry and Neurosurgery 20. Egyptian Journal of Anaesthesia	2. Saudi Arabia	1. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 2. Saudi Medical Journal 3. Hematology/ Oncology and Stem Cell Therapy 4. Journal of King Abdulaziz University, Earth Sciences 5. Journal of the Saudi Heart Association 6. Neurosciences 7. Journal of King Abdulaziz University, Marine Science 8. Scientific Journal of King Faisal University 9. Pan Arab Journal of Neurosurgery 10. King Fahd University of Petroleum and Minerals Research Institute Annual Catalysts in Petroleum Refining and Petrochemicals Symposium Papers To be continued----------

3. Tunisia	1.Tunisie Medicale 2. Archives de l'Institut Pasteur de Tunis	4. Morocco	1.Physical and Chemical News 2.Malta Medical Journal 3.Journal of Mediterranean Studies
5.Jordan	1.Advances in Environmental Biology 2.Jordan Journal of Mechanical and Industrial Engineering 3.Advances in Natural and Applied Sciences 4.International Arab Journal of Information Technology 5.American-Eurasian Journal of Sustainable Agriculture 6.Jordan Journal of Pharmaceutical Sciences 7.Jordan Medical Journal	6.United Arab Emirates	1.Iranian Red Crescent Medical Journal 2.Open Dentistry Journal 3.Current Aging Science 4.Recent patents on food, nutrition & agriculture 5.Current Molecular Pharmacology 6.International Journal of Diabetes and Metabolism 7.Open Neuroscience Journal 8.Open Chemical and Biomedical Methods Journal 9.Open Applied Mathematics Journal 10.Open Automation and Control Systems Journal 11.Open Mathematics Journal 12. Open Medical Devices Journal 13.Open Glycoscience, 14. Open Toxinology Journal
7.Kuwait	1.Kuwait Medical Journal 2.Arab Journal for the Humanities 3.Journal of the Social Sciences 4.gulf journal of oncology, The 5.Kuwait Journal of Science and Engineering	8.Lebanon	1.Middle East Journal of Anesthesiology 2.Journal Medical Libanais 3.Jamahiriya Medical Journal
9. Oman	1.Sultan Qaboos University Medical Journal 2.Journal of Engineering Research	10.Iraq	1.New Iraqi Journal of Medicine 2.Iraqi Journal of Veterinary Sciences 3.Arab Gulf Journal of Scientific Research
11.Qatar	1.Qatar Medical Journal	12.Sudan	1.Arab journal of nephrology and transplantation
13.Syrian Arab Republic	No journal reported	14.Bahrain	1.Bahrain Medical Bulletin 2.GeoArabia 3.Journal of the Bahrain Medical Society
15.Libyan Arab Jamahiriya	No journal reported	16.Yemen	No journal reported
Algeria	No journal reported	Palestine	No journal reported
Mauritania	No journal reported	Djibouti	No journal reported
Comoros	No journal reported		

Table 4: Shows the journals from different Arabic countries that are included in the Scopus index.

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Mirror of Research in Veterinary Sciences and Animals (MRVSA)

Original Article

Measurement of antibody titers against Newcastle disease vaccines by Elisa and hemagglutination inhibition test using different methods of administration in Broiler chicks

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Abstract

Poultry vaccines were widely applied to prevent and control contagious viral diseases. Antibody response produced by Newcastle diseases(ND) virus vaccine which have been given by different routes of administration in broiler chicks using haemagglutination inhibition (HI)test and an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) was determined in this study .One hundred ninety eight ,one day old ,unsexed Ross breed broiler chicks were used for this purpose. The birds were allocated into 6 equal groups ,one for control and the others were vaccinated at 7th day of age with Hitchner B1 and LaSota at 21st and 35th day old via drinking water with skimmed milk(SM), RO water ,aerosol ,intraocular, and intranasal routes respectively .All groups were vaccinated at 14th of age against Infectious Bursal Disease (IBD) .Ten blood samples have been collected from each group at 1st ,21st ,35th ,and 49th day of age .Serum has been separated and stored at-20 c^{0} until analysis. For all routes of the vaccine administration higher antibody titers were detected using ELISA technique than HI test .For both serological assays, the highest antibody titers detected when the vaccine was administered via drinking water route mixed with(SM) with significant level ($p<0.05$) compared to the control regardless to the age, followed by Ro group and intranasl route respectively. The 4th and 5th groups revealed, more or less, the same results. In conclusion, ELISA proved more accurate, sensitive and rapid, but more expensive than HI test when used for measuring of antibody response against NDV vaccines administered with different routes in broiler chicks.

Keywords: HI, Elisa, SM, RO, ND.

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Introduction

Poultry vaccines are widely applied to prevent and control contagious diseases. Their use in poultry production is aimed at avoiding or minimizing the emergence of clinical disease at farm level, thus increasing production (Marangon and Busani, 2006). Newcastle Disease (ND) is one of the most serious endemic diseases in a wide variety of birds. It always leads to considerable chicken death and economic losses in poultry production (Homhuan and prakongpan, 2007). Newcastle disease (ND) is a fatal and highly contagious disease of poultry (Alexander, 2003). It is enzootic in most countries, where it continues to cause serious losses despite the vaccination of industrialized poultry (Aldous and Alexander, 2001). The availability of standard sensitive serological test adapted to the condition in these countries would facilitate diagnosis and accurate monitoring of vaccination programs. Haemagglutination inhibition (HI) test is the most widely used for measurement of antibodies (Abs) against Newcastle disease virus (NDV) (Allan and Gouph, 1984; Burgh *et al.,* 1978). The test is simple to perform but difficult to standardize among laboratories (Beard and Wilkers, 1985). ELISA has also been employed for the detection of Abs against NDV (Synder *et al*, 1983; Adair *et al*.1989). Comparative studies between the two for-mentioned tests to monitor antibody response to NDV in chicken and other species sera had also been conducted and the results of both tests especially in broiler chicken sera were compared (Bozorghmehrifard and Mayahi, 2000; Tabidi *et al* 2004[a]).

Different strategies can be implemented to effectively prevent and control the spread of the disease, and control plans often include the use of vaccination. Vaccines are, in fact, an important component of poultry disease prevention and control worldwide. Vaccines and vaccination programs vary widely depending on several local factors (e.g. type of production, level of biosecurity, local pattern of disease ,status of maternal immunity ,vaccines available, costs and potential losses)(Marangon and Busani,2006). Route of administration exerts an important role in the bird immunity, after establishing the type of vaccine to be used, the method of vaccine administration must be defined in the vaccination program.

There are different vaccination programs for broiler to achieve a reasonable protection against NDV. The objective of the present study was to determine efficacy of field ND vaccination program in establishing solid immunity using different methods of vaccination measured by ELISA and HI tests.

MATERIAL AND METHODS

One hundred ninety eight, one-day old, unsexed Ross breed broiler chicks were randomly allocated into 6 equal groups. All birds were kept in separate pens and fed on a commercial ration *ad labium* .Chicks of 5 groups were vaccinated at 7^{th} day of age with Hitchner B1,and at 21^{st} and 35^{th} laSota vaccine has been used, whereas the 6^{th} group was left as control unvaccinated with NDV vaccines . Birds in all experimental groups were maintained in experimental units under similar management conditions and kept for 50 days. Chicks of all groups were similarly vaccinated against IBD at 14^{th} day of age via drinking water route. All vaccines were supplied by Lohman Animal LHA (Germany). Different methods of administration for NDV vaccines have been used as shown in the experimental design (Table 1) below (Awaad *et al*, 2010).

Blood samples were obtained through slaughtering each bird and collected individually in plain tubes. Collected blood was left slantwise over-night at room temperature to clot and then centrifuged at 1000 rpm for 10 min and serum was harvested and stored at-$20c^{0}$ until analysis. The presence of ND Abs in serum samples was measured using the HI method. The reciprocal of the last serum dilution showing an inhibition of hemagglutination of the 8 hemagglutinating

units of the laSota virus vaccine was considered as the HI antibody titer of the serum. Firstly, NDV antigen used in the test prepared from LaSota virus vaccine.

Group	Route of NDV vaccines administration
A	Drinking: Tap Water + Skimmed Milk(SM).
B	Drinking: Reverse Osmoses (RO water).
C	Aerosol: Distilled water.
D	Intraocular: Distilled water.
E	Intranasal: Distilled water.
F	Control: Unvaccinated with NDV vaccines.

Table1: Shows the experimental Design

- All groups (except control) were vaccinated With Hitchner B1 at 7^{th} and with LaSote at 21^{st} and 35^{th} day of age.
- Blood samples (about 2ml) were colleted from each bird (5 birds of each group) at 2wks interval after each administration as well as at the 1^{st} day of age from the control group.

The HI test was carried out according to Abdalla *et al*. (1999).Two-fold serial dilutions of serum samples were made with normal saline in micro titer plates. Volumes of 2.5µl of NDV antigen were added in each well of the plate. Two rows of wells were left as control, the first row contained NDV antigen alone (negative control) and the second row contained normal saline with RBCs (reagent control). The plate was left for 30 min at room temperature before the addition of 2.5 µl of chicken RBCs to each well. The plate was then rotated and left till a pattern of HA appeared.

The ELISA technique used in the present study was as described by Tabidi *et al*, (2004**[b]**). The diluted test sera with phosphate buffer were added into wells already coated with NDV, and the plate was incubated at $37C^0$ for 30 min. The contents of wells were aspirated and the plate was washed four times with washing buffer. A 100µl of conjugate was added to each well, and the plate was again incubated at $37C^0$ for 30 min. The plate was washed and 100µl of substrate was added and incubated at room temperature for 10 min. A100µl of stop solution was added and the reading was recorded by reading spectro-photometrically at 492nm as instructed by the manufacturer.

Duncan Multiple Range Test (DMRT) was used to determine the significance between groups of data obtained.

RESULTS AND DISCUSSION

Poultry viral diseases constitute one of the major problems facing the rapidly expanding poultry industries, these diseases cause considerable economic looses, such as ND. For this reason, veterinary authorities rely fairly on vaccination. The ideal vaccination regimen is depending basically on selecting the type of vaccination method (Hafez, 2005).

An effective vaccination plan should result in a general improvement of the health status and the productive performance of the vaccinated population .The efficacy of vaccine administration and the level of immunological response in vaccinated birds can be serologically monitored. Two methods are used to measure antibody titers: the HI and ELISA

(Alexander, 2003). Table 2 showed the immune response against NDV vaccines which have been administered via different routes in this study as detected by HI and ELISA.

Generally, higher Abs levels were noted using ELISA technique compared to those detected with HI. For both tests used, the highest Ab titers detected when mixed with skimmed milk followed by Ro water. These titers are significantly ($p<0.05$) high as compared to the control unvaccinated group.

Group	Method of vaccination	Age / Days					
		21		35		49	
		HI	ELISA	HI	ELISA	HI	ELISA
A	Drinking: Tap Water + SM	$4.420^{a} \pm 0.23$	$2330.000^{b} \pm 0.42$	$5.012^{c} \pm 0.11$	$5078.300^{d} \pm 0.42$	$6.000^{e} \pm 0.31$	$6385.700^{f} \pm 0.42$
B	Drinking: Ro water	$3.921^{b} \pm 0.53$	$1397.600^{c} \pm 0.53$	$4.321^{d} \pm 0.35$	$2383.200^{cd} \pm 0.24$	$5.013^{f} \pm 0.91$	$4135.800^{af} \pm 0.32$
C	Aerosol: Distilled water	$3.500^{ab} \pm 0.81$	$1200.200^{cb} \pm 0.72$	$3.801^{cd} \pm 0.93$	$2104.300^{e} \pm 0.51$	$4.501^{ef} \pm 0.54$	$3115.300^{cf} \pm 0.34$
D	Intraocular: Distilled water	$2.210^{c} \pm 0.91$	$1150.200^{d} \pm 0.81$	$2.551^{e} \pm 0.53$	$1655.400^{f} \pm 0.33$	$2.758^{cf} \pm 0.12$	$1840.500^{ef} \pm 0.19$
E	Intraocular: Distilled water	$2.310^{c} \pm 0.51$	$1136.500^{d} \pm 0.18$	$2.491^{e} \pm 0.72$	$1442.200^{f} \pm 0.31$	$2.685^{cf} \pm 0.63$	$1708.300^{ef} \pm 0.35$
F	Control: Unvaccinated	$1.100^{cb} \pm 0.33$	$258.000^{cd} \pm 0.34$	$0.995^{ce} \pm 0.11$	$166.000^{cf} \pm 0.18$	$0.758^{g} \pm 0.22$	$135.400^{e} \pm 0.22$

Table 2: Shows the average antibody titers against NDV vaccines as detected by HI and ELISA

- Log_2 antibody titers detected by HI test (geometric mean $\pm$ sd ,n =5 samples)
- Log_{10} antibody titers detected by ELISA(geometric mean $\pm$ sd , n =5 samples)
- HI and ELHSA titer at the 1st day of control group was 4.5 $\pm$ 0.23 and 2130.000$\pm$0.95 respectively.
- Figures with different superscripts in the vertical and horizontal columns were significantly differed at $p<0.05$.

The intranasal and intraocular routes showed non- consistent pattern between the two tests. Tabidi *et al* .(2004**b**) found that the Ab titers against NDV vaccine (Komorove strain) at 10 days of life was higher for both tests when the vaccine was administered via the aerosol route. This might be due to the type of vaccine, age of the birds as well as the condition of the drinking water .Alexander *et al* ,(2003) stated that it is important not to use chlorine treated tap water and powdered milk to neutralize the effects of the chlorine is necessary.

The HI titer gives an indication of immune status of the bird, a titer of $\log_2^{3}$ in indicative of protection and a titer of $\log_2^{6}$ or more suggests a recent infection by the virus. An acceptable Ig G titer measured by ELISA that is correlated to protection should be above 1,000. (Khalifeh *et al*., 2009), where as Wambura ,(2009) stated good protection ,should be 6.5-8.0. Group D and E were failed to reach these level of protection when measured with HI. This may due to the difficulty in application of vaccine to individual birds. An important comparison for the antibody titer with age of testing (21st ,35th and 49th)showed that the level of antibody production increased ($p<o.05$) significantly in group A in both tests followed by group B and C respectively, whereas group D and E showed no significant difference between each other . It is important to mention that the control group had a highly significant

decreased ($p>0.05$) in antibody titer from 1 to 49 day, whereas the vaccinated groups showed an opposite trend as indicated in table 1- These findings were in agreement with those of Ali *et al*.(2004) who stated that a solution containing as little as 500 ppm of egg yolk or powdered skim milk (P.S.M) greatly reduced the activity of both chlorine and quaternary ammonium based disinfectants .Al-Mayah *et al* .(2009) concluded that R.o water is a suitable diluents to be used as drinking in vaccination against ND without the addition of P.S.M. because of its purity . HI tested has been used in those studies. Allan *et al*. (1978);Tabidi *et al* .(2004[b])mentioned that higher Ab levels were noted for both HI and ELISA tests when the vaccine was administered via the aerosol route.

