

**RELATIONSHIPS BETWEEN EXAM PERFORMANCE AND SOME  
BIOCHEMICAL PARAMETERS****JWAN ABDULMOHSIN ZAINULABDEEN<sup>\*1</sup> AND MOHAMMED MAHDI SAMI<sup>1</sup>***<sup>1</sup>Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.***ABSTRACT**

Examination stress is an ordinary and prevalent phenomenon for students, so the aim of this study was to investigate the relation of exam performance and first semester score with some biochemical parameters in sera of undergraduate students affecting by temporarily examination stress. Fifty five apparently healthy undergraduate students were enrolled in this study, their age was ranging from 19 to 24 years and the mean values of BMI were  $(24.20 \pm 2.6 \text{ kg/m}^2)$ . A blood sample was collected in two times from each student, firstly was on the morning of a written exam (Exam day or E-day) and secondly was in the day that the students do not have any exam (Normal day or N-day). The serum cortisol, fasting blood glucose, amylase activity, and albumin were measured by spectrophotometry, while total serum copper, iron, and zinc ions were measured by flame atomic absorption technique. The results (comparing E-day with N-day) were indicated that the examination stress is related to the exam performance and first semester score; meanwhile there were relations between fasting blood glucose and copper levels with exam performance and also with first semester score.

**KEYWORDS:** Some biochemical parameters, Exam performance, First semester score.**JWAN ABDULMOHSIN ZAINULABDEEN**  
Department of Chemistry, College of Science, University of Baghdad,  
Baghdad, Iraq

## INTRODUCTION

Stress is a necessary and unavoidable definite of daily living, necessary because without a stress human would be listed and apathetic creature, and unavoidable because it relates to any external event, be it pleasurable or anxiety producing.<sup>1</sup> Stress occurs with an interaction between individuals and sources of wish within the environment and is affected by one's perception of the stressor either as adaptive or threatening to one's resources.<sup>2</sup> A stressor is any real physical, social, or psychological stimulus that causes human bodies to respond.<sup>3</sup> It may promote physiological or behavioral disturbances or both. A person's response towards stress depends on whether an event is estimated as a challenge or threat.<sup>4</sup> The pathway to response to stress is that the corticotropin-releasing hormone (CRH) is released into hypophysial portal vessels that access the anterior pituitary gland. Binding of CRH to its receptor on pituitary corticotropes induces the release of adrenocorticotrophic hormone (ACTH) into the systemic circulation. The principal target for circulating ACTH is the adrenal cortex, where it stimulates glucocorticoid synthesis and secretion from the zona fasciculata; glucocorticoids are the downstream effectors of the HPA axis and regulate physiological changes through ubiquitously distributed intracellular receptors.<sup>5,6</sup> Cortisol (a glucocorticoid hormone) exerts widespread physiological effects throughout the body, acting in concert with other chemical messengers to help direct oxygen and nutrients to the stressed body site and suppress the immune response, while influencing certain functions, such as appetite and satiety; arousal, vigilance, and attention; and mood.<sup>7</sup> Cortisol (hydrocortisone) is a steroid hormone that is produced mainly by zona fasciculata.<sup>8</sup> It is synthesized from pregnenolone the consequences from cholesterol. The structure of cortisol and all steroid hormone based on the cyclopentanoperhydrophenanthrene nucleus (C17) that include four rings attached three rings; with six carbons and the last one with five carbons. The conversion of or inactive forms cortisol hormone involves alteration of ring substituents rather than the ring structure itself.<sup>9</sup> Several factors play a fundamental role in determining the nature and consequences of the stress response; these factors include inherent features of a given type of stressor as well as the conditions under which the stressor is encountered,<sup>7</sup> meanwhile stressful occupations lead to different health problems.<sup>10</sup> Examination stress refers to the stress or effort that are experienced by students to perform well in final school or undergraduate examinations and competitive college entrance examinations,<sup>11</sup> meantime having examination stress is a common and widespread phenomenon for students,<sup>12</sup> and up to 30%-50% of student's has test-induced anxiety problems.<sup>13</sup> Every student wants to pursue academic success to achieve respect, family pride, and social mobility.<sup>14</sup> The test scores of students may decrease due to anxiety under pressure,<sup>15</sup> whilst if test anxiety is eliminated, student grades will increase,<sup>16</sup> so test anxiety is significantly correlated with low academic performance.<sup>17</sup> A mild degree of stress and strain can sometimes be beneficial, for instance, often feeling mild stressed when carrying out a project or

assignment compels us to do a good job, focus better and work energetically.<sup>18</sup>

