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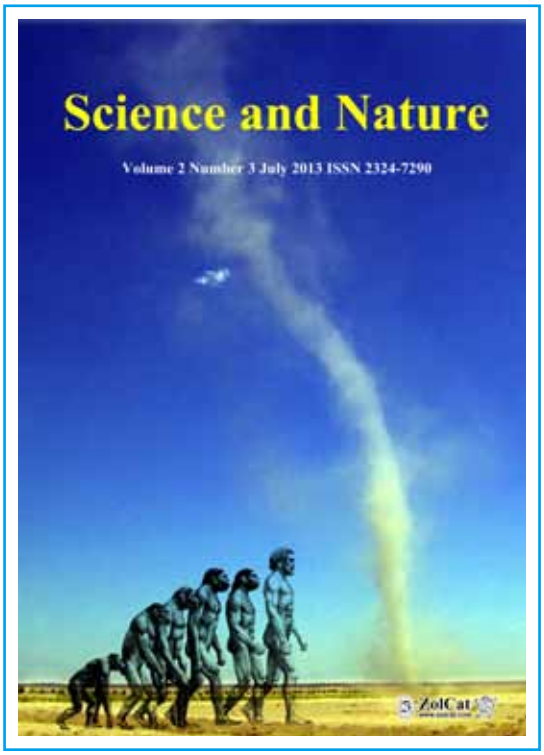

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Effect f Some Bio-Regulators on Growth and Yield of Some Wheat Varieties nder Newly Cultivated Land Conditions

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ABSTRACT

This investigation was conducted to study the effect of some bio-regulators, tryptophan, cysteine, thiamine, ascorbic acid and yeast extract on growth, yield and its components and some chemical constituents of some wheat varieties; Sakha93, Gemiza7 and Gemiza9 under newly cultivated land conditions. For this purpose two field experiments were carried out during two successive growing seasons (2008\2009 and 2009\2010) at the experimental farm of National Research Center at Nubaria, Egypt. Results revealed that foliar application of different applied growth bio-regulating substances, with different concentrations, significantly increased all growth criteria, plant height, number of tillers and leaves per plant, fresh and dry weight of plant. Grain and straw yield of wheat plants and yield components were also significantly increased as result of foliar application of the used bioregulators. On the other hand Gemiza-7 variety was most effective of all characters under study compared with the other two varieties Gemiza-9 and Sakha-93.

Key Words

Wheat, Bio-regulators, Growth, Pigments, Yield, Yield components.

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1 Introduction

Wheat is one of the major cereal crops with a unique protein, which is consumed by humans and is grown around the world in diverse environments (Mac Ritchie, 1994).Wheat provides approximately one fifth of the calories in the human diet and is an important source of vegetable protein and nutrients for a large proportion of the world's population. Modern wheat varieties differ in their grain concentrations of N, Zn and Fe. (Cakmak et al., 2000). (Sultan et al., 2000) reported, Wheat cultivars differed in yield and its components. Also Zaki, et al.,(2004)established that,grain, straw and biological yields and its components were significantly differed owing to varietal differences. In addition Sharaan, (2000) found that wheat varieties were significantly different in plant height, spike length, number of spikes, number of grains per spike and grain weight per spike Zaki et al., (2007)reported that, there were significant differences in all growth characters of wheat cultivars, as follows i.e. (Sakha93, Gemiza7 and Gemiza9) under their study.

Plant growth regulators (PGRs) are actively involved in a multitude of metabolic processes and play essential roles in plant growth and development under both stress and natural conditions. They also act as chemical messengers to modulate various processes or genes involved in plant growth and development (Morgan, 1990).

Plant growth regulators (PGRs) also play important roles in plant adaptationto stressful environments, including drought stress (Huang et al., 2008).

Under both wild and cultivated conditions, plants often experience a multitude of environmental stresses such as drought, salinity, waterlogging, extremes of temperature, and mineral toxicities and deficiencies. Environmental stresses are undoubtedly a major cause of food insecurity in many countries around the world, in particular, in developing countries where there is a major challenge to produce sufficient food. A large proportion of the world, agriculture depends on rainfall for irrigation, as good quality water supply is highly limited or unpredictable. In many of such regions, crops are often negatively affected by severe drought. It has been estimated that the countries that generate twothird of the world's agricultural product experience water-deficit conditions on a regular basis (Revenga et al., 2000).

In plants, amino acids fulfill a wide variety of functions. Their common role is to serve as building blocks of proteins, which exert manifold functions in plant metabolism, and as metabolites and precursors involved in plant defense, vitamin, nucleotide and hormone biosynthesis, and as precursors of a huge variety of secondary metabolites. One way or the other, as active catalysts or as precursors, amino acids are essentially involved in all metabolic, regulatory, and physiological aspects of plant metabolism(Buchanan et al., 2000).

Unlike humans, plants are capable of producing vitamins. In fact, the foods

Ali A El-Hosary, Gaber Y Hammam, Abdalla El-Morsi, Esmat A Hassan, Mohamed E El-Awadi, Yasser R Abdel-Baky. Effect of some bio-regulators on growth and yield of some wheat varieties under newly cultivated land conditions. Science and Nature (2013) 2(3):75-83. (ISSN 2324-7290) © ZolCat Academic House. www.zolcat.com

highest in vitamins are plant-based, such as raw fruits and vegetables. Vitamins could be considered as bio-regulators compounds which in low concentrations exerted a profound influence upon plant growth. In general, the energy metabolic pathway could be affected by one or another of these substances. Vitamins could serve as coenzyme in decarboxylation of a-keto acids, such as pyruvic acid and keto-glutamic acid which has its importance in the metabolism of carbohydrates and fats (Bidwell, 1979).

Vitamins are important cofactors for the transketolation reactions of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the reduced NADP required in various synthetic pathways (Kawasaki, 1992).

On the other hand, Amer, (2004) and Kurtzman and Fell (2005) demonstrated that, Active dry yeast is a natural safety biofertilizers causes various promotive effect on plants. It is considered as a natural source of cytokinins which simulates cell division and enlargement as well as the synthesis of protein, nucleic acid and vitamin-B. It also releases CO2 which reflected in improving net photosynthesis.

The objectives of this study was to evaluate the beneficial effect of some bio-regulators as foliar application on growth, yield and yield components and some chemical constituents of some wheat varieties grown under newly reclaimed soil conditions.

2 Materials and Methods

This investigation was carried out at the experimental farm of National Research Center at Nubaria, Egypt, during two successive seasons 2008\2009 and 2009\2010, to study the effect of some bio-regulators on growth development, yield and yield components of three cultivars of wheat plant. (Triticum aestivum L.).