The basic principle of this study was to compare between HI test and ELISA in detecting the antibody responses to the ND virus vaccines (Hitchner B1 at 7th day and Lasota at 21st and 35th day of age respectively).The results obtained revealed that ELISA can detect high levels of Abs to the vaccine virus and considered accurate and sensitive compared to HI test .This result is supported by the finding of other research workers published previously (Marguardt *et al* .,1985; Cadman et al .,1997; Tabidi *et al*., 2001[a])who stated that the test is proved more practical , sensitive and rapid for detection of Ab titer against NDV vaccines compared to the HI test. HI test was confirmed more cheaper than ELISA as no micro plate reader is required in addition to the cost of ELISA kit. Similar finding was also published by Bozorghmehrifardi and Mayahi (2000), they showed that HI test is more economic than ELISA kit used for detection of Ab levels against NDV.

The variations in the figures obtained for both tests in all groups of chickens could be attributed to the inherent characteristics of either the tests that the ELISA can detect all functional types of Abs whereas HI can only detect the haemagglutinating Abs (Tabidi *et al*., 2004[b]). Khalifeh *et al*.(2009) stated that HI test showed the differences between the antibody level result from different vaccination programs while the ELISA was not. However, ELISA detected maternal antibodies with significant titers in sera of commercial chicks during the first three weeks of age, which could prevent the early attacks in such flock.

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Original Article

Effect of some plant oils on some oxidant – antioxidant parameters in alloxan – induced diabetic male rats.

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Abstract

The present study aimed to evaluate the influence of plant oils pellet containing of black seed (10%), cinnamon oil (10%), olive oil (10%) and ginger oil (10%) on some oxidant – antioxidant parameters of male rats, these parameters included glucose, malondialdehyde, iron, transferrin, ceruloplasmin and albumin. Eighteen male rats divided into three groups, the first group was control intraperitoneally received 0.5 ml saline solution, fed *ad libitum* on normal commercial chow and had free access to water, the second group was diabetes rats fed with the same diet given in group (1) and the third group was diabetes animals fed with plant oils pellet containing black seed oil 10%, cinnamon oil, 10% olive oil 10%, ginger oil 10% respectively, daily for 5 weeks. The results indicated that alloxan and normal diet caused a significant increase in serum glucose, malondialdehyde, iron and ceruloplasmin, while the level of transferrin and albumin was a significant decreased in diabetic animal group compared with control group. The male rats were fed with plant oils pellet show a significant decrease in the level of serum glucose, malondialdehyde, iron, ceruloplasmin, while a significant increase in the level of serum transferrin and albumin in comparison to diabetic animal group.

Keywords: Diabetes, plant oils, oxidant – antioxidant parameters, rats.

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Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin that frequently results in severe metabolic imbalances and pathological changes in many tissues (Maritim *et al*., 2003). Dysfunction of the gastrointestinal tract is common among diabetic patients (Zhao *et al*., 2006).As many as 75% of patients visiting diabetes clinics report significant gastrointestinal symptoms

(Folwaczny *et al*., 1999). The intestinal mucosa is vulnerable to oxidative stress on account of the constant exposure to reactive oxygen species(ROS) generated by several conditions such as ischemia/ reperfusion, inflammatory bowel disease, surgical stress, and diabetes(Bhor *et al*., 2004). Increased oxidative stress is important in the development and progression of diabetes and related complications.

The protective effects of exogenously administered antioxidants have been extensively studied in animal models in recent years. Several studies have shown that consumption of antioxidant vitamin and nutrient rich antioxidant such as ginger decrease diabetic complications and improve the antioxidant system of the body (Bianca *et al*., 2000). Recent experimental studies have shown the therapeutic effects Black seed oil on diabetic animals (El-Dakhakhny *et al*., 2002; Alsaif, 2008). However its effects on blood glucose in human subjects are of an interest Although Najmi *et al*., (2008) had shown therapeutic effects Black seed oil on the metabolic syndrome including the blood glucose.

True cinnamon (*C. zeylanicum*) is among 300 species of Cinnamomum that belong to the Lauraceae family. The aromatic bark of the cinnamon tree is used worldwide for culinary purposes, but it is also used in Ayurvedic and traditional Chinese medicine for its hypoglycemic, digestive, antispasmodic, and antiseptic properties (Battaglia 1995). Animal studies have demonstrated that cinnamon, and its active constituent cinnamaldehyde, doses dependently improve glycaemic control and hyperlipidemia in normal and streptozotocin-induced diabetic rats (Kannappan *et al*., 2006; Kim *et al*., 2006; Subash Babu *et al*., 2006). Recently, Kwon and coworkers described that cinnamon oil protects from the streptozotocin-induced β-cell damage in vivo and in vitro and proposed inhibition of iNOS protein expression -mediated at the transcriptional level through the inhibition of NF-κB activation and iNOS transcription- as a plausible mechanism underlying this effect (Kwon *et al*., 2006). Human's clinical studies on low fat diets also show reversal of the disease. Many studies have shown low-fat diets to be effective in controlling diabetes (Barnad *et al*., 1983). Olive oil is considered as the pillar of the Mediterranean diet, since it improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile. Endothelial function, inflammation and oxidative stress are also positively modulated. Some of these effects are attributed beside the monounsaturated fatty acids (MUFA) to the minor components of virgin olive oil (Al-Jamal and Ibrahim, 2001). Hydrocarbons, polyphenols, tocopherols, sterols, triterpenoids and other components, despite their low concentration, non-fatty acid constituents may be of importance because studies comparing monounsaturated dietary oils have reported different effects on cardiovascular disease. Most of these compounds have demonstrated antioxidant, anti-inflammatory and hypolipidemic properties (Perona *et al*., 2006). Moreover, MUFA-rich diet prevents central fat redistribution and the postprandial decrease in peripheral adiponectin gene expression and insulin resistance induced by a carbohydrate-rich diet in insulin-resistant subjects (Paniagna *et al*., 2007). Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood testis barrier stability (Wolff *et al*., 1991; Baynes and Thorpe, 1999). Nowadays ginger rhizome (*Zingiber officinale R*., family: *Zingiberaceae*), is used worldwide as a spice. Both antioxidative (Palmeria *et al*., 2001) and androgenic activity of *Z. officinale* were reported in animal models. All major active ingredients of *Z. officinale*, such as *Zingerone, Gingerdiol, Zingibrene, and gingerols shogaols*, have antioxidant activity (Sexton and Jarow, 1997). Besides, other researches showed that ginger oil has dominative protective effect on DNA damage induced by H_2O_2 and

might act as a scavenger of oxygen radical and might be used as an antioxidant (Peluso, 2006). Therefore, the present study was carried out to assess the effects of these oils on some oxidant-antioxidants parameters in alloxan-induced diabetic male rats.

Materials and Methods

Animals

Eighteen male albino rats of the *Rattus norvegicus*, weighing (130-150 g) were included in the present study. The experimental animals were obtained from the animal house of Biology Department / College of Science, Thi-Qar University/ Iraq. The experimental animals housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (21±1°C) and 12:12 h light-dark cycle. Wister rats were fed *ad libitum* on normal commercial chow and had free access to water. The animals divided into three groups:-

- Group (1) were served as control, intraperitoneally received 0.5 mL saline solution, fed *ad libitum* on normal commercial chow and had free access to water .

- Group (2), diabetic rats were fed with the same diet given in group 1.

– Group (3), diabetic rats were fed with plant oils pellet containing black seed (10%), cinnamon oil (10%), olive oil (10%), ginger oil (10%) respectively, daily for 5 weeks.

Diabetes Induction

After fasting of 18 hours, the rats were intraperitoneally injected with alloxan (BDH, England) at a single dose of 125 mg/kg (body weight) in 1 ml saline solution. After injection, the rats had free access to food and water. Diabetes was allowed to develop and stabilize in these alloxan-treated rats over a period of seven days. Diabetes mellitus was defined in these rats using determination of fasting blood glucose levels. The rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and selected for the experimentation.

Biochemical Measurement

At the end of 5 weeks, the experimental animals were fasted for 12 hours, water was not restricted, and then blood samples were drawn from diethyl ether anaesthetized rats. Serum was obtained after the blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 15 minutes. Sera were then collected and stored in freezer till the determination time of the levels of glucose, malondialdehyde (MDA), iron (Fe), transferrin (Tf), ceruloplasmin (Cp), albumin(Alb).

Statistical Analysis

Data were statistically analyzed using Package Social Sciences (SPSS) for Windows version 12.0 software. All experimental data were expressed as mean ± standard deviation(SD). Statistical analysis was performed by the least significance difference (LSD) method. The $p < 0.01$ level of probability was used as the criteria of significance.

Results

Table (1) explained the effect of some plant oils on some oxidant-antioxidant parameters of diabetic male rats. The results indicated a significant increase ($p<0.01$) in concentrations of serum glucose in diabetic male rats group (2) in comparison with control group (1), while there was a significant decrease ($p<0.01$) in concentrations of serum glucose in diabetic male rats group(3) were fed with plant oils pellet in comparison with diabetic male rats group (3) daily for 5 weeks, figure (1). There was a significant increase ($p<0.01$) in concentration of serum malondialdehyde in group (2) in comparison with control group (1), whereas there was a significant decrease ($p<0.01$) in concentration of serum malondialdehyde in group (3) in comparison with group (2), figure (2).

Parameters	Group (1)	Group (2)	Group (3)	LSD
Glucose (mg/dl)	94.38 ± 4.99^{b}	224.78 ± 24.35^{a}	101.59 ± 4.76^{b}	47.12
MDA(nmol/ml)	13.24 ± 1.30^{c}	44.23 ± 3.85^{a}	26.98 ± 2.35^{b}	10.48
Iron (µmol/L)	20.53 ± 1.46^{c}	49.33 ± 3.95^{a}	31.47 ± 0.77^{b}	1.84
Transferrin(g/L)	3.17 ± 0.13^{a}	1.47 ± 0.49^{b}	2.9 ± 0.36^{a}	8.46
Ceruloplasmin (g/L)	5.68 ± 0.53^{b}	7.63 ± 0.15^{a}	5.44 ± 0.31^{b}	1.43
Albumin(g/L)	50.15 ± 0.81^{a}	41.89 ± 1.02^{b}	49.56 ± 0.72^{a}	3.70

Table.1: shows the effect of some plant oils on some oxidant-antioxidant in alloxan – induced diabetic rats. Each value represents mean ± SD values with non-identical superscript (a , b or c…etc) were considered significantly differences ($P<0.01$).

- Group (1) = Control rats received 0.5 ml saline solution , fed ad libitum on normal commercial chow and had free access to water .
- Group (2) = Diabetes rats were fed with the same diet given in group 1.
- Group (3) = Diabetes animals were fed with plant oils pellet containing 10% black seed oil,10% cinnamon,10% olive oil,10% ginger oil respectively. Daily for 5 weeks.

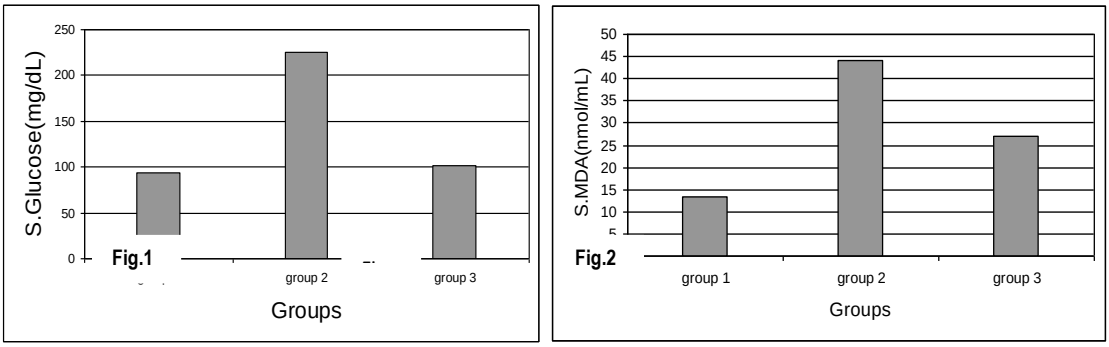

Figure.1: Shows the serum glucose levels in experimental groups.
Figure.2: Shows the serum MDA levels in experimental groups.

The results showed significant increase ($p<0.01$) in concentration of serum iron in group (2) in comparison with control group (1), while there was a significant decrease ($p<0.01$) in concentration of serum iron in group(3) in comparison with group (2), figure (3). The results indicated a significant decrease ($p<0.01$) in concentration of serum transferrin in group (2) in comparison with control group (1), while there was a significant increase ($p<0.01$) in concentration of serum transferrin in group (3) in comparison with group (3), figure (4).

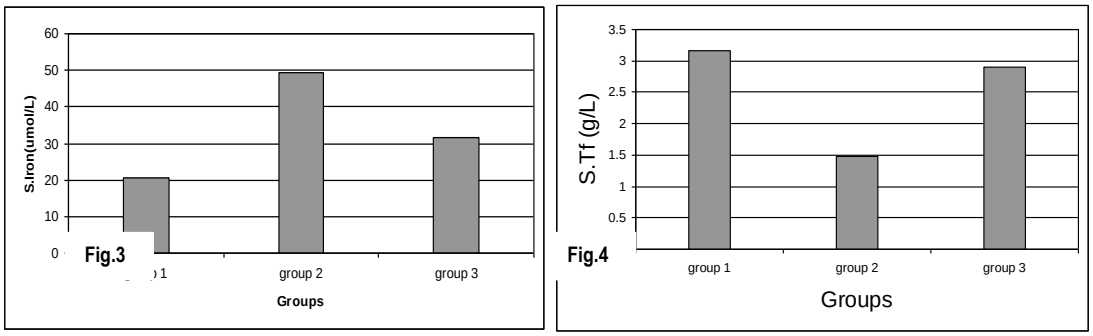

Figure.3: Shows serum Iron levels in experimental groups.
Figur.4: Shows serum Transferrin levels in experimental groups.

The results showed significant increase ($p<0.01$) in concentrations of serum ceruloplasmin in group (2) in comparison with control group (1), while there was a significant decrease ($p<0.01$) in concentration of serum ceruloplasmin in group (3) in comparison with group (2), figure (5). The results indicated a significant decrease ($p<0.01$) in concentrations of serum albumin in group (2) in comparison with control group (1), while there was a significant increase ($p<0.01$) in concentrations of serum albumin in group(3) in comparison with group(3), figure (6).

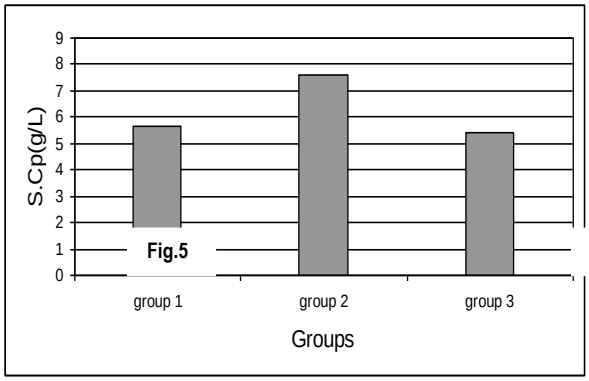

Fig.5

Fig.6

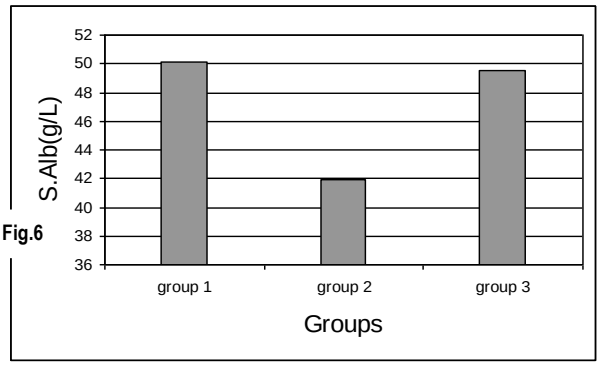

Figure.5: Shows serum Ceruloplasmin levels in experimental groups.
Figure.6: Shows serum Albumin levels in experimental groups.