## MATERIALS AND METHODS

Fifty five apparently healthy undergraduate students from University of Baghdad, college of science and also from Al-Esraa college were participated in this study. The age of these students ranged from 19 to 24 years, and BMI mean values was  $(24.20 \pm 2.6 \text{ kg/m}^2)$ . Blood samples were obtained from the students in two days; the first day was at the morning of normal day (N-day) and the second day was in the morning of written exam (E-day). These undergraduate students were sitting in a relatively quiet laboratory place, and they rested for (5-15) minutes before blood sample taken, after clotting, the clear serum was separated and stored at  $(-20 \text{ }^\circ\text{C})$ . At normal day, the collecting of samples from undergraduate students with abnormal feeling was postponed to another day. The students were grouped according to exam performance and the first semester score, also they sub grouped to pass, medium, and good, meanwhile, the results were compared E-day with N-day. The study was approved by Human Ethical Committee with reg. No. 3027/G - 27/10/2014 and No.3385/C - 23/11/2024. There were two samples of student excluded from study due to iron deficiency.

### (i) Determination of serum cortisol

serum cortisol quantitatively determined in sera of undergraduate students by Enzyme Linked Immune-Sorbent Assay (ELISA) method using commercial kit manufactured by monobind.

### (ii) Determination of serum fasting blood glucose (F.B.G)

serum glucose was measured according to Dennis's method<sup>19</sup> using commercial kit manufactured by randox

### (iii) Determination of serum $\alpha$ -amylase activity

serum amylase activity was determined by Caraway-Somogyi starch-iodine amylase method.<sup>20</sup>

### (iv) Determination of serum trace element (Cu, Fe, and Zn)

serum copper, iron, and zinc were determined using digestion method with flame atomic absorption technique.<sup>21</sup> Digestion solution prepared by mixing concentrated nitric acid ( $\text{HNO}_3$ ) with the concentrated perchloric acid ( $\text{HClO}_4$ ) (1:4) ratio.

### (v) Data analysis

the data were analyzed by Duncan's multiple range tests at  $p < 0.05$  was accepted as statistically significant, using the SPSS version 21.0 with independent t-test and analysis.

## RESULTS

In current study the relation of some biochemical parameters (cortisol, F.B.G,  $\alpha$ -amylase activity with its specific activity, Cu, Fe, and Zn) with exam performance and first semester score were investigated. As shown in table 1, the studied parameters were

indicated non-significant differences in all studied groups except in F.B.G (was showed significant increase(  $p<0.05$ ) for undergraduate student that got

good degree) , and also in copper (was indicates significant decreases ( $p<0.05$ )) in serum of all three groups in E-day comparing with N-day.

Table 1

**Mean values and  $\pm$ SD of studied biochemical parameters compared N-day with E-day in relation with exam performance.**