Plant Materials

Pure lines and uniform grains of three local cultivars of wheat plant (Triticum aestivum L.) Sakha93, Gemiza-7 and Gemiza-9. These cultivars were obtained from Wheat Research Department, Agricultural Research Center, Ministry of Agriculture, Egypt.

Chemical materials

Three types of chemical materials were used, tryptophan ($C_6H_4NH.CH:C$) and cysteine ($C_3H_7NO_2S$) as amino acids, ascorbic acid (Vitamin-C) ($C_6H_8O_6$) and thiamine (Vitamin-B1) as vitamins and baking yeast (Saccharomyces cerevisiae) extract.

The experiments were carried out under sandy soil conditions. Grains of the three cultivars were sown at 27 and 23 November for the first and the second season respectively. The experimental design was split plot design with three replications. Each experiment included 33 plots and the plot area was 10.5 m^2 (1/400 of feddan), three meters in length and three and half meters in width. Each plot contained twenty rows. Seeds were planted at the rate of 60 Kg/feddan using drilling and cross rows method, the spacing between the rows was 15cm. The treatments of the bio-regulators were distributed at random in the main plot, and the three varieties were assigned in the sub plot randomly too.

Nitrogen fertilizer was applied at the rate of 120 Kg N/feddan in the form of ammonium nitrate (33.5%N) and was divided in ten equal portions. The dosage was added before the irrigation.

Phosphorus fertilizer was applied before sowing (during land preparation) at rate of 50 Kg P_2O_5/feddan in the form of calcium super phosphate (15.5% P_2O_5).

Potassium sulphate was added at rate of 50 Kg per feddan, and applied one month after sowing.

Two concentrations from each bio-regulator (50 and 100 mgl^{-1} of tryptophan, thiamine and ascorbic acid) and 100 and 150 mgl^{-1} of cysteine as well as 1 and 2 gl-1 of yeast extract were added twice to the plants as foliar spray in addition to control plants which sprayed with distilled water. First spray was carried out at vegetative stage (30 days after sowing) the spray volume of this sprinkling was 200 liter per feddan, and the second spray was seven days later, its volume was 250 liters per feddan.

The sample was taken fifteen days after the second spray (at heading stage).

Growth and yield measurements

a. Plant height (cm).
b. Number of tillers per plant.
c. Number of leaves per plant.
d. Fresh weight of shoot per plant.
e. Dry weight of shoot per plant.
f. Flag leaf area (cm^2)

Yield and yield components characters

At harvest, ten plants from each plot were chosen randomly to estimate the following data.

a. Spike length (cm).
b. Number of spikes per plant.
c. Spike weight (g).
d. Number of grains per spike.
e. Grain weight per spike (g).
f. Grain yield per feddan (Kg) which was determined from the whole grain yield of each sub-plot and converted to tons of grains per feddan.

Photosynthetic pigments

Chlorophylls a, b and carotenoids were extracted from fresh leaf tissues and estimated, colorimetrically, according to method described by Wettstein (1957).

Statistical Analysis

Each experiment was statistically analyzed as a spilt plot design according to Snedecor and Cochran, (1980). Combined analysis of the two seasons was carried out whenever homogeneity of variance was detected for all studied traits. The Duncan multiple range test was used to compare the treatment means (Duncan, 1955). The MSTATC (1989) program was used in this connection.

3 Results

Growth characters

Effect of bio-regulators

According to data in Table (1), yeast extract at concentration of 1000 mgl-1 was the most effective treatment increasing wheat plant height followed by Cysteine treatment at concentration of 150 mgl-1. Data also show that there was no significant difference between the treatments 150 mgl^{-1} of cysteine and 1000 mgl-1 of yeast extract in the second season.

The highest number of tillers and leaves per plant was obtained by using tryptophan at concentration of 50 mgl^{-1}. Data show also that , the highest values of fresh weight of plant wereobtained by treatment of 1000 mgl-1 yeast in the second season and combined data of the two seasons. On the other hand treatment of 100 mgl^{-1} ascorbic acid recorded the highest value in the first season whereas, the untreated plants (control) recorded the lowest values of the fresh weight of plant during first, second season and their combined data.

It could be emphasized from the present data that, dry weight of wheat plant was significantly affected by all bio-regulating substances at different concentrations in both season and their combined data. Results show that, the effect was strikingly noticeable with the treatment of ascorbic acid 100 mgl^{-1} in the first season and tryptophan 50 mgl^{-1} in the second season and the combined data.

It is clear from Table (2) that, flag leaf area was significantly increased as a result of foliar application with the used bio-regulating substances at different concentrations.Ascorbic acid treatment at 100 mgl^{-1} recorded the highest value in this respect, while, the lowest value obtained by untreated plants (control).

Varieties performance

Data in Tables (1 and 2) revealed that, the examined varieties significantly varied in their responses to foliar spray with the different bioregulating substances. Gemiza-7 variety gave the highest values of plant height, fresh and dry weight of plant and flag leaf area. While, Gemiza-9 variety recorded the highest values of number of tiller and leaves per plant and these results were true for the two seasons and their combined data.

Effect of interaction

The results in Tables (3 and 4) cleared that, there were significant increasesof all growth characters under study in the two seasons and their combined data as affected by interaction between wheat varieties and bio-regulating substances.

The highest values of plant heightwere obtained from the treatment 1000 mgl^{-1} of yeast with Gemiza-7 variety, during the first, second season

Table 1. Plant height, number of tillers and number of leaves per plant, as affected by some growth bio-regulators treatments and varieties of wheat at heading stage during two growing seasons and their combined data.

Treatments		Plant height (cm)			No. of Tillers/plant			No. of leaves/plant		
		S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		55.97 d	58.40 h	57.19 e	2.334 e	3.008 f	2.671 f	11.17 g	12.33 g	11.75 h
Tryptophan	50 mgl^{-1}	74.70 ab	77.81 c-e	76.26 c	6.017 a	7.563 a	6.790 a	23.14 a	26.35 a	24.74 a
	100 mgl^{-1}	71.60 c	74.41 g	73.00 d	4.533 c	5.158 d	4.846 d	17.33 ef	18.90 f	18.11 g
Cystine	100 mgl^{-1}	70.74 c	76.43 f	73.58 d	3.691 d	4.250 e	3.971 e	18.03 de	20.78 de	19.40 f
	150 mgl^{-1}	75.73 ab	80.67 a	78.20 ab	5.034 bc	5.750 cd	5.392 c	21.49 b	22.43 c	21.96 c
Thiamine	50 mgl^{-1}	73.80 b		76.34 c	5.046 bc	5.456 cd	5.251 cd	19.75 c	20.58 e	20.17 e
	100 mgl^{-1}	69.51 c	77.09 d-f	73.30 d	4.670 bc	5.264 cd	4.967 cd	18.43 d	20.21 e	19.32 f
Ascorbic Acid	50 mgl^{-1}	75.10 ab	79.47 b	77.28 bc	4.612 bc	5.482 cd	5.047 cd	18.20 d	20.15 e	19.17 f
	100 mgl^{-1}	71.28 c	76.80 ef	74.04 d	5.209 b	6.509 b	5.859 b	20.09 c	21.74 cd	20.91 d
Yeast	1000 mgl^{-1}	76.26 a	80.77 a	78.51 a	5.123 bc	6.611 b	5.867 b	21.10 b	24.98 b	23.04 b
	1500 mgl^{-1}	69.99 c	78.27 b-d	74.13 d	4.867 bc	5.904 c	5.386 c	16.91 f	22.29 c	19.60 ef
Sakha-93		±1.31 cm	66.74 c	66.50 c	4.706 ab	5.642 a	5.174 a	17.72 c	19.97 c	18.84 c
Gemiza-7		±8.65 cm	85.39 a	81.16 a	4.432 b	5.275 b	4.853 b	18.65 b	20.50 b	19.58 b
Gemiza-9		±1.31 cm	76.68 b	73.75 b	4.808 a	5.708 a	5.258 a	19.72 a	22.46 a	21.09 a