Discussion

Intravenous injection of alloxan rapidly damages the β cells of the islets of langerhans in pancreas (Farjou and Lami, 1988; Koman *et al*., 1991). If the pancreatic arterial supply is clamped for five minutes after the injection, the islets are protected (Akhtar *et al*., 1984). The immediate effect of alloxan is the elevation of the blood glucose (Koman *et al*., 1991). This elevation can be prevented by simultaneous treatment with insulin (Akhtar *et al*., 1984). Prevention of the hyperglycemic phase does not prevent damage to the β cells, however, shortly after the initial hyperglycemic episode, there is a rapid drop of blood glucose to hypoglycemic levels, as a results of insulin released by the damaged β cells. Over the next few days, the blood glucose arises again and is usually maintained thereafter at elevated levels (Asyama *et al*., 1989). At this point, β cells degenerated, and the insulin content of the pancreas is reduced to very low levels.

Destruction of pancreatic beta-cells by alloxan may results from reaction with glutathione or other sulfhydryl groups of proteins which would inactivate essential enzymes or coenzymes of the cell, alloxan injection may also results in generation of free radicals which cause breaking of DNA stands of beta-cells. Alloxan has also been shown to inactivate Ca^{+2} -and calmodulin-dependent protein kinase, the activity of this enzyme was related to insulin secretion (Koman *et al*., 1991).

The fall in concentrations of serum glucose in group(3) which was reported in the present study agreement with the study of (AKhani *et al*., 2004) who found that treatment of streptozotocin – induced diabetic rats with ginger extract caused a significant decrease in the blood glucose and increased the insulin level (Kar *et al*., 1999) reported that, the inorganic part of a medicinal plant contains mainly mineral elements, which are responsible for the hypoglycemic activity. In support of this view, a number of essential minerals(Ca, Zn, K, Mn and Cr) are known to be associated with the mechanisms of insulin release and its activity in different animals and in human beings (Castro, 1998).

The increase of the malondialdehyde concentration in group (2) as reported in the present study, this agree with the results of (Coli *et al*., 2005). This rising in MDA level is directly associated with the degree of lipid peroxidation which is one of the most important measurement of oxidative stress in diabetes (Djeridane *et al*., 2006).

The decrease of the malondialdehyde concentration in group(3) may be due to the main pharmacological actions of ginger and compounds isolated there from include immumo-modulatory, anti-tumorigenic, anti-inflammatory, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free

radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ajay *et al*., 2007).

The increase in concentration of serum iron in group (2) which were reported in the present study agree with the finding of (Wood *et al*., 2004) and who showed that the toxic free radical types are superoxide radical anion (O^{-2}), the presence of the latter in high amount leads to releasing of free iron to circulatory system because O^{-2} attack to ferritin. While Lee *et al.* (2009) showed that the load of iron in patients with diabetes, and these findings was attributed to high oxidative stress in these patients to iron-derived free-radicals and to the patients diminished antioxidant reserve. An evidence suggests non-transferrin bound iron (NTBI) may be found in persons with diabetes (Hutchinson *et al*., 2004). This (NTBI) has been associated with oxidative stress and chronic disease (Lee *et al*., 2009).

The decrease in concentration of serum iron in group (3) which were reported in this study agree with the finding of (Golalipour *et al*., 2007) that reported the decrease of iron levels in patients with diabetes as a result of treatment by phenolic extract (as anti oxidants) can cause deficiency of oxidation processes, disruption of heme biosynthesis and low oxygen transfer might be resulted in a compensatory increase in the rate of red blood cell (RBC) production (Golalipour *et al*., 2007). On the other hand (Thephiniap *et al*., 2007) showed that phenolic compounds work by scavenging free radicals and quenching the lipid peroxidative chain. The hydroxy and phenoxy groups of phenolic compounds donate their electron to the free radicals and quench them. Besides, *in vitro* study of Thephiniap *et al*. (2007) had documented a protective effect of polyphenols like flavonoids on iron-induced oxidative stress.

The decrease in concentrations of serum transferrin in group (2) which were reported in the present study agree with study of Thabrew (2001) that concluded 86% of patients with diabetes develop hypoferremia. Besides, during diabetes, stimulation of phagocytes and activation of other immune complexes lead to further superoxide radical's production (Van Campenhout *et al*., 2003). The latter attacks ferritin (iron storage protein) leads to release iron by reductive process.

The increase in concentration of serum transferrin in group(3) this may be due to the decrease of serum iron levels after treatment, our results is compatible with the previous study of (Biessels *et al*., 2004) which showed that the decrease in serum iron levels associated with the increase of the binding capacity of transferrin to iron. Knekt *et al*., (2002), suggested that polyphenols have combined effect on free iron that caused a decreased iron level.

The increase in concentration of serum ceruloplasmin in group (2) was probably due to an increase in the proportion of younger red blood cells and the compensatory mechanism after increased oxidant stress (Rowe *et al*., 1984). Besides, it was found to be as a result of the increase in catalysis of the liver cells synthesis of Cp against iron overload status (Tobe *et al*., 2002) and elevation in serum copper level (Butric and Ashood, 1996) as a defiance function.

The decrease in concentration of serum ceruloplasmin in group(3), this may be due that plant polyphenols decrease an activity of numerous proteins associated with oxidative stress. Also the reduction in Cp concentration could be to counter balance of the ROS generation radicals generated in the lipid peroxidation processes and presence of iron or copper ions (Sirjwala *et al*., 2007).

In the present work, we focused on the antioxidant activity of albumin because oxidative stress is thought to play a significant role in the pathogenesis of many diseases, including diabetes. Wolff *et al*. (2001) reported that the concentration of serum albumin decreased in diabetes because albumin is a carrier protein of copper and diabetic patients exhibit elevated concentration of copper ions that have been shown to generate free radicals. These highly reactive species are able to induce oxidative degradation of protein (Pacifici and Davies, 2001). Vlassara *et al*. (2001) reported that these decreasing are due to the increasing in

synthesis of lipid peroxide and elevation formation of free radicals which result in increasing of membranes permeability and leaking the proteins outside the vascular system.

The results after treatment with plant oils pellet appear the increase in albumin levels, which is in agreement with the result of (Al-Hashem, 2009) who reported that polyphenols could induce decrease in lipid peroxidation processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human.

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Original Article

The Ameliorative Effect of Vitamin E on Electrocardiogram of Rabbits Exposed to Cadmium Chloride

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Abstract

This study was designed to study the effect of cadmium as an oxidant agent on electrocardiogram(ECG) component and the possible preventive role of vitamin E on deleterious effects of cadmium in adult male rabbits. Twenty adult male rabbits were divided randomly into 4 equal groups (5 animals /group) and treated daily for 84 days. The first group were received ordinary tap water and serve as control (C); the second group (T1) received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride; the third group T2 received (50ppb) of cadmium chloride in drinking water, in addition to intubation of vitamin E (40mg/Kg B.W.) orally, while the fourth group (T3) were intubated daily with 40mg/Kg B.W of vitamin E. Fasting blood samples were collected at 0, 21, 42, 63 and 84 days of the experiment to determine serum calcium concentration . The ECG was also recorded in all groups at the same interval of the experiment. The results revealed that administration of 50 ppb $CdCl_2$ in drinking water (T1 group) for 84 days caused a significant decrease($p<0.05$) in serum calcium concentration as compared to control. On other hand, the animals treated with vitamin E (T2 and T3) showed, no significant ($p>0.05$) differences in this parameter as compared to control and other groups. Analysis of ECG in Cadmium treated group (T1) showed significant ($p<0.05$) differences represented by significant prolongation of P wave, T wave, QRS complex and P-Q as well as Q-T interval, with a significant ($p<0.05$) decreased of heart rate as compared to the control and vitamin E treated groups (T2 and T3) which clarified non-significant ($p>0.05$) differences in ECG waves analysis. In conclusion, Cadmium effect on electrical conduction of heart was represented by abnormality in some of ECG component as well as the protective role of vitamin E as antioxidant in the cardiovascular system was also confirmed.

Key Words: Cadmium chloride, ECG, Calcium, Vitamin E.

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Introduction

The heavy metal cadmium (Cd) a pollutant associated with several modern industrial processes such as pigments, stabilizers, alloys ,electronic compounds, and especially in rechargeable nickel-cadmium batteries (Jarup ,2003;Cannino et al., 2009) . Human intoxication results mainly from cigarette smoking due to high concentrations of Cd in cigarettes (Tsutsumi et al., 2009). There has been increasing interest in the potential adverse cardiovascular effects of environmental exposures, including heavy metals (Weinhold, 2004; Bhatnagar, 2006; Houston, 2007). Cadmium has been reported to have cumulative effects on mortality, cardiovascular, neurologic, renal, and developmental diseases (ICEIT, 2009). Increased cadmium body burden is associated with lower aortic pulse wave velocity, lower pulse pressure throughout the arterial system, and higher femoral distensibility (Schutte *et al*, 2008). A long-term-Cd^{+2} exposure increased stroke volume (SV) and cardiac output (CO) (Ozturk *et al*,2009). A recent large USA study found an association between U-Cd and myocardial infarction (Zaslavina *et al*., 2007; Everett and Frithsen, 2008).

Reports have shown that antioxidants like vitamin C and Vitamin E have shown protection against cadmium induced toxicity in different animal models (Beytut et al., 2003; Ognjanovic et al., 2003). α-tocopherol can reduce Cd-induced oxidative stress and improve the glutathione level together with other biochemical parameters (Nemmiche et al .,2007).This study was designed to study the effect of chronic exposure to cadmium chloride on electrocardiograph of adult male rabbits and the protective role of vitamin E.

Materials and Methods

Twenty male rabbits were randomly divided into four equal groups (each group consist of five rabbits) and were treated for 84 days as follow: Group I (control), Group II: rabbits of this group were received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride, Group III: rabbits of this group were received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride and 40 mg/kg B.W. of vitamin E ((RRR-a-tocopherol) orally. ECGs were recorded by a direct writing electrocardiograph (Cardisuny type A; Fucuda M.E Kogyo Co., LTD Japan). This study was approved by research committee/ Department of Physiology and Pharmacology / College of Veterinary Medicine / Baghdad University, Iraq. All ECGs were standardized at 1 mV = 10 mm, with a chart speed of 50 mm/sec. Leads I, II, III, aVR, aVL and aVF were recorded at 0,21,42,63 and 84 and calculation was done according to Reisner and his coworkers (2006) and Serum calcium concentration was determined as described by Cali et al., (1972). Statistical analysis of data was performed on the basis of two- way analysis of variance (ANOVA) depending on the experimental design at each time .Specific group differences were determined using least significant differences (LSD) test (Steel and Terrie, 1980).

Results

Serum calcium concentration (mg/dl)

A significant (P<0.05) decrease in serum calcium concentration was recorded after different days of experiment (21, 42, 63 and 84 days) in Cd exposure group (T1) as compared to the control and other treated groups (T2 andT3). No significant (P>0.05) differences was observed in the mean value of this parameter in both vitamin E treated groups (T2 and T3) after the same duration of treatment (84 days) as compared to control group, as well as when they compared with each other (table.1) **.**

Days	Groups			
	(C) Control group.	**(T1)** 50 ppb of CdCl in drinking water	**(T2)** 50ppb of $CdCl_2$ + 40mg Vit.E.	**(T3)** 40mg Vit.E.
Zero	12.20±0.30 A a	12.70±0.10 A a	12.40±0.20 A a	12.20±0.22 A a
21	12.50±0.40 A a	10.90±0.10 B b	12.50±0.50 A a	12.10±0.20 A a
42	12.40±0.20 A a	9.80±0.10 B c	12.50±0.40 A a	12.22±0.22 A a
63	12.10±0.20 A a	8.80±0.10 B d	12.10±0.20 A a	11.80±0.20 A a
84	12.10±0.10 A a	7.50±0.10 B e	12.0±0.20 A a	12.10±0.40 A a

Table.1: Effect of cadmium chloride and vitamin E on Ca^{++}concentration (mg/dl) in serum of rabbits.
Values are expressed as mean ± SE, n = 5 each group , Capital letters denote differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control.

Electrocardiograph

P -wave interval and amplitude

After 42, 63 and 84 days of treatment with cadmium chloride (T1 group) a significant (P<0.05) differences in p- wave interval (p/sec) was observed comparing to control and treated groups (T2 andT3), while p- wave amplitude (p/mv) did not show significant (P>0.05) differences in T1 group as compared to control, T2 and T3 groups in the same duration of experiment (after 84 days). A significant (P<0.05) prolongation was observed in p/sec at the days at 84 of the treatment in rabbits received 50 ppb of $CdCl_2$(T1) as compared to the pretreatment period (table-2 and figure-2).

Groups	C		T1		T2		T3	
Days	**P/sec.**	**P/mv**	**P/sec.**	**P/mv**	**P/sec.**	**P/mv**	**P/sec.**	**P/mv**
Zero	0.042± 0.002 A a	0.10± 0.01 A a	0.046± 0.002 A a	0.08± 0.01 A a	0.040± 0.003 A a	0.10± 0.02 A a	0.042± 0.002 A a	0.09± 0.01 A a
21	0.044± 0.002 A a	0.08± 0.01 A a	0.052± 0.004 A ab	0.08± 0.01 A a	0.046± 0.004 A a	0.09± 0.01 A a	0.044± 0.002 A a	0.08± 0.01 A a
42	0.044± 0.002 A a	0.08± 0.01 A a	0.054±0 .004 B ab	0.09±0.01 A a	0.046±0.0 04 A B a	0.09±0.01 A a	0.044± 0.002 A a	0.09± 0.01 A a
63	0.042± 0.002 A a	0.08± 0.01 A a	0.056± 0.002 B b	0.07± 0.01 A a	0.040± 0.002 A a	0.09± 0.01 A a	0.044± 0.002 A a	0.08± 0.01 A a
84	0.042± 0.002 A a	0.08± 0.01 A a	0.066± 0.004 B c	0.07± 0.01 A a	0.044± 0.002 A a	0.08± 0.01 A a	0.044± 0.002 A a	0.09± 0.01 A a

Table .2: Effect of cadmium chloride and vitamin E on P- wave interval (second) and p-amplitude (mv) in ECG of rabbits. Values are expressed as mean ± SE, n = 5 each group , Capital letters denote differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control.

T- Wave interval (second) and T amplitude (mille volt):

Results in table (3)showed a significant(P<0.05) prolongation in T- wave interval (T/sec) at the days 21, 42, 63 and 84 of the treatment in rabbits received 50 ppb of $CdCl_2$ (T1) (figures 2,3) as compared with the control group and treated groups (T2 and T3). Also there were no significant differences (P>0.05) in the mean values of T/sec for T2 and T3 groups after 84 days of treatment when they compared to the control group as well as to each other. A significant (P<0.05) differences in T/sec were observed in (T1) treated group at the end of the experiment comparing to the pretreated period.