Parameters	pairs	Pass	p-value	Medium	p-value	Good	p-value
Cortisol (ng/mL)	E-day	12.55 $\pm$ 1.17	N.S	12.46 $\pm$ 1.26	N.S	12.21 $\pm$ 0.457	N.S
	N-day	12.31 $\pm$ 1.24		12.25 $\pm$ 0.94		12.31 $\pm$ 1.07	
F.B.G (g/dL)	E-day	104.5 $\pm$ 14.2	N.S	105.5 $\pm$ 14.7	N.S	99.01 $\pm$ 16.2	<0.05
	N-day	101.7 $\pm$ 16.4		99.4 $\pm$ 18.4		92.7 $\pm$ 13.6	
Amylase activity (U/L)	E-day	173.7 $\pm$ 48.2	N.S	225.1 $\pm$ 36.6	N.S	174.3 $\pm$ 42	N.S
	N-day	197.7 $\pm$ 45		193 $\pm$ 73.2		166.8 $\pm$ 71.4	
Amylase Sp.ac. (U/g)	E-day	20.3 $\pm$ 5.59	N.S	28.7 $\pm$ 6.06	N.S	21.5 $\pm$ 6.44	N.S
	N-day	24.5 $\pm$ 5.8		23.3 $\pm$ 8.75		21.42 $\pm$ 8.78	
Alubmin(g/dL)	E-day	3.49 $\pm$ 0.351	N.S	3.51 $\pm$ 0.44	N.S	3.47 $\pm$ 0.215	N.S
	N-day	3.64 $\pm$ 0.414		3.34 $\pm$ 0.606		3.45 $\pm$ 0.213	
Cu(mg/L)	E-day	0.337 $\pm$ 0.231	<0.05	0.369 $\pm$ 0.213	<0.05	0.285 $\pm$ 0.221	<0.05
	N-day	0.392 $\pm$ 0.298		0.44 $\pm$ 0.224		0.333 $\pm$ 0.205	
Fe(mg/L)	E-day	1.45 $\pm$ 0.521	N.S	1.3 $\pm$ 0.384	N.S	1.46 $\pm$ 0.457	N.S
	N-day	1.57 $\pm$ 0.227		1.54 $\pm$ 0.193		1.4 $\pm$ 0.413	
Zn(mg/L)	E-day	0.147 $\pm$ 0.027	N.S	0.155 $\pm$ 0.038	N.S	0.158 $\pm$ 0.036	N.S
	N-day	0.134 $\pm$ 0.031		0.15 $\pm$ 0.074		0.14 $\pm$ 0.017	

The results that listed in table 2 shows significant increase ( $p<0.05$ ) in F.B.G in sera of undergraduate students groups that got pass degree while the differences of other groups were non-significant in E-day comparing with N-day in relation with semester score . The same table also, were indicated, significant

decreases ( $p<0.05$ ) in serum copper of students group with good score while other groups were showed non-significant differences. Non-significant decreases in other studied biochemical parameters were found in E-day comparing with N-day in relation with semester score.

Table 2

**Mean values and  $\pm$ SD of studied biochemical parameters compared N-day with E-day in relation with exam first semester score**

Parameters	pairs	Pass	p-value	Medium	p-value	Good	p-value
Cortisol (ng/mL )	E-day	11.93 $\pm$ 1.1	N.S	12.5 $\pm$ 1.103	N.S	12.23 $\pm$ 1.28	N.S
	N-day	12.4 $\pm$ 1.29		12.1 $\pm$ 1.034		12.52 $\pm$ 1.047	
F.B.G (g/dL)	E-day	114.4 $\pm$ 15.81	<0.05	103.2 $\pm$ 16.72	N.S	96.2 $\pm$ 11.13	N.S
	N-day	104 $\pm$ 16.4		96.19 $\pm$ 15.88		94.5 $\pm$ 15.7	
Amylase activity (U/L)	E-day	183.2 $\pm$ 45.48	N.S	176.9 $\pm$ 47.7	N.S	193.5 $\pm$ 77.2	N.S
	N-day	197.9 $\pm$ 55.6		186.5 $\pm$ 40.13		168.1 $\pm$ 80.2	
Amylase Sp.ac. (U/g)	E-day	24.95 $\pm$ 5.42	N.S	21.72 $\pm$ 6.84	N.S	23.07 $\pm$ 7.1	N.S
	N-day	22.94 $\pm$ 5.87		24.29 $\pm$ 10.49		20.7 $\pm$ 10.49	
Alubmin(g/dL)	E-day	3.53 $\pm$ 0.3	N.S	3.51 $\pm$ 0.27	N.S	3.44 $\pm$ 0.346	N.S
	N-day	3.63 $\pm$ 0.23		3.41 $\pm$ 0.179		3.513 $\pm$ 0.562	
Cu (mg/L)	E-day	0.327 $\pm$ 0.194	N.S	0.292 $\pm$ 0.19	N.S	0.341 $\pm$ 0.138	<0.05
	N-day	0.381 $\pm$ 0.22		0.349 $\pm$ 0.141		0.393 $\pm$ 0.111	
Fe (mg/L)	E-day	1.19 $\pm$ 0.309	N.S	1.5 $\pm$ 0.432	N.S	1.41 $\pm$ 0.517	N.S
	N-day	1.21 $\pm$ 0.57		1.46 $\pm$ 0.341		1.57 $\pm$ 0.166	
Zn (mg/L)	E-day	0.147 $\pm$ 0.027	N.S	0.155 $\pm$ 0.038	N.S	0.158 $\pm$ 0.036	N.S
	N-day	0.134 $\pm$ 0.031		0.15 $\pm$ 0.074		0.14 $\pm$ 0.017	