S1 = first season, S2 = second season, Comb. = combined data.

Table 2. Fresh and dry weight per plant and flag leaf area, as affected by some growth bio-regulators treatments and varieties of wheat at heading stage during two growing seasons and their combined data.

Treatments		Fresh weight/plant (g)			Dry weight/plant (g)			Flag leaf area (cm^2) (Average two seasons)
		S1	S2	Comb.	S1	S2	Comb.	
Control		10.53 f	14.00 h	12.27 h	8.797 f	9.558 g	9.178 g	26.20 g
Tryptophan	50 mgl^{-1}	27.20 d	38.78 c	32.99 bc	17.76 b	23.00 a	20.38 a	41.60 b
	100 mgl^{-1}	22.58 e	31.42 e	27.00 f	12.24 e	15.28 ef	13.76 f	37.75 e
Cystine	100 mgl^{-1}	21.87 e	28.14 g	25.00 g	15.06 d	16.02 de	15.54 de	37.34 e
	150 mgl^{-1}	30.04 b	35.59 d	32.81 bc	16.36 c	21.04 b	18.70 bc	40.19 c
Thiamine	50 mgl^{-1}	29.27 bc	34.91 d	32.09 c	14.52 d	16.75 d	15.64 de	38.94 d
	100 mgl^{-1}	28.76 bc	29.75 f	29.26 e	14.68 d	15.85 d-f	15.26 de	41.67 b
Ascorbic Acid	50 mgl^{-1}	28.12 cd	32.63 e	30.37 d	16.99 bc	14.74 f	15.87 d	39.92 c
	100 mgl^{-1}	33.67 a	40.77 b	37.22 a	19.39 a	16.84 d	18.12 c	44.67 a
Yeast	1000 mgl^{-1}	32.42 a	43.62 a	38.02 a	19.01 a	19.81 c	19.41 b	38.14 e
	2000 mgl^{-1}	28.17 cd	38.64 c	33.41 b	14.50 d	15.51 ef	15.01 e	36.53 f
Sakha-93		23.03 c	27.83 c	25.43 c	14.07 c	5.642 a	13.72 c	33.11 c
Gemiza-7		29.27 a	38.39 a	33.83 a	19.09 a	5.275 b	18.12 a	45.73 a
Gemiza-9		27.51 b	34.21 b	30.86 b	17.13 b	5.708 a	16.40 b	36.52 b

S1 = first season, S2 = second season, Comb. = combined data.

and their combined data.

Treatment of 50 mgl^{-1} tryptophan with Gemiza-9 variety recorded the highest values of number of tillers in the first, second season and their combined data. While, 50 mgl^{-1} treatment gave the highest number of leaves per plant when used on Sakha-93 variety during the same periods. On the other hand, treatment of 150 mgl^{-1} cysteine with Gemiza-7 variety recorded the highest value of fresh weight of plant in the first season. While, ascorbic acid at 100 mgl^{-1} recorded the highest values of this character when used on Gemiza-9 variety during second season and combined data.

The treatment of 50 mgl^{-1} tryptophan was more effective in increasing dry weight of plant during the second season and the combined data. While ascorbic acid treatment at 100 mgl^{-1} was more effective in the first season when used on Gemiza-9 variety. The most effective treatments for increasing flag leaf area was 150 mgl^{-1} cysteine when used on Gemiza-7 variety followed by thiamine treatment at 100 mgl^{-1} on Gemiza-7 variety also which was no significant difference between them.

Yield and yield components

Effect of bio-regulators

Foliar application of all concentrations of bioregulating substances under study (Tables 5-6) resulted in significant increases in spike length, number of spike per plant, weight of spike, number of grains per spike, number of spikelet per spike, number of grains per spikelet, grains weight per spike, grain and straw yield of wheat plant, during the two growing seasons and their combined data compared with untreated plants (control).

Tryptophan treatment at 50 mgl^{-1} recorded the highest values of spike length in the combined data. While, concentration of 100 mgl^{-1} was the best treatment in the first season. On the other hand 100 mgl^{-1} thiamine treatment recorded the highest value in this character during second season. Data also show that, yeast extract at 1000 mgl-1 concentration gave the highest values of number of spike per plant during first and combined data while, the highest value was obtained by 50 mgl^{-1} tryptophan treatment in the second season.

Also, tryptophan treatment at 50 mgl^{-1} resulted in the highest values of weight of spike per plant and grains weight per spike during the two growing seasons and their combined data as well as grain yield and number of grains per spike during the first season and the combined data, whereas 1000 mgl^{-1} yeast application recorded the highest value in the second season. In addition there was no significant difference between the two treatments in the second season.

Straw yield was positively affected

Table 3. Plant height, number of tillers and number of leaves per plant, as affected by interaction between some growth bio-regulators treatments and varieties of wheat at vegetative growth stage during two growing seasons and their combined data.