P-Q interval (second) and Q-T interval (second)

Values of P-Q and Q-T interval for the treated groups T1, T2, T3 and the control were depicted in table (4). While there were no significant (P>0.05) differences in the mean values between experimental groups during pretreated period, a significant increase (P<0.05) in the mean value of these parameters of T1 group was observed (figures 2, 3) after 21,42,63 and 84 days of the experiment comparing with the control and treated groups (T2 and T3). The results also clarified that treatment of male rabbits with vitamin E (T2 and T3 groups) did not cause significant (P>0.05)

differences after 84 days of experiment as compared to the control. Moreover, no significant (P>0.05) differences between T2 group and T3 group were observed at the end of experiment. A significant (P<0.05) increase was manifested within the time in the mean values of P-Q/sec and Q-T/sec after treatment of adult male rabbits with cadmium chloride in drinking water (T1) as compared to pretreated period.

Groups	C		T1		T2		T3	
Days	**T/sec.**	**T/mv**	**T/sec.**	**T/mv**	**T/sec.**	**T/mv**	**T/sec.**	**T/mv**
Zero	0.056± 0.002 A a	0.16± 0.010 A a	0.056± 0.005 A a	0.16± 0.010 A a	0.055± 0.003 A a	0.17± 0.01 A a	0.060± 0.003 A a	0.16± 0.010 A a
21	0.056± 0.002 A a	0.15± 0.015 A a	0.080± 0.003 B b	0.18± 0.010 A a	0.060± 0.003 A a	0.18± 0.01 A a	0.060± 0.001 A a	0.16± 0.020 A a
42	0.056± 0.002 A a	0.15± 0.020 A a	0.082± 0.005 B b	0.17± 0.012 A a	0.058± 0.004 A a	0.18± 0.03 A a	0.060± 0.003 A a	0.15± 0.015 A a
63	0.058± 0.002 A a	0.15± 0.015 A a	0.090± 0.004 B c	0.16± 0.010 A a	0.058± 0.002 A a	0.16± 0.01 A a	0.056± 0.002 A a	0.17± 0.015 A a
84	0.058± 0.002 A a	0.16± 0.010 A a	0.098± 0.002 B d	0.18± 0.010 A a	0.060± 0.003 A a	0.16± 0.01 A a	0.060± 0.003 A a	0.16± 0.010 A a

Table -3: Effect of cadmium chloride and vitamin E on T wave interval (second) and T amplitude (mv) in ECG of rabbits. Values are expressed as mean ± SE, n = 5 each group , Capital letters denote differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control.

Groups	C		T1		T2		T3	
Days	**P-Q/ sec.**	**Q-T/ sec.**	**P-Q/ sec.**	**Q-T/ sec.**	**P-Q/ sec.**	**Q-T/ sec.**	**P-Q/ sec.**	**Q-T/ sec.**
Zero	0.058± 0.002 A a	0.128± 0.003 A a	0.058± 0.005 A a	0.124± 0.004 A a	0.060± 0.002 A a	0.130± 0.004 A a	0.060± 0.003 A a	0.130± 0.004 A a
21	0.058± 0.004 A a	0.126± 0.004 A a	0.072± 0.004 B b	0.148± 0.004 B b	0.062± 0.002 A a	0.128± 0.004 A a	0.058± 0.004 A a	0.126± 0.004 A a
42	0.060± 0.003 A a	0.126± 0.004 A a	0.074± 0.004 B b	0.154± 0.004 B bc	0.060± 0.003 A a	0.124± 0.004 A a	0.060± 0.003 A a	0.125± 0.005 A a

63	0.060± 0.003 A a	0.124± 0.004 A a	0.076± 0.002 B b	0.164± 0.004 B cd	0.058± 0.002 A a	0.128± 0.005 A a	0.060± 0.003 A a	0.123± 0.004 A a
84	0.060± 0.003 A a	0.126± 0.004 A a	0.078± 0.022 B b	0.168± 0.004 B d	0.058± 0.002 A a	0.126± 0.004 A a	0.060± 0.004 A a	0.124± 0.004 A a

Table.4: Effect of cadmium chloride and vitamin E on P-Q interval (second) and Q-T interval (second) in ECG of rabbits. Values are expressed as mean ± SE, n = 5 each group , Capital letters denote differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control.

QRS complex interval (second)

The results showed no significant differences (P>0.05) in the mean value of QRS/sec of control and treatment groups at 0 ,21 ,42, and 63 days of the experiment when compared to each other(table-5) . On the other hand, a significant (P<0.05) increase was recorded in mean value of this wave in cadmium treated group (T1) at 84 day (figure-3) as compared to the control and vitamin E treated groups (T2 and T3). The results have clarified that the mean value of QRS/sec in T2 group and T3 group did not show significant differences (P>0.05) comparing to the control group as well as to each other in the same duration of the experiment. Within the time T1 group showed a significant (P<0.05) prolongation in QRS wave interval at day 84 of experiment as compared to the pretreatment period.

Heart rate

A significant decrease (P<0.05) in mean value of heart rate was detected at days 42 ,63 and 84 in Cd exposed group (T1) comparing to the control ,T2 and T3 groups . Depending on the statistical results, each of vitamin E treated groups (T2 and T3) did not show significant (P>0.05) differences in the mean value of heart rate when they compared to control group as well as to each other(table-6)

Groups / Days	(C) Control group.	T1 50 ppb of $CdCl_2$ in drinking water	T2 50ppb of $CdCl_2$ + 40mg Vit. E.	T3 40mg Vit.E.
Zero	0.034±0.004 A a	0.032±0.033 A a	0.030±0.003 A a	0.032±0.002 A a
21	0.032±0.002 A a	0.034±0.002 A a	0.032±0.003 A a	0.034±0.002 A a
42	0.034±0.002 A a	0.038±0.002 A ab	0.034±0.002 A a	0.034±0.002 A a
63	0.034±0.002 A a	0.038±0.003 A ab	0.034±0.002 A a	0.034±0.002 A a
84	0.034±0.002 A a	0.044±0.002 B b	0.032±0.002 A a	0.034±0.002 A a

Table (5): Effect of cadmium chloride and vitamin E on QRS complex interval (second) ECG of rabbits. Values are expressed as mean ± SE, n = 5 each group , Capital letters denote

differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control.

Groups / Days	C **Control group.**	T1 **50 ppb of $CdCl_2$ in drinking water**	T2 **50ppb of $CdCl_2$ + 40mg Vit. E.**	T3 **40mg Vit.E.**
Zero	288.8±6.90 A a	288.8±6.90 A a	288.8±6.90 A a	284.4±10.2 A a
21	294.4±5.61 A a	290.4±8.90 A a	294.4±5.60 A a	294.4±5.60 A a
42	291.4±5.70 A a	270.8±8.40 B b	288.8±6.90 A a	288.8±6.90 A a
63	288.4±5.30 A a	252.0±2.010 B c	288.8±6.90 A a	288.8±6.90 A a
84	291.4±5.70 A a	242.8±7.90 B c	288.8±6.90 A a	288.8±6.90 A a

Table.6: Effect of cadmium chloride and vitamin E on Heart rate (beat/mint) of rabbits.
Values are expressed as mean ± SE, n = 5 each group , Capital letters denote differences between groups, P<0.05 vs. control. , Small letters denote differences within group, P< 0.05 vs. control

DISCUSSION

A significant decrease in Calcium ions concentration after cadmium treatment observed in the present study could be attributed to the similarity between hydrated radius of Cd +2a Ca+2, which lead to inhibition of receptor and voltage operated calcium channels as well as all types of Ca-ATPases pumps (Zhang et al., 1990; McNulty and Taylor, 1999; Saderholm et al., 2000 and Baldisserotto et al.,2004). Cadmium can interfere for uptake with essential metal ions including calcium (Ca), zinc (Zn) and copper (Cu), it is affect Ca^{2+} signaling in hepatic cells (Blazka and Shaikh 1992, Dundjerski *et al*. 2000, Baker *et al*. 2003). Biagioli *et al.*, (2008), observed that cadmium decreased agonist-evoked endoplasmic reticulum (ER) Ca^{2+} signals and caused a 40% inhibition of sarcoplasmic–ER calcium ATPases activity leading to depression in serum calcium concentration. The enhancement effect of vitamin E on the elevation of calcium concentration might be attribute either to a direct increase in the entry of Ca^{2+} through voltage - dependent Ca^{2+} channels or secondary effects resulting from, for example, modulation of K^{+} channels with the consequent alteration in plasma membrane potential (Yang and Wang , 2008). The results of the present study showed significant prolongation of P wave, T wave, QRS complex and P-Q as well as Q-T interval, with a significant (p<0.05) decreased of heart rate as compared to the control and vitamin E treated groups (T2 and T3).

P-wave analysis has long been used to study the atrial electrical activity (depolarization and repolarization) in the heart (Birkbeck *et al*., 2006, Dilaveris and Stefanadis, 2009; Davey, 2010). Prolonged P-wave duration is a useful predictor of atrial fibrillation (AF) development (Ciaroni *et al*., 2000, De *et al*., 2007, Dilaveris and Stefanadis, 2009). Atrial repolarization starts during the PQ (PR) segment and continues into the QRS complex (Ihara *et al*., 2006). A long PR interval reflects slow conduction through the atrioventricular (AV) node and bundle of His, and may indicate a disease of the conducting tissue predisposing to bradyarrhythmia through high-grade AV block (Davey, 2010).

The T wave is generated by myocardial voltage gradients during the repolarization phase of cardiomyocyte action potentials (Yan and Antzelevitch, 1998; Antzelevitch, 2006). Myocardial ischaemia may cause T wave changes and abnormally tall T waves (Rowlands , 2002). Clinical studies have shown that long QT intervals predispose people to malignant ventricular arrhythmias and sudden death (Bednar *et al*., 2001). Prolonged QT interval is associated with blood pressure; left ventricular mass, prevalent coronary artery disease (Festa *et al*., 2000).As clarified in table (4-5) cadmium treated group (T1) showed a significant depression in serum calcium concentration. In hypocalcaemia, the T wave morphology remains normal and QT interval is prolonged (Sype and Khan, 2005). Prolongation of the QT interval may be a consequence of an unfavorable balance between sympathetic and parasympathetic activity. It has been noted that imbalance in cardiac autonomic function (increased or decreased sympathetic activity) shortens or prolongs the QT interval of the electrocardiogram (Karjalainen *et al*., 1997 , Bednar *et al*., 2001).

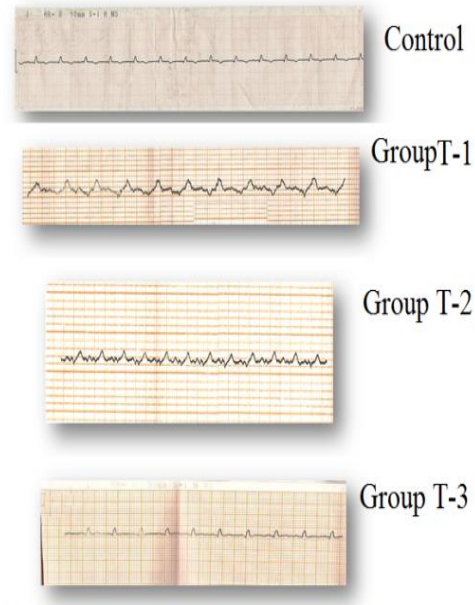

Figure.1: Lead I of electrocardiograph for male rabbits in control, T1, T2, and T3 groups at the end of experiment.

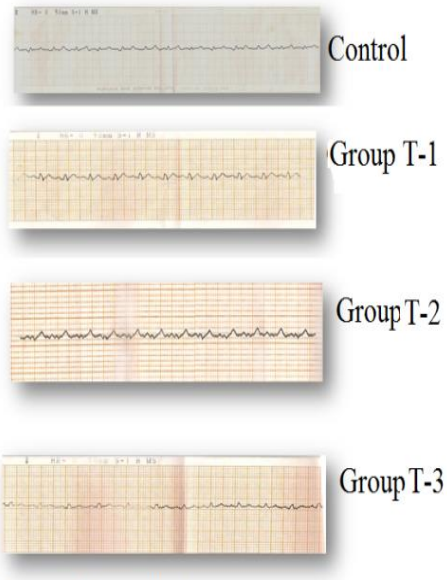

Figure. 2: Lead II of electrocardiograph for male rabbits in control, T1, T2, and T3 groups at the end of experiment.

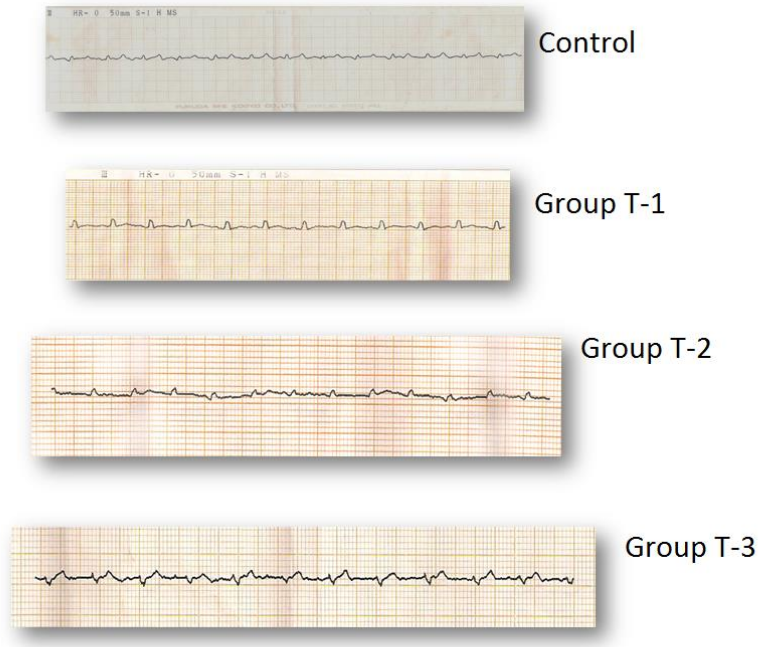

Figure. 3: Lead III of electrocardiograph for male rabbits in control, T1, T2, and T3 groups at the end of experiment.

Cadmium induced decreases in both intracellular potassium and reduced glutathione concentrations (Chan and Cherian, 1992). The proposed hypokalaemia after cadmium exposure may associated with cardiac arrhythmias and prolongation of the QRS duration, and an increase in P wave amplitude and duration (Rowlands , 2002). Wide QRS complexes is a definite diagnosis of atrial fibrillation (AF) (Gulamhusein *et al*., 1985), also a broad QRS usually reflects a disease of the right, the left or both bundle branches (Davey, 2010).

Heart rate dependency of QT interval is well known (Malik et al., 2008), QT interval and heart rate, however, are highly negatively correlated with each other (Rautaharju and Zhang, 2002). Similar to the QT interval, the PQ interval and QRS width are rate dependent (Malik *et al*., 2008).

Several mechanisms were placed on deciphering the Cd toxicity, including induces reactive oxygen species (ROS) and oxidative stress (Lopez *et al*., 2006; Gems and Partidge, 2008). Many studies conducted in the last decade have illustrated increased biological oxidative pathways during cardiovascular disease (CVD) in animals and humans. Thus, increased production of reactive oxygen species may be a unifying mechanism in CVD progression, and antioxidants may have therapeutic value in this setting (Wattanapitayakul and Bauer, 2001). The cardioprotective effects of vitamin E are attributed to its antioxidant properties. Vitamin E is able to extinguish single oxygen species as well as to terminate free radical chain

reactions (Giugliano, 2000). In conclusion, this study showed that the Cadmium effect on electrical conduction of heart was represented by abnormality in some of ECG component as well as the protective role of vitamin E as antioxidant in the cardiovascular system was also confirmed.