The following (figure 1) appears the significant results of the studied biochemical parameters in relation with

exam performance and first semester score in E-day in comparison with N-day.

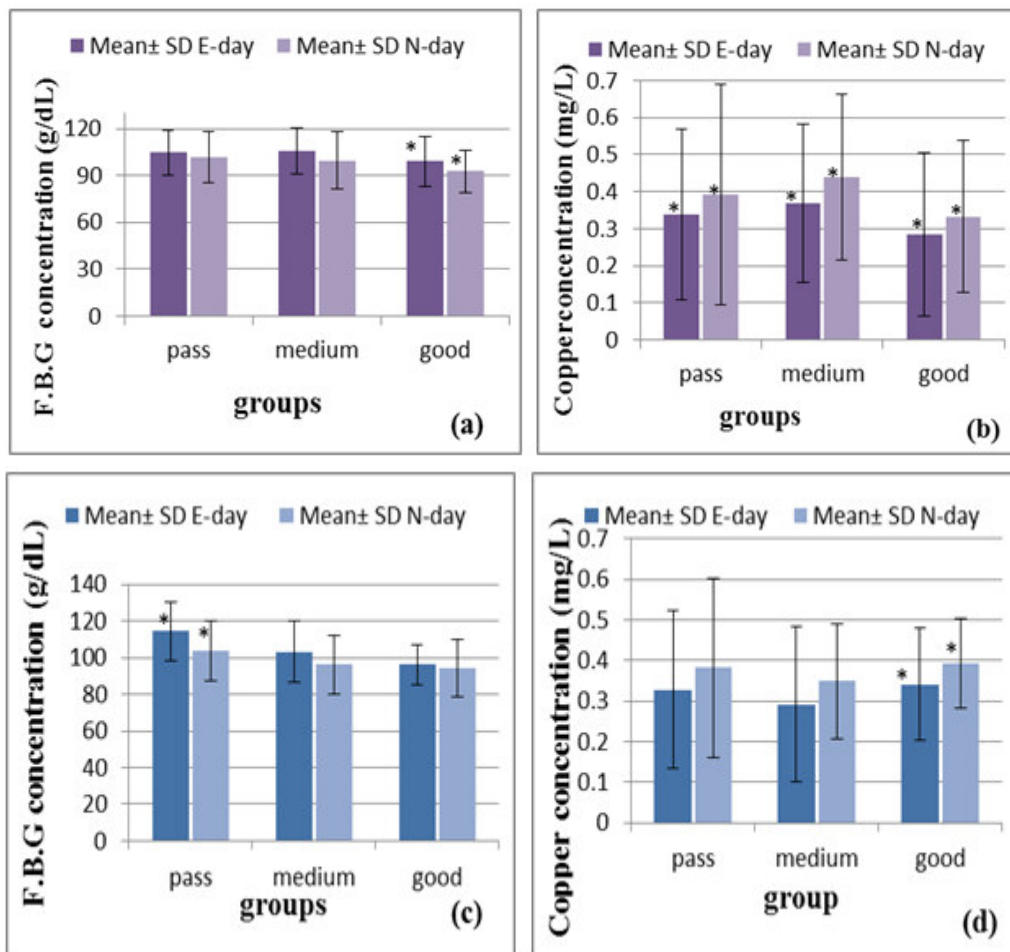


Figure 1

Mean values and  $\pm$ SD of (a): F.B.G (b): Copper concentrations comparing N-day with E-day in relation with exam performance. (c): F.B.G (d): Copper concentrations comparing N-day with E-day in relation with first semester score.

## DISCUSSION

Recently selected biochemical parameters were evaluated in sera of Iraqi academic students in E-day comparing with N-day,<sup>22</sup> this is in agreement with the results of the present study appeared non-significant differences in its level in E-day comparing with N-day in relation with exam performance and also with the first semester score for all three groups, (Table 1&2), that may be because the adaptation of these students with stress.<sup>22</sup> In relation with exam performance the same table was indicated significant increasing of serum F.B.G in sera of undergraduate students that got good performance and non-significant values recorded for other groups (pass and medium). there were previous studies that refer to increasing of F.B.G as results of stress.<sup>23,24</sup> That may be because of increasing of mental activity which is associated with increased glucose metabolisms in brain,<sup>25,26</sup> that means, higher levels of blood glucose are associated with higher levels of glucose in brain.<sup>27-29</sup> Previous study was demonstrated that improving the memory performance, in which the number of words recalled correlated significantly with blood glucose levels from a 50-gram glucose versus a placebo(non-glucose drink) drink in young healthy adult participants and the low blood glucose within the normal range, can negatively affect

memory performance.<sup>30,31</sup> As mentioned above it can say that the increasing serum glucose is a response to increasing its metabolism by brain due to increasing mental activity that causes by exam (thinking about the exam and try to remember some information that related to the exam), that may be the reason for our result. Increasing serum glucose in group with good performance due to stress<sup>32</sup> and mental activity<sup>24</sup> not because of fear of exam but it is due to competition with other colleagues to get a higher grade.<sup>33,34</sup> Table 1 was showed non-significant differences in serum amylase activity with its specific activity in all three groups comparing E-day with N-day. There were studies reported that the salivary amylase activity increased significantly in examination stress comparing E-day with N-day.<sup>35,36</sup> Differences in our results comparing with previous studies may be due to the differences in sources of synthesis and secretion of serum and salivary amylase, in which salivary amylase synthesis in salivary gland specifically in parotid gland,<sup>37</sup> while serum amylase synthesis and secreted mainly in pancreatic acinar cells,<sup>38</sup> also 80% of amylase that synthesized in pancreatic acinar cells stored in salivary gland,<sup>39</sup> however nutritional deficiencies compromise salivary gland function,<sup>40</sup> in addition, there is no study refer to the response of the pancreatic acinar cell to the psychological stress. Significant decrease in serum copper were indicated in pass and medium groups