Treatments			Plant height (cm)			No. of Tillers/plant			No. of leaves/plant		
			S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		Sakha-93	37.1 n	35.5 q	36.3 o	1.07 l	1.60 kl	1.33 n	5.32 no	5.83 mn	5.58 o
		Gemiza-7	42.7 m	36.8 p	39.7 n	1.33 l	1.20 l	1.27 n	5.15 o	5.34 n	5.24 o
		Gemiza-9	37.5 n	41.3 o	39.4 n	1.75 l	2.40 jk	2.07 m	6.68 n	6.90 m	6.79 n
Tryptophan	50 mgl-1	Sakha-93	53.9 c-e	53.3 d	53.6 c	4.47 ab	5.15 ab	4.81 ab	15.92 b-d	18.85 b	17.38 bc
		Gemiza-7	57.4 ab	56.9 a	57.2 a	3.53 c-h	4.33 b-e	3.93 c-g	11.12 i-l	11.40 jk	11.26 jk
		Gemiza-9	48.9 f-i	50.9 f	49.9 ef	3.97 ad	5.00 ab	4.48 a-c	12.26 hi	15.82 ef	14.04 fg
	100 mgl-1	Sakha-93	42.9 m	42.5 n	42.7 m	2.60 ik	3.03 g-j	2.81 j-l	10.51 j-m	11.24 j-l	10.88 j-l
		Gemiza-7	49.1 f-h	52.8 d	50.9 e	2.80 h-k	3.57 e-i	3.18 h-l	9.877 lm	10.67 j-l	10.27 lm
		Gemiza-9	45.1 l	44.4 lm	44.8 l	2.93 g-k	4.16 b-g	3.55 d-i	11.06 i-l	11.87 j	11.47 j
Cysteine	50 mgl-1	Sakha-93	49.7 fg	46.5 jk	48.1 g-i	2.57 jk	2.68 ij	2.62 lm	9.253 m	10.07 l	9.66 m
		Gemiza-7	54.1 cd	55.9 a-c	55.0 b	2.47 k	3.49 e-j	2.98 i-l	11.17 i-l	13.80 hi	12.49 hi
		Gemiza-9	46.3 j-l	44.8 lm	45.6 kl	2.90 g-k	3.87 c-h	3.38 f-k	12.09 hi	14.47 gh	13.28 gh
	100 mgl-1	Sakha-93	52.0 e	49.3 gh	50.7 e	3.80 a-f	4.10 b-g	3.95 c-g	12.59 g-i	15.54 fg	14.06 fg
		Gemiza-7	57.8 a	56.6 ab	57.2 a	3.47 d-h	4.04 b-g	3.75 d-h	14.50 d-f	16.27 ef	15.38 e
		Gemiza-9	48.5 f-i	45.5 kl	47.0 ij	3.87 a-e	4.56 a-e	4.21 b-d	19.77 a	20.24 a	20.01 a
Thiamine	50 mgl-1	Sakha-93	48.6 f-i	52.1 de	50.3 e	4.30 a-c	4.80 a-d	4.55 a-c	15.21 c-e	17.70 cd	16.45 d
		Gemiza-7	55.8 bc	52.9 d	54.3 bc	3.40 d-i	3.20 f-j	3.30 g-l	11.47 i-k	11.30 j-l	11.38 jk
		Gemiza-9	49.9 f	48.3 hi	49.1 fg	3.60 c-h	3.86 c-h	3.73 d-h	14.80 de	16.39 ef	15.60 e
	100 mgl-1	Sakha-93	46.0 j-l	50.3 fg	48.1 g-i	3.00 f-k	3.17 f-j	3.08 h-l	10.23 j-m	11.78 j	11.01 j-l
		Gemiza-7	53.3 de	51.3 ef	52.3 d	2.87 g-k	2.80 h-j	2.83 j-l	9.77 lm	10.35 kl	10.06 lm
		Gemiza-9	47.3 h-k	47.3 ij	47.3 hi	3.68 b-g	3.60 e-i	3.64 d-i	13.97 e-g	15.90 ef	14.93 ef
Ascorbic Acid	100 mgl-1	Sakha-93	46.9 i-l	49.4 gh	48.1 g-i	3.97 a-d	4.07 b-g	4.02 c-f	12.47 hi	14.40 hi	13.43 gh
		Gemiza-7	54.4 cd	54.9 c	54.7 bc	4.00 a-d	4.37 b-e	4.18 b-e	13.13 f-h	16.80 de	14.9 ef
		Gemiza-9	100 mgl-1	44.1 m	44.7 l	3.43 d-h	3.60 e-i	3.52 e-i	11.77 h-j	13.41 hi	12.59 h
	100 mgl-1	Sakha-93	45.3 kl	48.0 i	47.2 i	4.30 a-c	4.90 a-c	4.60 a-c	16.42 bc	19.10 b	17.76 b
		Gemiza-7	58.6 a	56.4 ab	57.5 a	4.10 a-d	4.80 a-d	4.45 a-c	15.32 c-e	18.39 bc	16.85 cd
		Gemiza-9	47.7 g-j	50.1 fg	48.9 fg	4.58 a	5.50 a	5.04 a	19.70 a	20.36 a	20.03 a
Yeast	1000 mgl-1	Sakha-93	47.2 h-k	49.5 gh	48.4 gh	3.13 e-k	3.70 d-i	3.42 f-j	10.10 k-m	13.20 i	11.65 ij
		Gemiza-7	52.6 de	56.8 a	54.7 bc	4.00 a-d	4.23 b-f	4.11 c-e	16.92 b	19.30 ab	18.11 g
		Gemiza-9	45.4 kl	46.6 jk	45.9 jk	3.33 d-j	3.84 c-h	3.59 d-i	14.54 d-f	15.96 ef	15.25 e
	2000 mgl-1	Sakha-93	47.1 h-l	150 mgl-1	45.9 jk	2.53 jk	2.90 h-j	2.72 kl	9.60 lm	11.01 j-l	10.31 lm
		Gemiza-7	53.7 de	100 mgl-1	54.6 bc	3.60 c-h	2.87 h-j	3.23 h-l	9.86 lm	11.03 j-l	10.45 k-m
		Gemiza-9	45.1 l	74.41 g	45.3 kl	3.13 e-k	3.04 g-j	3.09 h-l	12.31 hi	14.37 g-i	13.34 gh

S1 = first season, S2 = second season, Comb. = combined data.

by 100 mgl^{-1} ascorbic acid treatment during the first, second season and their combined data compared with the untreated plants (control).

Varieties performance

The examined cultivars significantly varied in their response to different bio-regulators in the yield and yield component characters (Tables 5-6). In comparison between varieties, Gemiza-7 variety significantly exceeded in spike length, weight of spike in the two growing seasons and their combined data, number of grains per spike in the second season, grain weight of spike and grain yield in the two growing season and their combined data.

Gemiza-9 variety recorded the

Table 4. Fresh and dry weight of plant, as affected by interaction between some growth bio-regulators treatments and varieties of wheat at vegetative growth stage during two growing seasons and their combined data.