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Original Article

Effect of vitamin E on Some Blood Parameters Related to Cardiovasacular Diseases in Cadmium Chloride- Treated Rabbits

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Abstract

This study was designed to investigate the effect of cadmium as an oxidant agent on cardiovascular system and some blood parameters and the possible preventive role of vitamin E on deleterious effects of cadmium in adult male rabbits. Twenty adult male rabbits were divided randomly into 4 groups (5 animals /group) and treated daily for 84 days. The first group were received ordinary tap water, serving as control (group C); the second group (T1) received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride; the third group T2 received (50ppb) of cadmium chloride in drinking water in addition to intubation of vitamin E (40mg/Kg B.W.) orally, while the fourth group (T3) were intubated daily with 40mg/Kg B.W of vitamin E. Fasting blood samples were collected at 0, 21, 42, 63 and 84 days to determine: platelet count, partial thromboplastin (PTT), prothrombin time (PT), serum concentration of total cholesterol TC, and glutathione (GSH). Sections of heart & aorta were also assessed for histopathological changes. The results revealed that administration of 50 ppb $CdCl_2$ in drinking water (T1) for 84 days caused a significant increase ($p<0.05$) in platelet count and serum TC, with a significant decrease($p<0.05$) in PT, PTT and serum concentrations of GSH as compared to control and T2 and T3 groups which showed significant ($p<0.05$) elevation in GSH concentration. Histological sections of heart and aorta of Cd treated (T1) group revealed congestion of blood vessels. Neutrophils and cells vacuolation of cardiac muscle were also seen. Atheromatus lesions characterized by hyperplasia of intima, vacuolation in subintima and proliferation of fibrous connective tissues with the appearance of foamy cells in the subintima layer, were seen in aorta. In conclusion, this study approved the deleterious effect of Cadmium on some aspect of cardiovascular system and the cardioprotective role of vitamin E as antioxidant.

Key word: Cardiovascular Diseases, Cadmium chloride, Vitamin E, Antioxidant.

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Introduction

Cadmium is a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chloride (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide) (ATSDR,2008). Food and cigarette smoke are the biggest sources of cadmium exposure for people in the general population (Willers et al,2005). For nonsmokers, food constitutes the principal environmental source of cadmium. Acute toxicity may be resulted from the ingestion of cadmium through contaminated foods and beverages (Pratap et al,2007; Yoon et al,2008). Workers of industries exposed to cadmium in air from the smelting and refining of metals, batteries products, coatings, plastics or when soldering and welding metal that contains cadmium at high risk for inhalation exposure (ATSDR ,1999; Hogervorst et al,2007). Apart from smoking, inhalation and pollution sources such as coal-fired power plants and municipal waste incinerators, shellfish, liver, and kidney meats are other routes of Cd entry, causing cardiovascular anomalies (ATSDR ,1999; Navas-Acien et al,2005). Cadmium's influence on the cardiovascular system remains controversial; studies investigating cardiovascular effects in humans after oral exposure to cadmium have concentrated on the relationship between blood pressure and biomarkers of cadmium exposure such as cadmium levels in the blood, urine or other tissues (WHO ,2000; Varoni et al,2003). Recently, peripheral arterial disease has been reported to might be associated with cadmium, thus suggesting that cadmium is involved in arterial dysfunction (Navas-Acien et al,2004; Navas-Acien et al,2005).

Vitamin E is an important natural antioxidant, and protects biologic membranes from lipid peroxidation and its most common and biologically active form is α-tocopherol (Horvath et al,2006; Nusret , 2009).The different isoforms of vitamin E possess important physiological roles beyond their antioxidant activities including hypocholesterolemic, antithrombotic, anti-inflammatory, and anti-proliferative effects (Fuchs et al, 2003). This study was designed to study the effect of chronic exposure of adult male rabbits to cadmium chloride on antioxidant status and some parameter related to cardiovascular disease, as well as, the protective role of vitamin E.

Materials and Methods

Twenty male rabbits were randomly and equally divided into four groups (each group consist of five rabbits) and were treated for 84 days as follow:

Group I (control) , Group II: rabbits of this group were received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride , Group III: rabbits of this group were received *ad libitum* supply of drinking water containing(50ppb) cadmium chloride and 40 mg/kg B.W. of vitamin E ((RRR-a-tocopherol) orally.

Blood samples were collected from fasting animals (8-12 hrs) by cardiac puncture technique using disposable medical syringes (5ml). Blood sample were divided into 3 parts: 1-Part of Whole blood was kept in EDTA tube for platelet count. 2-A portion of blood sample was mixed 9 parts of freshly collected blood with 1 part of sodium citrate (0.11mol/L), centrifuged immediately for 10 minutes at 3000 rpm and plasma was stored in capped plastic test tubes at 2 to $4C^0$ for measurement of Prothormbin time (PT) and Partial Thromboplastin time (PTT). Tests were done within 3 hours after blood collection (Biggs, 1972). 3-Serum was isolated from another part of blood by centrifugation of blood sample (3ml) at 2000-2500rpm for 15 minutes and frozen at -18 C^0 till use.

The following biochemical tests were done at different intervals (0, 21, 42, 63 and 84 days): Total Platelets Count (platelet / $m.m^3$.) as described by (Becton-Dickinson,1996), Prothormbin Time (PT) as described by (Loeliger et al,1985). Partial Thromboplastin Time (PTT) according to (Biggs,1972) and (Hoffmann and Neulendijk, 1978).Serum Total Cholesterol (TC) Concentration (mg/dl), using enzymatic kits (Allain et al,1974) and (Richmond,1992) and Serum Reduced glutathione concentration (GSH) according to (Burtis and Ashwood, 1999).Besides ,histopathological changes were studied in heart and aorta and several tissues section were prepared and stained with Henatoxyllin-Esoin (H&E) stains according to (Bancroft and Stevens,1982) method. Statistical analysis of data was performed on the basis of two- way analysis of variance (ANOVA) depending on the experimental design at each time specific group differences were determined using least significant differences (LSD) test (Steel and Terrie, 1980).

Results

During treatment period (after 21,42,63 and 84 days) , the exposure of rabbits to 50 ppb of cadmium chloride in drinking water group (T1) caused significant ($P<0.05$) elevation in the mean values of platelet count (table-1) and significant depression in prothrombine time (table-2) and thromboplastin time (table-3) as compared to control group and treated group (T2 and T3) . While treatment of male rabbits with vitamin E alone (group T3) or in combination with cadmium (group T2) for 84 days caused correction of previous parameters with significant elevation in prothrombine and thromboplastin time and significant depression in platelets count (table 1 and 2).

Groups Day	C Control group	T1 50 ppb CdCl2	T2 50ppb CdCl2 + 40mg Vit.E.	T3 40 mg Vit.E.
Zero	62.0±1.20 A a	66.0±1.90 A a	64.4±3.60 A a	62.0±4.30 A a
21	60.0±1.10 A a	110±6.30 B b	64.0±2.90 A a	63.0±1.00 A a
42	61.4±1.09 A a	148±3.80 B c	62.0±1.70 A a	63.2±1.02 A a
63	66.0±1.90 A a	174±3.20 B d	67.6±1.90 A a	68.0±1.20 A a
84	65.6±2.23 A a	176±4.01 B d	65.6±1.80 A a	64.8±2.20 A a

Table.1: Effect of cadmium chloride and vitamin E on Platelets count (cells/ mm3) in blood of male rabbits .Values are expressed as mean ± SE, n = 5 each group ,Capital letters denote differences between groups, $P<0.05$ vs. control., Small letters denote differences within group, $P< 0.05$ vs. control.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	8.54±0.60 A a	8.22±0.30 A a	8.39±0.40 A a	8.52±0.40 A a
21	8.08±0.40 A a	7.19±0.40 B b	8.53±0.30 A a	8.06±0.30 A a
42	8.52±0.20 A a	5.61±0.40 B c	8.29±0.30 A a	8.25±0.24 A a
63	8.09±0.20 A a	4.04±0.20 B d	7.81±0.40 A a	7.73±0.20 A a
84	7.95±0.10 A a	3.97±0.20 B d	7.59±0.20 A a	7.67±0.20 A **a**

Table. 2: Effect of cadmium chloride and vitamin E on Prothrombin time (seconds) in plasma of male rabbits. Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control, Small letters denote differences within group, P< 0.05 vs. control.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	24.45±0.60 A a	24.42±0.60 A a	24.72±0.80 A a	23.85±0.90 A a
21	24.30±0.60 A a	22.28±0.70 B b	24.10±0.70 A a	24.61±0.60 A a
42	24.03±0.40 A a	19.33±0.30 B c	23.92±0.60 A a	24.47±0.50 A a
63	23.96±0.70 A a	17.40±1.20 B d	24.44±0.70 A a	23.95±1.50 A a
84	24.04±0.20 A a	14.64±0.20 B e	23.71±0.30 A a	23.85±0.20 A a

Table. 3: Effect of cadmium chloride and vitamin E on Partial thromboplastin time (seconds) in plasma of male rabbits. Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control. Small letters denote differences within group, P< 0.05 vs. control.

Serum Total Cholesterol (TC) Concentration (mg/dl)

Table (4) showed a significant increase (P<0.05) in serum TC concentration at days 21, 42, 63 and 84 of the treatment in T1 group compared with control group and treated groups (T2 andT3). The result also indicated that vitamin E treated groups (T2 and T3) normalized the values near that of the control**.**

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	129.6±2.3 A a	127.8±1.60 A a	130.5±1.7 A a	127.2±5.2 A a
21	130.1±1.8 A a	153.4±3.70 B b	128.8±3.3 A a	126.7±1.7 A a
42	132.6±0.7 A a	170.4±2.10 B c	124.8±7.3 A a	129.1±1.2 A a
63	126.2±2.5 A a	187.1±4.90 B d	128.1±2.2 A a	129.4±2.7 A a
84	128.6±2.0 A a	217.4±7.14 B e	136.1±3.2 A a	132.2±2.5 A a

Table .4 : Effect of cadmium chloride and vitamin E total Cholesterol concentration (mg/dl) in serum of rabbits.Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control,Small letters denote differences within group, P< 0.05 vs. control.

Serum Reduced Glutathione (GSH) Concentration (µmol/l)

During the treatment period (after 84 days) a significant (P<0.05) reduction in serum GSH concentration were detected in Cd treated group (T1) at day 84 comparing to control group in the same period (table-5) The results have also clarified that vitamin E oral intubation to rabbits in T2 and T3 treated groups caused a significant (P<0.05) elevation in serum GSH.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	11.9±0.50 A a	11.6±o.40 A a	12.4±0.40 A a	12.1±0.40 A a
21	11.9±0.40 A a	10.1±o.20 B a	16.0±0.60 C b	16.8±0.80 C b
42	12.1±0.70 A a	5.8±0.30 B b	20.9±0.40 C c	19.9±0.90 C c
63	12.4±0.40 A a	5.2±0.10 B bd	27.2±0.80 C d	31.8±0.30 D d
84	12.4±0.40 A a	3.8±0.50 B d	27.2±.070 C d	36.2±0.70 D e

Table .5: Effect of cadmium chloride and vitamin E on GSH concentration (µmol/l) in serum of rabbits. Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control, Small letters denote differences within group, P< 0.05 vs. control.

Histological findings of heart and aorta:

Light microscopic study of adult male rabbit heart and aorta related to T1 group which received 50 ppb of $CdCl_2$ in drinking water for 84 days showed histological changes represented by congestion of blood vessels of the heart (Figure -1),and neutrophil appeared in

their lumen with muscles fiber together as well as vacuolation of muscles cell comparing to control (Figure-2) and vitamin E treated groups T2 (Figure-3) ,T3(Figure-4) . As well as, the aorta of same group (T1) clarified hyperplasia of intima with vacuolation in subintima (Figure-5) and foamy cell appeared in subintima layer and inflammatory cell infiltration as compared to control (Figure-6), T2 group (Figure-7) and T3 group (Figure-8).

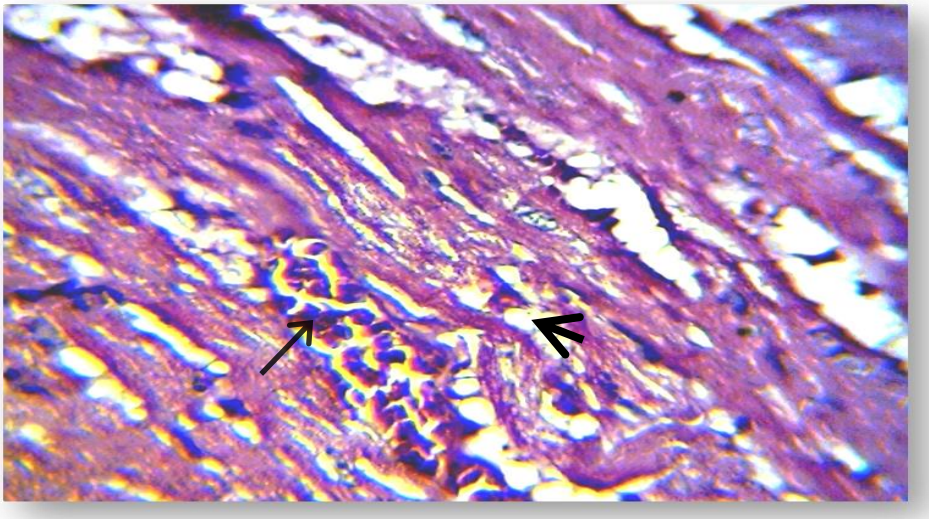

Figure (1): Histological section in the heart of animal at 84 days post treated with CdCl2 (50 ppb) in drinking water (T1) showed congestion of blood vassals between muscles fiber with inflammatory cell in their lumen (→) with vacuolation of muscles cell (➢).