comparing E-day with N-day, Table 1. The students with high level of serum copper had a cognitive problem as well as low academic achievement,<sup>41</sup> also the association between high serum copper levels and low cognitive performance in older adults.<sup>42</sup> Copper may be linked to working memory through its effects on attention, copper tends to accumulate to a higher level in brain areas associated with attention, that means, decreasing of serum copper may be a type of response to improvement of working memory.<sup>43</sup> The students with high level of serum copper scored lower working memory than with the normal level of serum copper.<sup>44</sup> Additionally the mechanism of cognitive impairment lead to low working memory and high examination stress related to high copper levels. That may be due to copper's toxicity to brain function, especially, the redox-active Cu(II) facilitates amyloid- $\beta$  peptide (A $\beta$ )-mediated oxidative damage to brain cells and thus potentiates neurotoxicity exhibited by A $\beta$ .<sup>45</sup> The same table showed non-significant in serum iron and zinc in comparison E-day with N-day in all three groups. These results disagree with only a study that measured serum zinc in examination stress,<sup>46</sup> which reported that serum zinc was significantly decreased comparing exam day to control, and also this study reported that the cortisol level increased, and zinc serum decrease, when E-day compared with N-day in undergraduate students, also another study<sup>47</sup> reported that the serum zinc decreased, and cortisol increased after exposure to psychological stress for 7 to 14 days in a rat model. Additionally there was a study that proved the inhibitory effect of increasing serum zinc on cortisol secretion.<sup>48</sup> In the current study, the level of serum cortisol and zinc is not affected significantly, this can attribute to the level of stress that was not enough to promote significantly change in serum cortisol and hence zinc. It is difficult to relate these results which have been obtained from E-day compared with N-day to the first semester score, because of the length of the semester versus of just two samples (normal and exam day) of each student. It is possible that the students during the semester are exposed to sick or anxiety and stress at university, family and/or personal life, which may give the results; do not reflect the truth of study dependent of the semester score. The relation between results of biochemical parameters in the current study with exam

performance is more acceptable than the relation of the first semester score, and its better reflect the truth, because the biochemical parameters that adopted in the current study are affected directly by a stressor that its source is exam compared to control. So studying the relation between the semester score with some biochemical parameters need more specific designated study with more follow-up of the samples under study during the semester to give results that reflect more reality of this study, however we will investigate and discuss the relation that obtained in the current study. As shown in table 2 there was significant increase in serum F.B.G comparing E-day with N-day in group of undergraduate students that got pass score, also there was a significant decrease in serum copper in a group of undergraduate students that got the good score, while the other two groups showed non-significant differences and this results descibed previously. The rising of F.B.G may be due to anxiety output of thinking of this group of students about how to pass exams and avoid the failure and shame in front of colleagues and family (mental activity)<sup>49</sup> and that's causes to increasing of serum glucose due to increasing its metabolism by brain.<sup>24</sup> Accordingly, it is possible to reach the following most important conclusion :there were relations between F.B.G and copper levels with both exam performance and the first semester score, also the students groups with pass and good exam performance were more affected, however the comparison for the first semester score may be less important because of the length of the semester versus of just two samples of students under study.

## CONCLUSION

Fasting blood glucose and copper levels were in relation with both exam performance and first semester score, while there were no relation between serum cortisol, amylase activity, albumin and zinc levels with them. Meanwhile the students with good exam performance and pass semester score were more affected as indicated from the results of fasting blood glucose, while copper levels were varied in all three exam performance groups and in good group for semester score.

## REFERENCES

- Gross R, Psychology-The Science of Mind and Behaviour. 3<sup>rd</sup> Edition. Hodder & Stoughton, London. 1996-135-150 & 871-872.
- Long B, Stress in the workplace: ERIC Clearinghouse on Counseling Greensboro NC, Canadian Guidance and Counseling Foundation Ottawa (Ontario). 1995-00-55, ED414521.
- Glanz K, and Schwartz M. Stress, Coping and Health Behavior. University of Eglin. 2008-22-54.
- Lazarus R S, and Folkman S. Stress, Appraisal and Coping New York. 1984-Springer.
- Munck A, Guyre PM, and Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.*, 1984-5-25-44.
- Bamberger CM, Schulte HM, and Chrousos GP. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocr Rev.* 1996-17-245-261.
- Hymie A, and Zul M. Understanding Stress: Characteristics and Caveats. 1999-23-4, 242.
- Baulieu EE. Dehydroepiandrosterone (DHEA): a fountain of youth? *Journal of Clinical Endocrinology and Metabolism* 1996-81-31-47.
- Thomas MD, and John WS. Textbook of Biochemistry with Clinical Correlations. seventh edition 2011-433,434-916, 917.
- Saha A, Sahu S, and Paul G. Evaluation of Cardio-Vascular Risk Factor in Police Officers. *International Journal of Pharma and Bio Sciences.* 2010-1-263-271.