Treatments			Fresh weight/plant (g)			Dry weight/plant (g)		
			S1	S2	Comb.	S1	S2	Comb.
Control		Sakha-93	5.71 q	6.770 s	6.240 o	0.973 q	1.577 n	1.275 o
		Gemiza-7	6.62 pq	7.467 s	7.043 o	1.160 pq	1.727 mn	1.443 o
		Gemiza-9	6.95 pq	9.150 r	8.052 n	1.490 op	2.088 lm	1.789 n
Tryptophan	50 mgl-1	Sakha-93	17.42 b	20.97 b	19.20 b	3.777 bc	4.467 bc	4.122 bc
		Gemiza-7	15.92 bc	19.62 c	17.77 d	3.530 cd	4.357 bc	3.943 c
		Gemiza-9	15.27 cd	16.10 g-i	15.68 e-g	3.173 de	3.207 f-i	3.190 d
	100 mgl-1	Sakha-93	10.05 k-n	10.66 pq	10.35 l	1.950 j-n	2.083 lm	2.017 l-n
		Gemiza-7	11.84 g-k	12.62 mn	12.23 jk	2.083 j-m	2.717 jk	2.400 i-k
		Gemiza-9	11.32 h-k	12.12 no	11.72 k	2.363 h-k	2.750 i-k	2.557 h-j
Cysteine	50 mgl-1	Sakha-93	8.680 no	9.390 r	9.035 m	1.863 l-o	1.957 l-n	1.910 mn
		Gemiza-7	13.98 d-f	15.49 hj	14.73 gh	2.380 h-j	3.533 ef	2.957 d-f
		Gemiza-9	10.24 j-n	10.47 q	10.35 l	1.627 no	2.687 k	2.157 k-m
	100 mgl-1	Sakha-93	12.33 f-i	16.91 fg	14.62 h	2.300 h-l	3.600 ef	2.950 d-f
		Gemiza-7	16.33 bc	21.42 b	18.88 bc	3.703 c	4.053 cd	3.878 c
		Gemiza-9	13.32 e-g	17.05 fg	15.19 f-h	2.390 h-j	3.797 de	3.093 d
Thiamine	50 mgl-1	Sakha-93	15.83 bc	16.27 gh	16.05 ef	2.200 i-m	3.783 de	2.992 de
		Gemiza-7	11.72 g-k	13.76 kl	12.74 i-k	1.920 k-o	3.043 g-k	2.482 h-j
		Gemiza-9	15.56 cd	15.24 ij	15.40 f-h	1.953 j-n	3.293 f-h	2.623 g-i
	100 mgl-1	Sakha-93	11.38 h-k	13.87 kl	12.62 i-k	2.187 i-m	3.033 g-k	2.610 g-i
		Gemiza-7	9.453 l-o	11.51 op	10.48 l	1.830 m-o	2.680 k	2.255 j-l
		Gemiza-9	12.17 f-i	14.54 jk	13.35 i	2.753 e-h	3.080 g-k	2.917 d-g
Ascorbic Acid	100 mgl-1	Sakha-93	10.88 i-l	13.83 kl	12.36 i-k	2.677 f-h	3.157 f-j	2.917 d-g
		Gemiza-7	13.06 f-h	17.56 ef	15.31 f-h	3.850 bc	4.087 cd	3.968 c
		Gemiza-9	9.387 l-o	11.95 no	10.67 l	2.070 j-n	2.193 l	2.132 k-m
	100 mgl-1	Sakha-93	12.15 f-j	18.56 d	15.36 f-h	2.730 f-h	3.260 f-h	2.995 de
		Gemiza-7	19.36 a	22.75 a	21.06 a	4.720 a	5.127 a	4.923 a
		Gemiza-9	17.64 b	18.64 d	18.14 cd	3.803 bc	4.210 b-d	4.007 c
Yeast	1000 mgl-1	Sakha-93	10.60 i-m	15.03 j	12.81 ij	2.937 e-g	3.410 e-g	3.173 d
		Gemiza-7	14.94 c-e	18.30 de	16.62 e	4.187 b	4.547 b	4.367 b
		Gemiza-9	11.53 g-k	13.17 lm	12.35 i-k	2.547 g-i	2.883 h-k	2.715 e-h
	2000 mgl-1	Sakha-93	8.817 m-o	11.48 op	10.15 l	2.717 f-h	2.617 k	2.667 f-i
		Gemiza-7	12.03 g-j	13.49 lm	12.76 i-k	3.013 ef	2.917 h-k	2.965 d-f
		Gemiza-9	7.990 op	11.46 op	9.727 lm	1.770 m-o	2.917 i-k	2.265 j-l

S1 = first season, S2 = second season, Comb. = combined data.

Table 5. Spike length, number of spikes per plant and weight of spike of some wheat varieties, as affected by some growth bio-regulating substances during two growing seasons and their combined data.

Treatments		Spike length (cm)			No. of Tillers/plant			weight of spike (g)		
		S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		7.15 c	6.87 e	7.01 e	1.75 e	2.33 d	2.044 e	1.84 e	2.77 e	2.30 h
Tryptophan	50 mgl-1	9.21 ab	12.14 a	10.68 a	3.92 a-c	5.56 a	4.74 a	4.39 a	6.42 a	5.40 a
	100 mgl-1	9.43 a	10.77 cd	10.10 bc	3.41 b-d	4.33 bc	3.87 b-d	3.81 b	5.42 cd	4.62 c-e
Cystine	100 mgl-1	8.55 b	11.33 bc	9.94 cd	2.82 d	3.78 c	3.30 d	3.36 cd	5.79 b-d	4.57 d-f
	150 mgl-1	9.04 ab	10.65 d	9.85 cd	3.17 cd	4.00 c	3.58 cd	3.13 d	5.34 d	4.23 g
Thiamine	50 mgl-1	9.26 ab	11.04 cd	10.15 bc	3.50 b-d	4.42 bc	3.96 b-d	3.11 d	5.63 b-d	4.37 e-g
	100 mgl-1	8.86 ab	12.19 a	10.52 ab	3.61 b-d	3.94 c	3.78 b-d	3.79 b	5.95 a-c	4.87 bc
Ascorbic Acid	50 mgl-1	8.89 ab	10.82 cd	9.85 cd	3.41 b-d	3.94 c	3.68 cd	3.80 b	6.12 ab	4.96 b
	100 mgl-1	8.68 ab	11.88 ab	10.28 a-c	4.04 ab	4.78 a-c	4.41 ab	3.23 d	5.66 b-d	4.44 d-g
Yeast	1000 mgl-1	8.77 ab	11.65 ab	10.21 a-c	4.44 a	5.22 ab	4.83 a	3.52 c	5.90 a-c	4.71 b-d
	1500 mgl-1	8.51 ab	10.62 d	9.56 d	3.72 a-c	4.50 bc	4.11 bc	3.33 cd	5.24 d	4.29 fg
Sakha-93		8.09 c	9.96 c	9.023 c	3.56 a	4.33 ab	3.94 a	2.75 c	5.02 c	3.88 c
Gemiza-7		9.81 a	12.31 a	11.06 a	3.30 a	3.98 b	3.64 b	3.84 a	6.09 a	4.96 a
Gemiza-9		8.38 b	10.45 b	9.416 b	3.46 a	4.45 a	3.95 a	3.59 b	5.32 b	4.46 b

S1 = first season, S2 = second season, Comb. = combined data.

highest values of number of spikes per plant in the second season and the combined data, number of grains per spike in the first season and the combined data and straw yield in the two growing season and their combined data. Whereas, Sakha-93 variety was exceeded in number of spikes per plant in the first season.