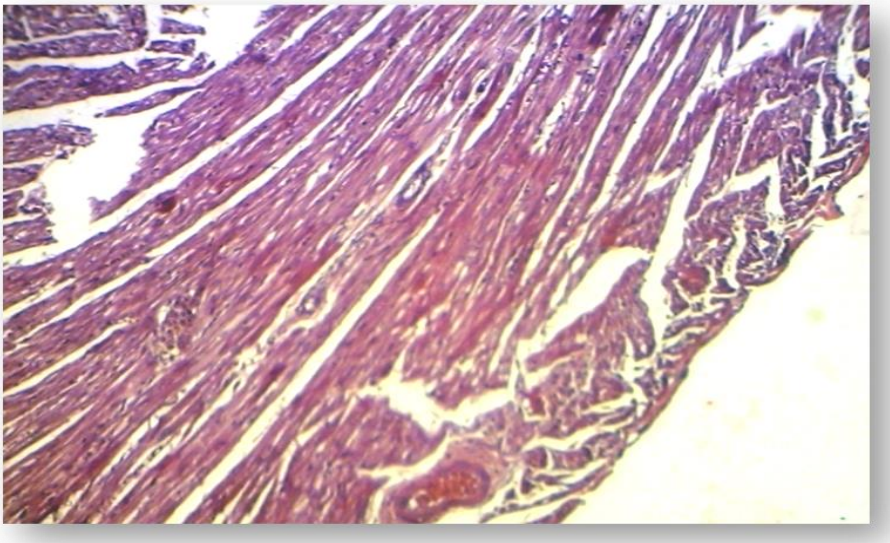

Figure (2): Histological section in the heart of animal belong to control group showed normal structure of heart (H & E 40 X)

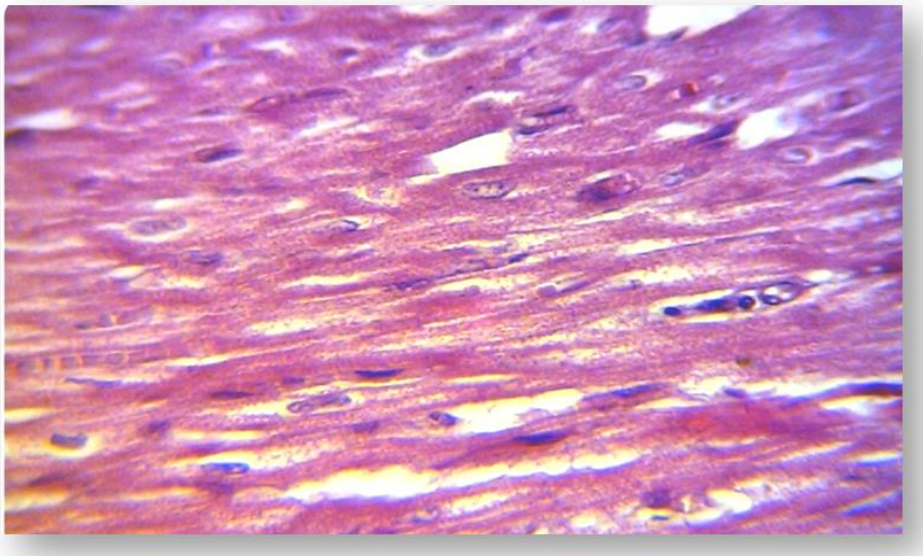

Figure (3): Histological section in the heart of animal belongs to group post treated with CdCl2 (50 ppb) in drinking water plus vitamin E (40 mg/kg B.W) (T2) showed no clear pathological lesion (H & E 40 X).

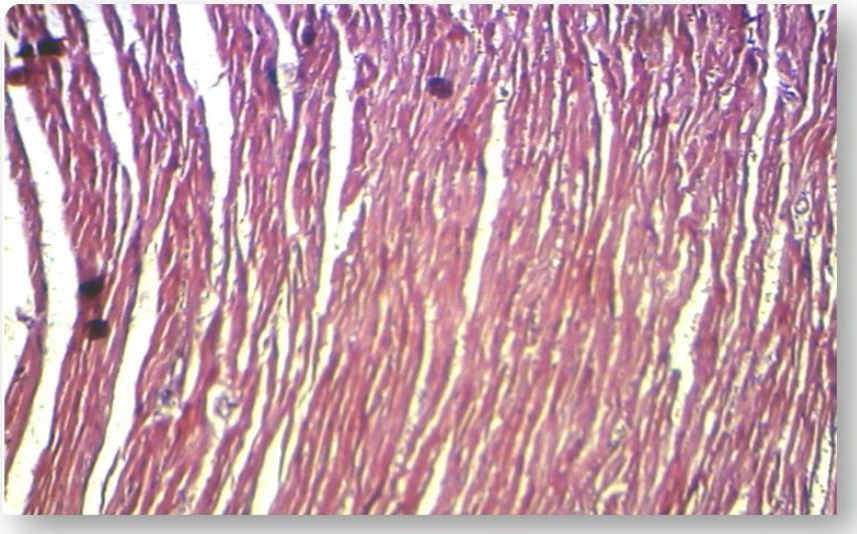

Figure (4): Histological section in the heart of animal belong to group post treated with vitamin E (40 mg/kg B.W) (T3) showed no clear pathological lesion (H & E 40 X).

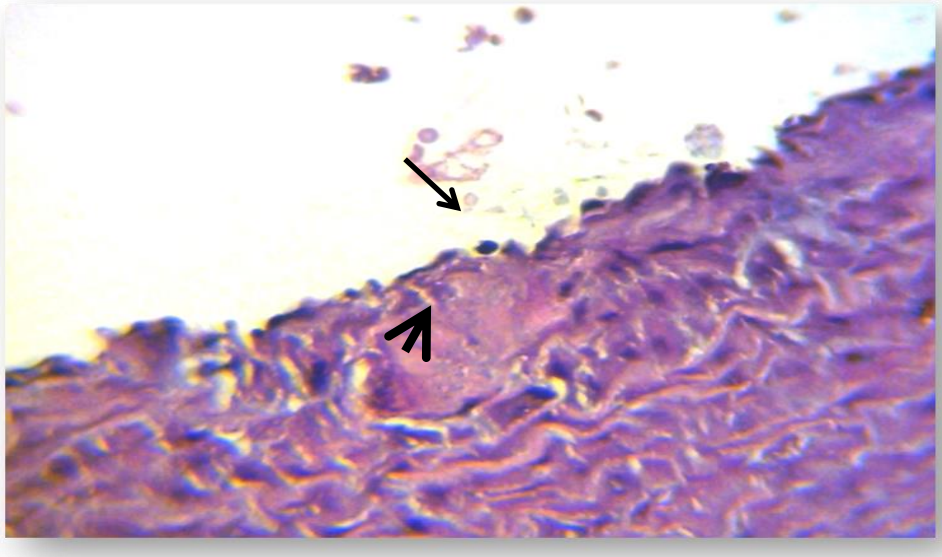

Figure (5): Histological section in the aorta of animal at 84 days post treated with CdCl2 (50 ppb) in drinking water (T1). Showed opaque area in subintimal layer (➔) with inflammatory cell attachment to intimal layer (⟶) (H & E 40 X)

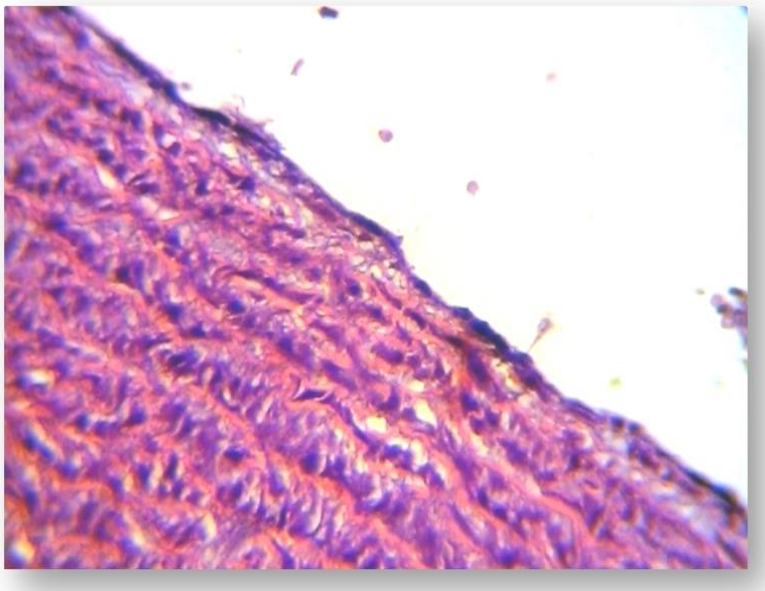

Figure (6): Histological section of the aorta of the control group showed normal structure of aorta.

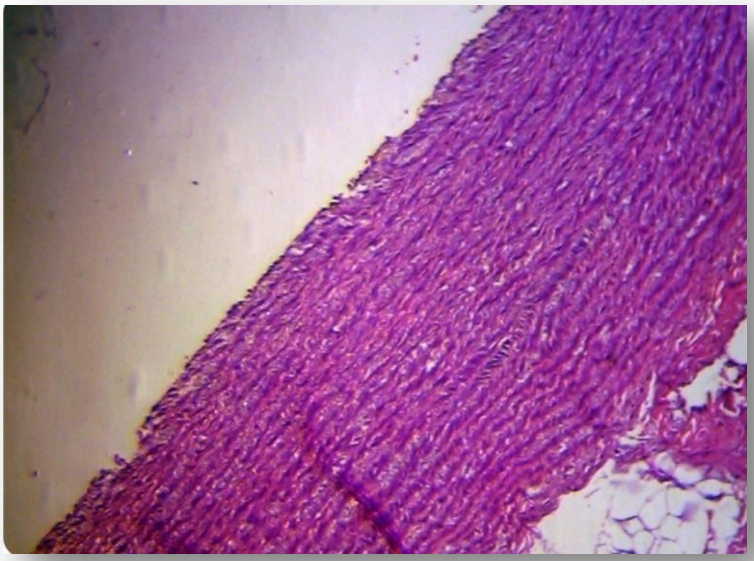

Figure (7): histological section of the aorta of the T2- group post treatment with CdCl2 (50 ppb) in drinking water plus vitamin E (40 mg/kg B.W), showed normal stature (H & E 40 X).

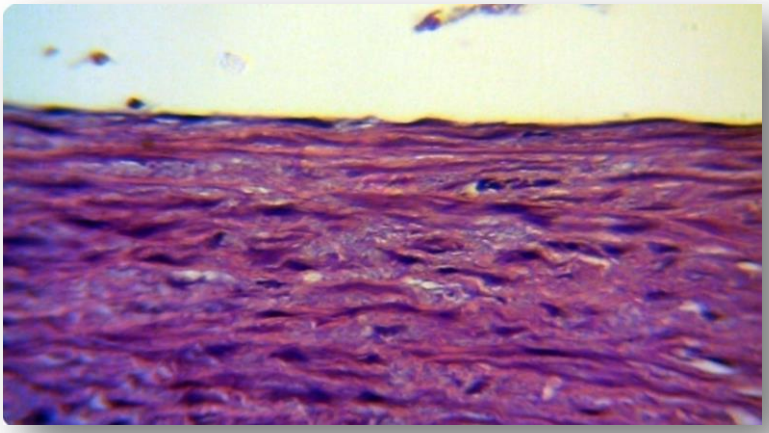

Figure (8): histological section in the aorta of T3- group post treatment with vitamin E (40 mg/kg B.W) showed no clear pathological lesion (H & E 40 X).

Discussion

The present study pointed to significant increase in platelets count with significant decrease in plasma PTT and PT which may be related to oxidative stress induced by cadmium .Cadmium (Cd) is a divalent transitional metal ion, which has been closely associated with

oxidative stress in adult tissue (Borges et al,2008; Kaplan et al,2008; Ognjanovic et al,2008; Shukla and Kumar 2009).), and in embryonic tissue (Warren et al,2000; Paniagua-Castro et al,2008). Oxidative stress led to increase some of blood coagulation mechanisms represented by decreased prothrombin time and partial thromboplastin time with elevation in platelet count and increased risks of blood clot formation (Al-Shami ,2003). Supplementation of antioxidant vitamins including vitamin E could cause platelets aggregation (Salonen et al,1999; Mabile et al,1999).This effect might be due to its antioxidant and cardioprotective actions(Hassan and Awad, 2007; Nusret, 2009) .

Significant increase in serum cholesterol concentration in T1 treated group indicated the hypercholesterolemic effect of cadmium. In Wistar rats' subcutaneous and intra-peritoneal (i.p.) injection of cadmium (Cd) caused significant changes in lipid profile in serum (Murugavel and Pari, 2007) and brain microsomes (Modi and Katyare,2009) . Because the liver plays a pivotal role in lipid homeostasis in addition to glucose homeostasis, the accumulation of Cd in liver could be responsible for the dysfunction of the liver and the observed alterations of lipid profiles (Murugavel and Pari, 2007; Larregle et al,2008). Generally, heavy metals induce changes in the activity of hydroxy 3-methylglutaryl-coenzyme A (HMG-CoA) reductase , which alters cholesterol as well as all lipid metabolisms. The inflammatory cytokines (tumor necrosis factor [TNF- α] and interleukin [IL] 1β) have been reported to increase the expression of cholesterogenic enzymes including HMG-CoA reductase (HMGR) and suppressed cholesterol 7α-hydroxylase (CYP7A1), a catabolic enzyme of cholesterol in the liver (Hardardottir et al, 1994; Kojima et al,2004). Several studies have also shown that cytokines are involved in increasing serum TG levels and VLDL production by stimulating hepatic lipogenesis and suppressing fatty acid oxidation (Memon et al, 1993; Nachiappan et al, 1994). In agreement with these reports, Cd exposure markedly increased the levels of inflammatory cytokines such as TNF-α and IL-1β in the liver (Kayama et al, 1995; Harstad and Klaassen,2004), which might be responsible for hypercholesterolemic effect of cadmium.

Vitamin E supplementation lowered the elevated cholesterol concentration in T2 group. Vitamin E down-regulates the expression of the cholesterol scavenger receptors SR-A (Teupser et al,1999) via mechanisms that appear independent of protein kinase C and antioxidant activity. Regulation of these sterol receptors occurs at the level of transcription, suggesting that α -tocopherol acts through specific receptors or tocopherol-responsive transcription factors (Azzi et al,2001). Both α- and γ-tocopherol diminished endogenous cholesterol synthesis as well as apolipoprotein-AI-(apo-AI)-mediated cholesterol efflux. These effects were the consequence of a tocopherol-mediated down-regulation of several genes implicated in endogenous cholesterol synthesis (Landriera et al.,2010).

A significant decrease in serum GSH concentration T1 group are in accordance with (El-Maraghy et al,2001) and (Eybl et al,2004) who suggested that cadmium toxicity can cause oxidative stress by an interaction with –SH groups of major intracellular defender glutathione and that lipid peroxidation is an early and sensitive consequence of acute Cd exposure. The intravenous administration of cadmium chloride (2 mg/kg bw/day) resulted in a pronounced increase of lipid peroxidation in the liver of rat accompanied by a depletion of hepatic GSH (Nemmiche et al,2007). As well as, cadmium accumulation in liver and kidney of rats due to chronic dietary intake, is associated with alteration of enzymatic (SOD, CAT and GST-px) and non enzymatic antioxidants (GSH, vitamins C, E) (El-Sharaky et al,2007; Ognjanovic et al,2008). This may possibly be due to the excessive formation of FRs, which led to deteriorations of biological molecules (Stohs et al,2001; El-Maraghy et al,2001). Vitamin E appears to be the most effective lipid soluble antioxidant in biological systems (Nagel et al,1997). It inhibits lipid peroxidation and regenerates reduced vitamin C and glutathione

(GSH) (Upston et al,1999). Cadmium induces an oxidation of cellular lipids and proteins and administration of α-tocopherol can reduce Cd-induced oxidative stress and improve the glutathione level (Satarug and Moore,2004; Nemmiche et al,2007).

Histological changes observed after cadmium treatment may indicate disturbance in cardiovascular function. A large number of studies have suggested a possible link between exposure to Cd and the development of atherosclerosis and hypertension (Navas-Acien et al,20005; Kaji , 2004) and there is evidence suggesting that the vascular endothelium may be intimately involved in mediating these effects of Cd (Szuster-Ciesielska et al,2000). Cd can cause the release of a variety of proinflammatory mediators from endothelial cells that would facilitate the inflammatory component of the atherosclerotic process (Szuster-Ciesielska et al,2000; Mlynek and Skoczynska,2005; Gryg et al,2002). Vitamin E is lipophilic and has been shown to inhibit the oxidative modification of low density lipoprotein (LDL), a process thought to be of crucial importance in atherogenesis (Helzlsouer et al,2000) and prevention of cardiovascular diseases. Inhibition of monocytes adhesion (Areds,2001),cytokine expression (Nedeljkovic et al,2003) and maintenance of vascular endothelial and smooth cells integrity (Vivekananthan et al,2003; Lonn et al,2005;64)due to vitamin E antioxidant effect could also be claimed. In conclusion, this study approved that Cadmium has a deleterious effect on some aspect of cardiovascular system while vitamin E has antioxidant cardioprotective roles.