11. Lee M, and Larson R W. The Korean Examination Hell: Long hours of studying, distress, and depression. *Journal of Youth and Adolescence*. 2000-29-249- 272.
12. Kyriacou C, and Butcher C. Stress in Year 11 school children, *Pastoral Care in Education* 11, Survey Schedule for Children. *Journal of Child Psychology and Psychiatry*. 1993-30-775-784.
13. Beidel DC, Turner SM, and Taylor Ferreira JC. Teaching study skills and test-taking strategies to elementary school students. *Behavior Modification*. 1999-1-23-630-692.
14. Gow LJ, Balla, D, Kember KT, Hau. The learning approaches of Chinese people: A function of socialization processes and the context of learning? In the handbook of Chinese psychology, ed. M.H.bond, Hong Kong: Oxford University press. 1996-109-123.
15. Worthy DA, Markman, AB, and Maddox WT,.Choking and excelling under pressure inexperienced classifiers. *Attention, Perception, & Psychophysics journal* 2009-71-4-924-935.
16. Ramirez G, and Beilock SL. Writing about testing worries boosts exam performance in the classroom. *Science*, 2011-331-211-213.
17. McCarthy JM, and Goffin RD. Selection test anxiety: Exploring tension and fear of failure across the sexes in simulated selection scenarios. *International Journal of Selection and Assessment*. 2005-13-4-282-295.
18. Archana K. Examination stress, and anxiety: A study of college students *global journal of multidisciplinary studies*. 2014-4-31-40.
19. Dennis LK, and Eugene B. Anthony SF, Stephen H, Dan LL, *Principles of Internal Medicine*. 16th edition. 2005-956-61.
20. Cheesbrough M. *District Laboratory Practice in Tropical Countries*. 2009-360-363.
21. Gerardo J, Marianne L, and Leonisa Y. Determination of the sensitivity range of biuret Test for undergraduate biochemistry experiments. *journal of science & technology*, 2011-6-5-77-83.
22. Zainulabdeen JA, and Sami MM, Effect of temporarily examination stress on some biochemical parameters in iraqi Academic students. *advances in environmental biology*, in publish. 2016.
23. Zardooz H, Veservi MKG, and Hedayati M. Effect of chronic restraint stress on carbohydrate metabolism in rat. *Physiology and Behavioral journal*, 2006-89-373-378.
24. Rand JS, Kinnaird E, Baglioni A, Blackshaw J, and Priest J. Acute stress hyperglycemia in cats is associated with struggling and increased concentration of lactate and nor epinephrine. *journal of veretinary international medicine*. 2002-16-123-132.
25. Benton D, Ruffin P, Lassel T, Nabb S, Messaoudi M, Vinoy S, Deor D, and Lang V. The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans. *Psychopharmacology*. 2003-166-86-90.
26. Fellows LK, Boutelle M G, and Fillenz M. Extracellular brain glucose levels reflect neuronal activity: a microanalysis study in awake freely moving rats. *Journal of Neurochemistry*. 1991-59-: 2141-2147.