Effect of interaction

The effect of interaction between foliar application of bio-regulating substances and the three wheat varieties on yield and its components was significant and highly increased all yield components compared with the untreated plants (Tables 7-8).

Data indicated that, the highest

Table 6. Number of grains per spike, grainsweight per spike, grain and straw yield per feddanof some wheat varieties, as affected by some growth bio-regulating substances during two growing seasons and their combined data.

Treatments		No. of grains/spike			Grains weight(g)/spike			Grain yield (Tons/feddan)			Straw yield (Tons/feddan)		
		S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		30.01 g	29.63 d	29.82 e	1.33 f	1.94 e	1.64 g	1.61 f	2.00 f	1.80 f	1.82 g	2.37 f	2.09 h
Tryptophan	50 mgl-1	55.48 a	69.44 ab	62.46 a	2.85 a	5.18 a	4.01 a	2.59 a	3.02 a	2.80 a	4.26 bc	4.95 b	4.60 b
	100 mgl-1	44.93 d	59.47 c	52.20 cd	2.44 bc	4.14 d	3.29 c-e	2.37 bc	2.73 b	2.55 b	3.49 e	4.02 d	3.75 e
Cystine	100 mgl-1	45.47 cd	72.14 a	58.80 b	2.22 cd	4.81 a-c	3.51 bc	1.83 e	2.30 e	2.06 e	2.88 f	3.61 e	3.25 g
	150 mgl-1	38.61 f	60.08 c	49.34 d	1.83 e	4.14 d	2.98 f	2.33 bc	2.46de	2.40 cd	4.19 c	4.43 c	4.31 c
Thiamine	50 mgl-1	41.07 e	65.04 bc	53.05 c	2.01 de	4.56 b-d	3.29 c-e	2.17 d	2.50 c-e	2.34 d	3.76 de	4.29 cd	4.02 d
	100 mgl-1	50.44 b	67.73 ab	59.08 b	2.61 ab	4.91 ab	3.76 ab	2.32 c	2.63b-d	2.48 bc	4.53 b	5.04 b	4.79 b
Ascorbic Acid	50 mgl-1	47.81 c	70.35 ab	59.08 b	2.42 bc	4.96 ab	3.69 b	2.11 d	2.47 de	2.29 d	4.00 cd	4.51 c	4.25 c
	100 mgl-1	40.34 ef	64.92 bc	52.63 c	2.09 c-e	4.36 cd	3.23 d-f	2.42 b	2.69 bc	2.56 b	4.97 a	5.63 a	5.30 a
Yeast	1000 mgl-1	47.14 cd	65.32 bc	56.23 b	2.20 cd	4.82 a-c	3.51 b-d	2.32 c	3.14 a	2.73 a	2.99 f	4.05 d	3.52 f
	1500 mgl-1	41.28 e	60.69 c	50.99 cd	1.90 de	4.20 d	3.05 ef	2.09 d	2.52 b-d	2.31 d	2.80 f	3.43 e	3.12 g
Sakha-93		42.97 b	61.51 a	52.24 b	1.84 b	3.89 c	2.86 c	1.95 c	2.36 b	2.15 c	3.54 a	4.23 a	3.88 b
Gemiza-7		42.63 b	62.63 a	52.63 b	2.41 a	5.00 a	3.71 a	2.42 a	2.73 a	2.57 a	3.58 a	4.03 b	3.81 b
Gemiza-9		46.02 a	62.62 a	54.32 a	2.26 a	4.21 b	3.24 b	2.22 b	2.67 a	2.45 b	3.70 a	4.37 a	4.04 a

S1 = first season, S2 = second season, Comb. = combined data.

Table 7. Spike length, number of Spikes per plant and weight of spike of, as affected by interaction between some growth bio-regulating substances treatments and wheat varieties during two growing seasons and their combined data.