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Original Article

The Pathology of Experimental Rhoodococcus equi infection in foals

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Abstract

The pathology of experimental *Rhodococcus equi (R .equi*) infection in 2-8 weeks-old-foal is studied. For this purpose, twenty foals were divided into three groups, and given *R. equi* intratracheally (1st group), through gastric route (2nd group) and through umbilicus by contamination (3rd group). A control group of foals were given a Phosphate buffered Saline (PBS). Pulmonary and intestinal lesions were seen in foals of all infected groups. Grossly, there were multiple, variable-sized abscesses diffusely scattered throughout the lung parenchyma, in addition to the presence of different stages of pneumonia with variable-sized areas of consolidation and emphysema. Intestinal lesions were evident as engorgement of mesenteric blood vessels, subserosal hemorrhages seen along the intestinal tract especially the small intestine, in addition to enlargement of lymph nodes (mesenteric, bronchial and mediastinal). Some lymph nodes were edematous, have circular foci of caseous necrosis and some of them were filled with yellowish, thick creamy pus.
The microscopic lesions were basically similar in all foals of the experimental groups, but varied depending on the time of death or euthanasia and included: acute pulmonary congestion, acute suppurative broncho-pneumonia, chronic pyogranulomatous pneumonia, and emphysematous and atelectatic area. There were focal necrosis of the pulmonary parenchyma and numerous bacterial colonies seen free or as aggregates within the cytoplasm of many histiocytes. Also there were focal interstitial thickening of the alveolar septae. The pleura and interlobular septae were thickened due to cellular infiltration.

Keywords: *Rhodococcus equi*, foals, histiocytes, umbilicus, pneumonia

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Introduction

Rhodococcus equi is recognized chiefly as a cause of suppurative bronchopneumonia in the foals, but ulcerative entercolitis is another, although less common manifestation of the infection in young horses (Barton and Hughes, 1980; Elissaldi et al., 1980; Meijer and Prescott, 2004; Özsoy, and Haziroglu, 2009). Generalization of infection from the lung occasionally leads to suppurative arthritis, hepatic or splenic abscesses, renal infarcts, vertebral abscesses or hypopyon (Wilson, 1955; Barton and Hughes, 1980). Cellulitis and ulcerative lymphangitis have been reported following skin injury by nematode larvae (Dewes, 1972; Dewes, 1989; Barton and Hughes, 1980; Etherington et al., 1980).

The pulmonary lesions caused by *R.equi* usually take the form of a chronic suppurative bronchopneumonia often with prominent abscesses. The relationship between the development of the intestinal and pulmonary lesions is not fully understood. Many authors consider the intestinal whether clinical or inapparent as usual initiator of the pulmonary abscesses (Bull, 1924; Bain, 1963; Gary Muscatello, 2012; Giguère et al, 2012; Noah Cohen, 2012). It is thought that dissemination of infection from the gut occurs via intestinal lymph nodes to the pulmonary circulation. The distribution of the pulmonary lesions in some naturally-infected foals, which suggests an embolic spread throughout the lungs, has been tendered as supportive evidence for this hypothesis (Bull, 1924; Bain, 1963; Giguère et al, 2012). Barton and Hughes, (1980) suggested that spread of the disease via umbilical route is not an important factor in its pathogenesis, while Martens et al, (1982), suggested that umbilicus is a common route of entry for many pathogens in the neonate. Transmission studies involving umbilical deposition of *R. equi* are not reported to the best of our knowledge. Johnson et al 1983 a,b described gross and histopathological changes in foals following intrabrochial and intragastric inoculation of *R.equi*. They found that all foals in the intrabronchial group developed a severe bronchopneumonia in the inoculated lung. In 8 to 9 days old lesion, the alveoli were filled with macrophages, neutrophils and multinucleated giant cells and most contained numerous *R. equi*. The few foci of alveolar necrosis were associated with aggregates of bacterial-laden macrophages undergoing degeneration. In addition, there were extensive parenchymal destruction, little fibrous tissue reaction, hyperplastic bronchiolitis, pulmonary edema, perivascular lymphocytic cuffs and a pyogranulomatous lymphadenitis in bronchial lymph nodes. In case of intragastric inoculation, severe ulcerative colitis, typhlitis and lymphadenitis of colonic and cecal nodes were seen.

An aim of the present work was to study the gross and histopathological changes in foals experimentally infected with *R.equi* through different routes (intratracheal, intragastric and navel infections of foals).

Materials and methods

Twenty 2-8 week-old- clinically normal Arabian foals were individually housed with their dams. The foals were randomly divided into three groups as follow:

- Group.1 (intratracheal inoculation)

Foals in this experiment were randomly numbered and placed in either infected or control group. Foals, number 1-4 were given $6.5X10^{10}$CFU *R.equi* in 40 ml PBS intratracheally. Foals numbered 5-8 were reared in a separate building acted as control and given 40 ml sterial PBS intratracheally.

- Group .2 (intragastric inoculation)

Foals in the second group were randomly divided in either infected or control group. The infected group (9-12) received 6.5X1010CFU *R.equi* in 75 ml PBS by stomach tube daily for five consecutive days. The stomach tube subsequently flushed with 400 ml of tap water before removing through the nose. While control group (13-16) received 75ml of sterile PBS using stomach tube.

- Group .3 (Navel infection)

Two foals (17-18) from the third group were infected through the umbilicus (navel) during the first few hours after birth by bacterial culture. This was done by contaminating the umbilicus of the newborn foal by *R.equi* culture. The other two foals (19-20) were acted as control foals.

Foals showed clinical signs of *R.equi* infection during the course of observation. At the peak of the clinical disease, foals were euthanized by intravenous injection of 10 ml sodium pentobarbital to perform necropsy. Pieces of the following tissue were collected in 10% neutral- buffered formalin for histopathological examinations: lungs, spleen, liver, kidney, brain, lymph nodes (bronchial, mediastainal and mesenteric) and several sites in the small and large intestine. After fixation tissues were processed routinely for paraffin embedding and 4-6 µm thick sections were cut and stained with hematoxylin and eosin. This study was approved by the research committee/College of veterinary medicine / university of Baghdad.

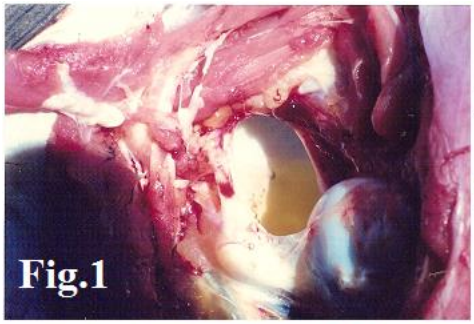
Results

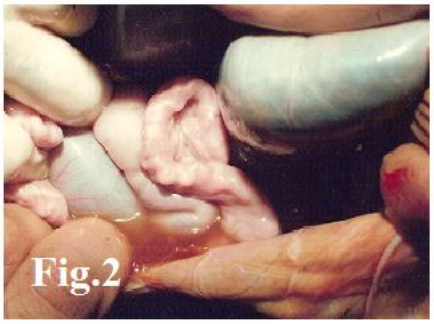
Gross lesions

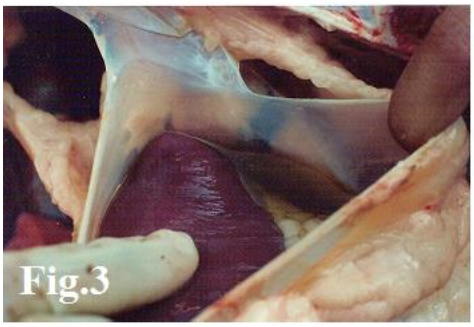
The most prominent gross lesions seen in foals of the intratracheally-infected group were the followings: In all foals, the eyes had petechial hemorrhages seen in the conjunctivae of both eyes and there was a slight to moderate increase in the amount of synovial fluid of the hip and knee joints (Figure.1). The fluid was serous in nature. The abdominal cavity had hydroperitoneum with a fluid volume ranging from 80-150ml. The fluid was turbid and slightly thick in consistency (Figure. 2). The chest cavity had straw-colored, watery fluid ranging in amount from 15-25 ml, with some fibrin strands seen in it. In two foals No.3, 4 of this group there was an increased amount of serous fluid in the pericardial sac (Figure.3), with the heart of these two foals showing subepicardial petechial hemorrhages in the left ventricle, together with gelatinous atrophy of the subepicardial adipose tissue seen in all foals (Figure.4). Focal subendocardial hemorrhages mostly seen as ecchymoses in the left ventricle were seen in foal No.4. Both lungs of infected foals had multiple abscesses but severity of the lesion seemed to be more extensive in the right lung. Besides, dorsal surface of both lungs was more involved (Figure.5). The left lung of all foals had diffuse consolidation of the caudal lobe together with variable –sized, raised focal emphysematous areas. Two foals (No.1 and 3) showed greyish hepatization while the other two foals showed dark red consolidations together with focal petechial hemorrhages seen throughout the dorsal surface and especially the uppermost part of the caudal lobe of left lung. Multiple nodular grayish areas (suppurative foci) were seen extensively scattered through the pulmonary parenchyma of the ventral surface of left lung in all foals. Cut section of these nodules revealed suppurative exudate which was yellowish to greyish in color and creamy in consistency (Figure.6) in two foals (No.1 and 3) a well- developed suppurative foci (abscesses) were seen since these foci were walled off by fibrous connective tissue capsule. The abscesses were approximately 2mm in diameter, and embedded in the pulmonary parenchyma. In all foals emphysematous areas are seen adjacent to the consolidation areas. In one foal (No.4), cut section of the left caudal

lobe revealed a totally consolidated lung tissue with tissue with wet foci from which too much exudate was oozing.
The right lung was extensively involved. In foal (No.4), the cranial lobe had almost two thirds of its surface area being involved, while the caudal lobe had almost one-half of it being consolidated. The lesions seen in the right lung of two foals (2and 4) were in the form of greyish areas of consolidation, ranging in diameter from 2-8 mm (Figure.7). Also there were dark red, firms, nodular lesion. The cut section of the right caudal lobe revealed foci of consolidation, with meaty to firm consistency dark red color and were well-delineated by a zone of congestion. Occasionally, focal areas of emphysema and atelectasis were also seen. The right lungs of two foals (No.1 and 3) have large foci of atelectasis surrounded by multi focal emphysemateous areas. In addition to the previous lesion, two foals (No.1, and No. 3) had bronchiectasis with the reason for widening was the large amount of suppurative exudate in the airways. In all foals subpleural emphysematous areas were seen on the dorsal aspect of the caudal lobes and some areas of congestion were present. Tracheal mucosa was slightly congested with little amount of mucopurulent exudate seen in the lumen of trachea was severely congested and contained large amount of mucopurulent exudate. Lymph nodes were enlarged in all foals. In three foals (1, 2 and 4) the bronchial, mediastinal and mesenteric lymph nodes were so enlarged, edematous and have ecchymoses in its cortex. Besides, there was no clear demarcation between cortex and medulla. In one foal (No.3) there was suppurative lymphadenitis as evidenced thick greyish to yellow creamy pus (Figure. 8). Mesenteric blood vessels were engorged in all foals and in one foal (1) whitish to grey, nodular to elongated elevations varying in size from 0.3-2.5 cm were seen along the caecum, colon, rectum and mesentery.
Other gross pathological lesions were: congested liver rounded edges and focal areas of whitish to yellowish discoloration in two foals (2 and 4). Kidney was occasionally slightly congested. Adrenal gland had hemorrhages with multifocal greyish areas scattered through its cortex and medulla. The cut section of the foci caused oozing of blood-stained serous fluid. Spleen was slightly enlarged and congested.

All infected foals in the intragastric inoculation appeared in a poor nutritional status, severely emaciated with eyes had bilateral mucopurulent discharge. There was severe hyperemia and petechiation of the conjunctivae. Evidence of dehydration and diarrhea was seen in all foals. There was a serious atrophy of the subcutaneous adipose tissue with severe engorgement of the subcutaneous vessels. All foals showed also the same gross pathological lesions that appeared in the foals of the intratracheal group in addition to attachment of the large intestine and severe engorgement of mesenteric blood vessels (Figure.9). Mesenteric lymph nodes were edematous and approximately four times the normal size. The cecal and colonic lymph nodes contained circular foci of caseous necrosis and some lymph nodes filled with yellowish, thick, creamy pus.Some foals showed 4mm in diameter whitish grey focal area (micro abscesses) on the surface of the liver.
All foals in navel infected group were emaciated with congested mucous membranes and engorged conjunctival capillaries. There was a pale yellowish nasal discharge. Foals also showed hydroperitoneum, congested liver, enlargement of mesenteric lymph nodes, hydrothorax , hydropericardium and flappy heart . Both lungs showed well-delineated variable sized, multiple abscesses distributed throughout lung parenchyma in addition to other lesions that appeared on the lungs, bronchia intestine, liver and lymph nodes.







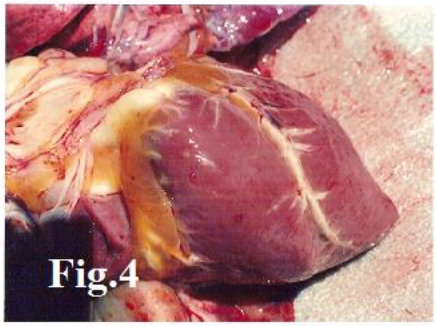
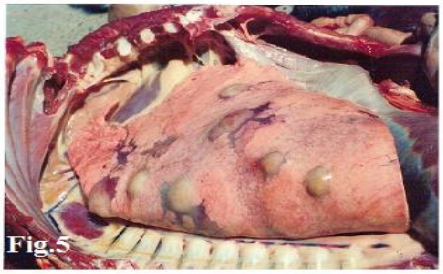
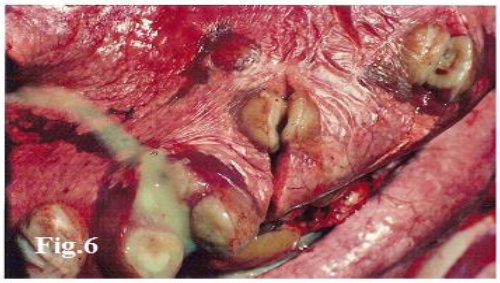


Figure.1: Shows the increase in the amount of synovial fluid of the hip joint.
Figure.2: shows the hydroperitoneum with a fluid volume ranging from 80-150ml.
Figure.3 shows the hydropericardium
Figure.4: Shows subepicardial petechial hemorrhages in the left ventricle, together with gelatinous atrophy of the subepicardial adipose tissue.