27. Fellows LK, and Boutelle KG. Rapid changes in extracellular glucose levels and blood level in the striatum of the freely moving rat. *Brain Research*, 1993-60-4-225-231.
28. Kandel ER, Schwartz JH, and Jessell T. *Principles of Neural Science*. 2000-4th Edition (New York: McGraw-Hill).
29. Lund-Andersen H. Transport of glucose from blood to brain *Physiological Review*, 1979-59-305-359.
30. Donohoe RT, and Benton D. The effects of nutrients on mood. *Public Health Nutrient*. 1999-2-3A-403-9.
31. Benton D, and Owens D. Is raised blood glucose associated with the relief of tension? *Journal of Psychosomatic research*. 1993-37-1-13.
32. Torres ILS, Gamaro GD, Silveria Cucco SN, Michalowski MB, Correa JB, Perry MLS. , and Dalmaz C. Effect of acute and repeated restraint stress on glucose oxidation to CO<sub>2</sub> in hippocampal and cerebral cortex slices. *Brazilian Journal of Medical and Biology Research* 2000-34-111-116.
33. Fairbrother K, and Warn, J. *Workplace Dimensions, Stress and Job Satisfaction*. *Journal of Managerial Psychology*. 2003-18-1-8-21.
34. Abouserie R. Sources and levels of stress in relation to locus of control and selfesteem in university students. *Educational Psychology*. 1994-14-3-323-330.
35. Anda H, van S, Oliver T, and Wolf MK. Salivary alpha amylase and cortisol responses to different stress tasks : impact of sex. *international journal of psychophysiology*. 2008-69-33-40,.
36. Kelly A, John E, Buen KJ, Bohan J, and Maye P. Determining the relationship of acute stress anxiety, and salivary  $\alpha$ -amylase level with performance of students nurse anesthetists during human – based anesthesia simulator training. *american association of nurse anesthetists Journal*. 2010-780-4-301-309.
37. Berk JE, Hayashi S, Searcy R L, and Hightower NC. Differentiation of parotid and pancreatic amylase. *the american journal of digestive diseases*. 1966-11-695.
38. Fridhandler, L, Berk JE, and Ueda M. Isolation and measurement of pancreatic amylase of human serum and urine. *Clinical Chemistry*. 1972-18-1493.
39. Astle D, and Castle A. Intracellular transport and secretion of salivary proteins. *Critical reviews in oral biology and medicine*. 1998; 9, 4-22.
40. Mahadevan K and Velavan S. Analysis of Salivary Proteins as the Biochemical Indicators of Nutritional Status and Salivary Gland Function. *International Journal of Pharma and Bio Sciences*. 2013-4-2-B-689 – 694 .
41. Salustri C, Barbati G, Ghidoni R, Quintiliani L, Ciappina S, Binetti G, and Squitti R. Is cognitive function linked to serum free copper levels? A cohort study in a normal population. *clinical and neurophysiology*. 2010-121-502-507.