Treatments			Spike length (cm)			No. of Spikes/plant			weight of spike (g)		
			S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		Sakha-93	6.91 m	6.80 m	6.85 p	1.67 h	2.67 g-i	2.17 lm	1.69 o	2.79 l	2.24 n
		Gemiza-7	7.41 j-m	7.07 m	7.24 p	1.93 gh	2.33 hi	2.13 lm	2.19 mn	2.68 l	2.44 n
		Gemiza-9	7.14 lm	6.73 m	6.94 p	1.67 h	2.00 i	1.83 m	1.65 o	2.83 l	2.24 n
Tryptophan	50 mgl-1	Sakha-93	8.68 f-i	12.56 cd	10.62 e-g	3.25 b-f	4.50 b-f	3.87 c-k	3.78 d-g	6.78 ab	5.28 b-d
		Gemiza-7	10.24 ab	12.33 de	11.28 b-d	4.00 a-e	5.17 b-e	4.58 b-f	4.60 ab	6.29 b-f	5.28 b-d
		Gemiza-9	8.72 f-i	11.53 ef	10.13 f-i	4.50 ab	7.00 a	5.75 a	4.78 a	6.19 b-g	5.48 ab
	100 mgl-1	Sakha-93	8.93 e-g	9.67 i-l	9.30 k-n	3.33 b-f	3.50 f-h	3.42 i-k	3.25 hi	5.30 hi	4.27 ij
		Gemiza-7	10.16 bc	11.34 f	10.75 d-f	3.06 c-g	4.00 c-g	3.53 g-k	4.12 c-e	5.50 f-i	4.81 d-h
		Gemiza-9	9.21 c-f	11.30 f	10.26 f-h	3.83 a-e	5.50 bc	4.67 b-e	4.07 c-e	5.48 f-i	4.77 e-h
Cysteine	50 mgl-1	Sakha-93	7.97 g-l	10.57 f-j	9.27 k-n	3.25 b-f	4.00 c-g	3.62 f-k	3.45 g-i	5.04 ij	4.24 ij
		Gemiza-7	9.84 b-e	12.82 b-d	11.33 b-d	2.33 f-h	3.50 f-h	2.92 kl	3.44 g-i	6.46 a-d	4.95 c-f
		Gemiza-9	7.84 h-m	10.61 f-i	9.23 k-n	2.89 e-g	3.83 d-g	3.36 i-k	3.18 ij	5.88 c-i	4.53 f-i
	100 mgl-1	Sakha-93	8.62 f-i	9.567 kl	9.09 l-n	3.33 b-f	4.17 c-g	3.75 d-k	2.58 k-m	4.46 jk	3.52 lm
		Gemiza-7	10.19 bc	12.77 cd	11.48 bc	3.17 c-g	4.00 c-g	3.58 g-k	3.61 e-i	6.28 b-f	4.95 c-f
		Gemiza-9	8.32 f-k	9.60 j-l	8.96mn	3.00 d-g	3.83 d-g	3.42 i-k	3.21 ij	5.27 hi	4.24 ij
Thiamine	50 mgl-1	Sakha-93	8.85 e-h	9.66 i-l	9.25 k-n	4.00 a-e	5.00 b-f	4.50 b-h	2.27 l-n	5.07 ij	3.67 kl
		Gemiza-7	11.14 a	12.53 d	11.84 ab	3.50 b-f	4.27 c-f	3.88 c-k	3.54 f-i	6.57 a-c	5.06 b-e
		Gemiza-9	7.80 i-m	10.93 f-h	9.36 k-n	3.00 d-g	4.00 c-g	3.50 i-k	3.51 f-i	5.26 hi	4.39 h-j
	100 mgl-1	Sakha-93	8.37 f-j	10.58 f-j	9.47 i-m	3.50 b-f	3.83 d-g	3.67 f-k	3.63 e-i	5.29 hi	4.46 f-j
		Gemiza-7	9.95 b-d	14.87 a	12.41 a	4.17 a-e	4.33 b-f	4.25 c-j	3.90 c-g	6.84 ab	5.37 a-c
		Gemiza-9	8.26 f-k	11.12 fg	9.69 h-l	3.17 c-g	3.67 e-h	3.42 i-k	3.84 d-g	5.72 d-i	4.78 e-h
Ascorbic Acid	100 mgl-1	Sakha-93	7.43 j-m	10.06 h-k	8.74 n	3.42 b-f	4.00 c-g	3.71 e-k	2.76 jk	5.62 e-i	4.19 ij
		Gemiza-7	10.40 ab	11.57 ef	10.98 c-e	3.11 c-g	3.50 f-h	3.30 jk	4.38 a-c	7.12 a	5.75 a
		Gemiza-9	8.84 e-h	10.82 f-h	9.83 j-n	3.71 b-e	4.33 b-f	4.02 c-j	4.26 b-d	5.62 e-i	4.94 c-f
	100 mgl-1	Sakha-93	7.70 i-m	11.14 fg	9.42 j-n	4.03 a-e	5.00 b-f	4.52 b-g	2.70 kl	5.39 g-i	4.04 jk
		Gemiza-7	9.72 b-e	13.49 bc	11.60 bc	3.83 a-e	4.17 c-g	4.00 c-j	3.75 e-h	6.02 b-h	4.88 d-g
		Gemiza-9	8.61 f-i	11.01 f-h	9.81 h-k	4.25 a-d	5.17 b-e	4.71 b-d	3.24 i	5.57 e-i	4.41 g-j
Yeast	1000 mgl-1	Sakha-93	8.21 f-k	10.16 g-k	9.18 k-n	5.00 a	5.83 ab	5.42 ab	1.90 no	5.34 hi	3.62 kl
		Gemiza-7	9.03 d-f	13.73 b	11.38 b-d	4.17 a-e	4.50 b-f	4.33 c-i	4.67 ab	6.81 ab	5.74 a
		Gemiza-9	9.06 d-f	11.06 fg	10.06 g-j	4.17 a-e	5.33 b-d	4.75 bc	3.98 c-f	5.53 f-i	4.76 e-h
	2000 mgl-1	Sakha-93	7.32 k-m	8.75 l	8.03 o	4.33 a-c	5.17 b-e	4.75 bc	2.19 mn	4.16 k	3.17 m
		Gemiza-7	9.83 b-e	12.84 b-d	11.34 b-d	3.00 d-g	4.00 c-g	3.50 h-k	4.01 c-f	6.38 a-e	5.19 b-e
		Gemiza-9	8.37 f-j	10.26 g-k	9.32 k-n	3.83 a-e	4.33 b-fj	4.08 c-j	3.79 d-g	5.19 h-j	4.49 f-j

S1 = first season, S2 = second season, Comb. = combined data.

value of spike length was obtained by thiamine treatment when used at 50 mgl^{-1} concentration in the first season and 100 mgl^{-1} in the second season and combined data when used with Gemiza-7 variety. The highest value of number of spikes per plant in the first season had been resulted by the interaction between 1000 mgl^{-1} yeast extract with Sakha-93 variety, while it had been resulted from interaction between 50 mgl^{-1} of tryptophan with Gemiza-9 variety during the second season and the combined data analysis.

The most effective treatment in the mean values of spike weight was that of 50 mgl^{-1} tryptophan with Gemiza-9 variety in the first season. On the other side ascorbic acid treatment at 50 mgl^{-1} was more effective on the weight of spike when used on Gemiza-7 during the second season and the combined data analysis. Results showed that, the most effective treatment on grains weight per spike in the first season was that of 50 mgl^{-1} tryptophan on Gemiza-7 variety, while, ascorbic acid was more effective in the second season and the combined data when used at 50 mgl^{-1} concentration on the same variety.

Foliar application of 50 mgl^{-1} tryptophan on Gemiza-9 variety recorded the highest values in grain yield in the first, second season and their combined data.

The best treatments of straw yield

Table 8. Number of grains per spike, grains weight per spike and grain and straw yield as affected by interaction between some growth bio-regulators treatments and varieties of wheat, during two growing seasons and their combined data.