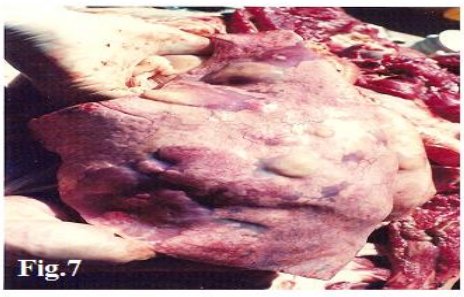


Figure.5: Shows multiple abscesses distributed on the surface of the lungs
Figure.6: shows the cut section of the nodules that revealed suppurative exudate which was yellowish to greyish in color and creamy in consistency
Figure.7: Shows greyish areas of consolidation, focal areas of emphysema and atelectasis
Figure.8: Shows suppurative adenitis that contained thick greyish to yellowish creamy pus

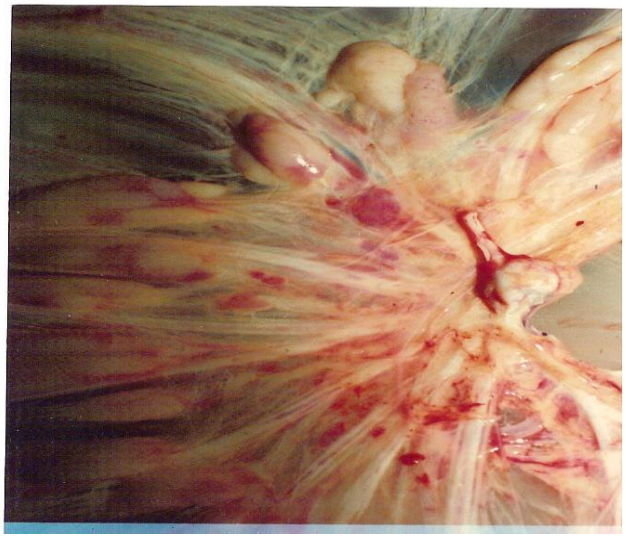


Figure.9: Shows severe engorgement of mesenteric blood vessels in addition to enlargement of mesenteric lymph nodes.

Microscopical findings

The microscopic appearance of lung lesions was similar in animals of the experimental group with slight differences. The most salient features of the lesions were briefly as follows: Acute suppurative bronchopneumonia was diagnosed and characterized by cellular filling of the alveolar spaces. The inflammatory cells present were PMNS and MN-cell type. Occasionally minimal amount of proteinaceous seepage was seen in the alveolar lumen together with some fibrinous networks (Figure. 10) .Lesion of the airways was characterized by degenerative changes of its mucosa with loss of cilia, focal loss of epithelial lining, migration of acute inflammatory cells toward the lumen and presence of suppurative exudate in their lumen. Multiple, variable- sized abscesses were seen in various stages of development but usually with no complete fibrous tissue encapsulation but definitely a starting to well-developed fibroplasia was seen adjacent to a pool of necrotic and living inflammatory cells (PMNS) (Figure.11). Morphological diagnosis of the more advanced lesion was chronic suppurative granulomatous pneumonia ¨pyogranulmatous response˝ (Figure.12 A&B), which was characterized by a diffuse mononuclear, polymorphonuclear cellular infiltration predominantly neutrophils and histiocytes together with lymphocytes, plasma cells and fibroblasts. Numerous multinucleated giant cell formations were also seen, these giant cells were variable in size and the number of their nuclei, associated with these lesions was focal areas of necrosis of the pulmonary parenchyma with numerous dots of phagocytized bacteria seen free or within cytoplasm of many inflammatory cells as basophilic granular structures (Figure.13&14). Also there were multifocal areas of emphysema and atelectasis and acute pulmonary congestion characterized by severe dilatation of the alveolar capillaries and occasionally frank hemorrhages were seen. Focal interstitial thickening of the alveolar wall, Pleura and interlobular septae thickening were seen due to cellular infiltrations, exudation and congested blood vessel.

Small intestine of all foals revealed acute necrotizing enteritis with necrotic eosinophilic masses, congestion and edema of the submucosa of ileum together with mild to moderate cellular infiltration predominantly eosinophils and lymphocytes. There was focal soughing of the superfacial epithelium of the gastric mucosa with slight MN type cells infiltration

including macrophages, plasma cells and few lymphocytes were seen in the lamina proparia. Submucosa had edema and numerous MN cells mainly macrophages but with some eosinophils. Adrenal glands showed vacuolation of cells of the zona reticularis. The cells were swollen and had fine vacuolation together with degenerative changes of their nuclei (karyorrhexis). Spleen showed congestion and focal mild hemosiderosis. Kidneys showed acute mild tubular degeneration. Liver had focal MN cellular infiltrations in the portal areas and in the lobular parenchyma, in addition to acute hepatocellular degeneration with eosinophilic infiltrations (chronic necrotizing hepatitis). Small intestine of all foals revealed acute necrotizing enteritis with necrotic eosinophilic masses, congestion and edema of the submucosa of ileum together with mild to moderate cellular infiltration predominantly eosinophils and lymphocytes. There was focal soughing of the superfacial epithelium of the gastric mucosa with slight MN type cells infiltration including macrophages, plasma cells and few lymphocytes were seen in the lamina proparia. Submucosa had edema and numerous MN cells mainly macrophages but with some eosinophils. Adrenal glands showed vacuolation of cells of the zona reticularis. The cells were swollen and had fine vacuolation together with degenerative changes of their nuclei (karyorrhexis). Spleen showed congestion and focal mild hemosiderosis. Kidneys showed acute mild tubular degeneration. Liver had focal MN cellular infiltrations in the portal areas and in the lobular parenchyma, in addition to acute hepatocellular degeneration with eosinophilic infiltrations (chronic necrotizing hepatitis).

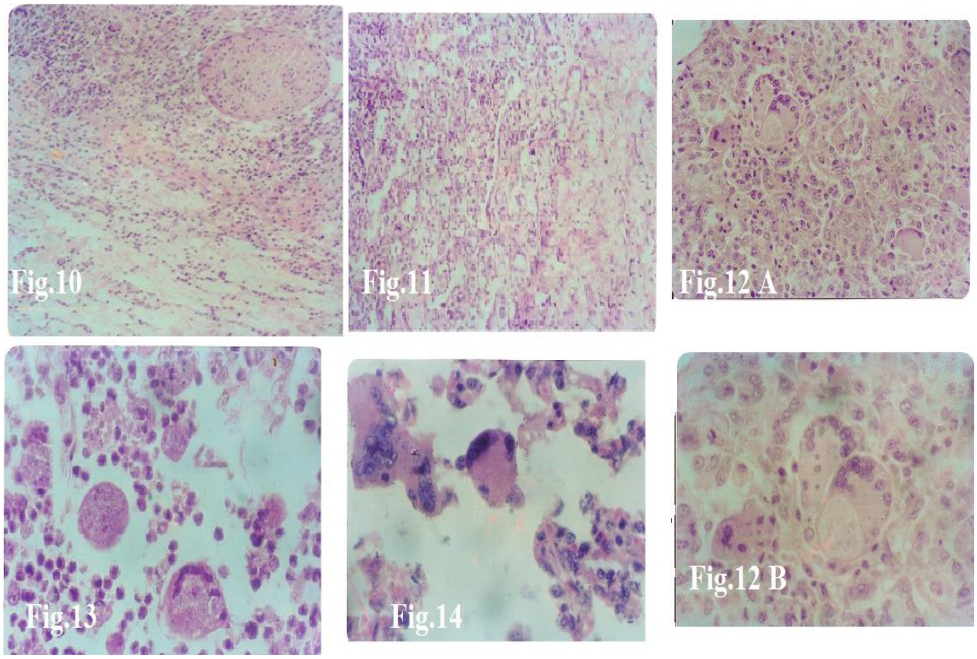


Figure.10: Shows lung of an intratracheal infected foal. Cellular filling of the alveolar space (PMNs and MN-cell type) with minimal amount of proteinaceous seepage together with an intravascular fibrinous network (H&E X100)
Figure.11: lung section shows suppurative exudate (right side) was developed fibroplasia. (H&E X100)
Figure.12: shows chronic suppurative granulomatous pneumonia ،،pyogranulmatous response،، (A.H&E X200, B. H&E X400)
Figure.13: Lung of a foal from intratracheal inoculation group.Numerous dots of phagocytized bacteria seen within cytoplasm of histiocyte. (H&E X400).
Figure.14. shows multinucleated giant cells.

Discussion

The pneumonia associated with *R. equi* infection in the foal is described most commonly as a suppurative bronchopneumonia with prominent abscess formation (Bain, 1963; Barton and hughes 1980; Barton and hughes 1984; Elissalde et al 1980;Meijer and Prescott, 2004; Özsoy, and Haziroglu, 2009). In this study all foals in the intratracheal group developed severe and extensive suppurative bronchopneumonia with large areas of caseous necrosis. The encountered lesions resembled those of natural infection of foal in which the disease could run a more fulminating course than usual, besides, this result is in agreement with previous experimental work (Johnson et al, 1983 a; Perkins,et al 2001: Jacks, et al 2007). The high concentration of bacteria in the inoculum and the manner of deposition (directly into the lung) no doubt contributed to the extensive tissue destruction. Histologically, lung lesions were characterized by massive cellular infiltration into the alveolar space. The majority of these cells were large macrophages, most probably representing macrophages both from resident proliferating alveolar macrophages and from inflammatory monocytes recruited from the circulating blood.

Multinucleated giant cells were a prominent component of the cellular exudate, even in the early lesions. These polykaryons were thought to be inflammatory cells of monocyte-macrophage lineage rather than syncytial giant cells of epithelial origin. One of the mechanisms of formation of inflammatory polykaryons is simultaneous endocytosis, where by several macrophages attempting to phagocytize the same particles and fuse in the process (Chambers, 1977). Inflammatory polykaryons once formed have a low phagocytic capability (Chambers,1978). Since most of the multinucleated giant cells in the *R. equi* experimental lesions (including our findings), had large phagocytic vacuoles containing numerous bacteria, it is likely that simultaneous endocytosis was the mechanism for their formation.

The presence of giant cells, as well as the predominance of macrophages among, the other things has established the lesions of *R.equi* as granulomatous in nature (Sippel et al, 1968; Reuss, et al 2009). Microscopic intestinal lesions typical of *R. equi* infection were present in all foals, intestinal lesions often accompanied pulmonary ones in the naturally- occurring diseases although the foal may not develop diarrhea (Hutchins et al, 1980). It has been suggested that the intestinal lesions develop from swallowing infectious expectorate. The naturally occurring *R. equi* entercolitis, in which there is no accompanying pneumonia (Bull, 1924; Sippel, 1968; Cimprich and Rooney 1977). In this study we produced typical intestinal lesions, in foals given the organism on five consecutive days. A single dose of the organism was not effective, emphasizing the dose dependence of lesion generation. The interval between infection and development of enteric lesions was approximately three weeks.

A single dose of organism was insufficient to alter the balance of the normal flora or to breach the intestinal epithelium in sufficient numbers to cause gross lesions, although microscopic lesions were seen in foals after infection. During the period of establishment of *R. equi* in the normal foal intestinal tract, transient microscopic and sometimes macroscopic lesions may develop in the mucosa and in the draining lymph nodes (Hutchins, et al, 1980: Jacks, et al 2007). Such macroscopic lesions are occasionally described as incidental necropsy findings in foals which die of other causes (Mahaffey, 1962). Whether foals develop severe lesions and the associated clinical sign (diarrhea) probably depends upon the size of the bacterial challenge and individual foal factors, including the animal's immunological status (Woolcock et al., 1980; Perkins, et al 2001: Jacks, et al 2007). The gross lesions in experimental foals were typical of those seen in naturally- infected foals described in the literature. The earliest microscopic lesion in the intestinal tract of the experimental foals indicated that *R.equi*

penetrates the specialized epithelium lying over the payer's patches. This route of entry has been demonstrated for other enteric pathogens, such as Salmonella (Carter and Collins, 1974), and would explain the predilection of the *R. equi* lesion for the gut-associated lymphoid tissue. Within the payer's patches, failure of the phagocytes to eliminate the organism generates the typical pyogranulomatous inflammatory response described in the natural disease (Elissalde et al, 1980; Falcon, et al 1985; Zink, et al 1986; Takai, et al 2000; Noah, 2012). Villous atrophy has been described in the small intestine of both acute and chronic cases of naturally-occurring *R.equi* enteritis (Cimprich and Rooney, 1977). Microcopic lesions in the mesenteric lymph nodes from the natural disease were also pyogranulomatous and usually so extensive that the architecture of the node is obliterated. Where the structure of the node has not been destroyed, the microscopic picture suggests that viable *R. equi* are transported to the node within phagocytic cells and are arrested in the interfollicular cortex, where the earliest pyogranulomatous lesions occurred.

Foals in the navel- infected group showed both pulmonary and enteric lesions. The umbilicus is a common route of entry for many pathogens in the neonate. However, transmission studies involving umbilical deposition of *R. equi* have not been reported (Martens et al, 1982). The development of pulmonary and enteric lesions in the navel- infected foal in this study means that this route is an important one in the development of the disease. Comparisons have been done between the microscopic lesions of *R. equi* and human mycobacterial infections, particularly lepromatous leprosy (Sippel et al 1968; Elissalde et al , 1980; Gansert et al 2003). *R. equi* furthermore, causes a lymphoadenitis in cattle and pigs which is indistinguishable grossly and microscopically from tuberculosis (Densen and Mandell 1980). The presence of mycolic acids in the cell wall of *R. equi*, Mycobacterium , and Nocardia spp (Barton and Hughes ,1980; Prescott,1991; Gansert et al 2003) may account for the similarity of tissue response which is typical of a facultative intracellular parasite. Facultative intracellular parasites are characterized by their ability to survive and to multiply within a phagocytic cell by evading the usual destruction that follows upon ingestion (Densen and Mandell 1980; Jacks, et al 2007). The suggestion has been made that *R. equi* behaves in such a manner (Hutchins et al, 1980). In the experimentally induced lesions, the bacteria are strongly cell associated. Indeed that's what we encountered, since few bacteria appeared to be multiplying freely in the alveolar space or in the necrotic material and many of them were intracellular. The bacteria retain their Gram-positive staining characteristics within the phagocytic vacuoles indicating that the cell wall is undamaged. Furthermore, the earliest indication of necrosis in the lung parenchyma in the experimentally-induced lesions was associated with groups of degenerate macrophages which primarily contained many bacteria. It is likely that the bacteria were responsible for the degeneration of the phagocytes. The histopathology of advanced natural and experimental *R. equi* lung lesions showed caseous necrosis as the predominant lesion. The tissue destruction may be due to either to bacterial, toxins, products of the inflammatory response, or to a combination of both factors. One potential *R. equi* toxin is the phospholipase C which could acts synergistically with a product of *C. pseudotuberculosis* to lyse sheep erythrocytes (Bernheimer et al, 1980; Prescott, 1991). It is doubtful, however, that this toxin is important in vivo, lysosomal enzyme, chiefly acid hydrolases, which have the capacity to degrade a wide range of natural tissue substrates probably are responsible for much of the tissue destruction in *R.equi* lesions. The enzymes can diffuse into the tissue destruction in *R.equi* lesions. The enzymes can diffuse into the tissue, not only from disintegrating macrophages and neutrophils but also from live cells engaged in phagocytosis.

In conclusion, this study approved that experimental infection of foals by *R. equi* ,via intratracheal, intragastric and navel infection lead to develop Pulmonary and intestinal lesions in foals of all infected groups.

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