42. Zecca L, Stroppolo A, Gatti A, Tampellini D, Toscani M, Gallorini M, Giaveri G, and Arosio P. The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. Proceeding and national academy sciences USA, 2004-101-9843-9848.
43. Alloway T P, Gathercole S.E. Kirkwood H, and Elliott J. The cognitive and behavioral characteristics of children with low working memory” Child Development.2009-80-606–621.
44. Huang X, Cuajungco MP, Atwood CS, Hartshorn M A, Tyndall JD, Hanson GR, Stokes KC, Leopold M, Multhaup G, and Goldstein LE. Cu (II) potentiation of Alzheimer A- neurotoxicity correlation with cell-free hydrogen peroxide production and metal reduction. journal of biology and chemistry. 1999-274-37111–37116.
45. Demir N, Yaşar D, Yildirim AO , Irfan K, and Ebubekir B. The effect of examination stress on the leve of  $Zn^{+2}$  , total protein , albumin in serum and carbonic anhydrase isoenzymes in erythrocytes. Turkish journal of chemistry. 1996-20-289-294.
46. Huang X, Moir RD, Tanzi RE, Bush AI, and Rogers JT. Redox-active metals, oxidative stress, and Alzheimer’s disease pathology. Annals of the new York academy of sciences. 2004-1012-153-163.
47. Liping T, Yuanyuan Z, Zhilei S, Yingjie, Xue T, Xiao D, and Jianxin Q, Hui S. Psychological stress-induced lower serum zinc and zinc redistribution in rats. Biological trace element research. 2013-155-65-71.
48. Espanani HR, Shirani K, Sadeghi L, and Yousefi BV. Investigation of the zinc oxide nanoparticles effect on testosterone, cholesterol and cortisol in rats. Research journal of recent sciences, 2014-3-4-14-19.
49. Alloway TP, Gathercole SE, Kirkwood H, and Elliott J. The cognitive and behavioral characteristics of children with low working memory. Child Development, 2009-80-606–621.