Treatments			No. of grains/spike			Grains weight(g)/spike			Grain yield (Tons/feddan)			Straw yield (Tons/feddan)		
			S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.	S1	S2	Comb.
Control		Sakha-93	37.63 l	32.67 h	35.15 m	1.13 o	2.28 m	1.71 n	1.55 o	1.88 m	1.71 q	1.50 m	2.04 r	1.77 p
		Gemiza-7	27.00 n	25.47 h	26.23 n	1.29 no	1.72 m	1.50 n	1.72 l-o	2.06 lm	1.89 o-q	1.75 lm	2.09 r	1.92 p
		Gemiza-9	25.40 n	30.75 h	28.08 n	1.58 k-o	1.82 m	1.70 n	1.55 o	2.07 k-m	1.81 q	2.22 k-m	2.97 q	2.59 o
Tryptophan	50 mgl-1	Sakha-93	54.12 bc	73.83 a-d	63.97 ab	2.69 a-e	5.80 ab	4.24 ab	2.35 g	2.64 e-i	2.49 d-i	4.46 a-d	5.00 a-f	4.73 c-f
		Gemiza-7	53.00 b-d	58.17 fg	55.58 c-i	3.08 a	4.70 d-i	3.89 b-f	2.66 a-c	2.91 c-f	2.78 bc	4.17 b-e	4.56 d-j	4.36 e-i
		Gemiza-9	59.33 a	76.33 a	67.83 a	2.78 a-c	5.04 b-f	3.91 b-f	2.75 a	3.51 a	3.13 a	4.15 b-e	5.28 a-d	4.71 c-g
	100 mgl-1	Sakha-93	41.73 i-k	59.67 e-g	50.70 i-k	1.97 g-m	3.83 j-l	2.90 j-l	2.08 hi	2.30 h-l	2.19 k-n	3.29 e-i	3.63 l-q	3.46 k-m
		Gemiza-7	46.53 e-h	57.83 fg	52.18 g-j	2.67 a-f	4.43 e-j	3.55 d-h	2.35 fg	2.48 f-k	2.42 g-j	3.39 e-i	3.59 m-q	3.49 k-m
		Gemiza-9	46.53 e-h	60.92 e-g	53.72 d-j	2.67 a-f	4.17 g-j	3.42 f-i	2.67 ab	3.40 ab	3.03 a	3.78 d-g	4.83 c-h	4.31 e-j
Cysteine	50 mgl-1	Sakha-93	48.20 ef	71.23 a-e	59.72 b-e	1.81 i-n	3.85 i-l	2.83 j-l	1.68 m-o	2.06 lm	1.87 pq	3.16 f-j	3.89 j-p	3.52 k-m
		Gemiza-7	46.53 e-h	75.19 a-c	60.86 bc	2.51 a-g	5.60 a-c	4.06 b-d	1.93 h-k	2.57 f-j	2.25 j-n	2.77 h-k	3.68 k-q	3.23 l-n
		Gemiza-9	41.67 i-k	70.00 a-f	55.83 c-i	2.35 b-i	4.97 b-g	3.66 d-g	1.88 i-l	2.27 i-m	2.07 m-p	2.71 h-k	3.27 o-q	2.99 m-o
	100 mgl-1	Sakha-93	38.53 kl	53.08 g	45.81 kl	1.64 j-o	3.32 kl	2.48 lm	1.97 h-j	2.19 j-m	2.08 m-p	4.25 b-e	4.70 d-i	4.47 d-h
		Gemiza-7	39.50 j-l	67.58 a-f	53.54 e-j	2.08 f-l	5.14 b-e	3.61 d-h	2.61 a-d	2.66 e-i	2.63 c-g	4.12 b-e	4.20 g-n	4.16 f-j
		Gemiza-9	37.80 l	59.57 e-g	48.68 j-l	1.76 i-n	3.97 h-k	2.87 j-l	2.42 d-g	2.53 f-j	2.47 e-j	4.19 b-e	4.38 e-l	4.29 e-j
Thiamine	50 mgl-1	Sakha-93	47.27 e-g	62.42 c-g	54.84 c-j	1.40 m-o	3.94 h-l	2.67 k-m	2.13 h	2.24 i-m	2.19 k-n	4.13 b-e	4.36 e-l	4.25 e-j
		Gemiza-7	37.13 l	65.39 a-g	51.26 h-k	2.33 b-i	5.55 a-c	3.94 b-e	2.44 d-g	2.99 c-e	2.72 cd	3.34 e-i	4.09 h-n	3.72 j-l
		Gemiza-9	38.80 kl	67.31 a-f	53.05 f-j	2.30 b-i	4.20 f-j	3.25 g-j	1.95 h-k	2.26 i-m	2.10 l-o	3.80 d-g	4.40 e-k	4.10 h-j
	100 mgl-1	Sakha-93	53.00 b-d	67.50 a-f	60.25 b-d	2.53 a-g	4.34 e-j	3.43 e-i	2.36 fg	2.55 f-j	2.45 f-j	4.20 b-e	5.02 a-f	4.61 c-h
		Gemiza-7	48.33 ef	67.11 a-f	57.72 b-h	2.74 a-d	5.61 a-c	4.17 a-c	2.60 a-e	3.00 c-e	2.80 bc	4.58 a-d	5.00 a-f	4.79 b-e
		Gemiza-9	50.00 de	68.57 a-f	59.28 b-f	2.55 a-g	4.79 c-h	3.67 d-g	2.00 h-j	2.34 g-l	2.17 k-n	4.81 a-c	5.11 a-e	4.96 a-d
Ascorbic Acid	100 mgl-1	Sakha-93	41.69 i-k	70.08 a-f	55.89 c-i	1.89 h-m	4.16 g-j	3.02 i-k	1.82 j-m	2.24 i-m	2.03 n-p	3.47 e-h	4.27 f-n	3.87 i-k
		Gemiza-7	45.82 f-h	75.58 ab	60.70 bc	2.88 ab	6.20 a	4.54 a	2.40 e-g	2.67 e-i	2.54 d-h	4.60 a-d	4.95 b-g	4.78 b-e
		Gemiza-9	55.93 b	65.39 a-g	60.66 bc	2.48 a-h	4.53 d-j	3.50 e-i	2.11 h	2.49 f-k	2.30 i-m	3.93 c-f	4.31 f-m	4.12 g-j
	100 mgl-1	Sakha-93	31.63 m	69.58 a-f	50.61 i-k	1.84 i-n	3.92 i-l	2.88 j-l	2.12 h	2.54 f-j	2.33 h-l	5.24 a	5.65 ab	5.45 a
		Gemiza-7	42.97 h-j	62.08 d-g	52.53 g-j	2.24 c-j	4.98 b-g	3.61 d-h	2.55 b-f	2.78 d-f	2.66 c-f	4.77 a-c	5.51 a-c	5.14 a-c
		Gemiza-9	46.42 e-h	63.08b-g	54.75 c-j	2.21 c-j	4.19 f-j	3.20 g-j	2.61 a-d	2.77 d-g	2.69 c-e	4.90 ab	5.73 a	5.31 ab
Yeast	1000 mgl-1	Sakha-93	45.20 f-i	62.28 d-g	53.74 d-j	1.81 i-n	4.21 f-j	3.01 i-k	1.76 k-n	3.14 a-d	2.45 f-j	2.26 j-m	4.01 i-n	3.13 l-o
		Gemiza-7	43.80 g-i	68.90 a-f	56.35 c-i	2.61 a-f	5.75 ab	4.18 a-c	2.73 ab	3.22 a-c	2.97 ab	2.99 g-k	3.52 n-q	3.25 l-n
		Gemiza-9	52.43 b-d	64.78 a-g	58.60 b-g	2.16 d-k	4.49 d-j	3.32 g-j	2.47 c-g	3.06 b-e	2.76 bc	3.72 d-g	4.60 d-j	4.16 f-j
	2000 mgl-1	Sakha-93	33.63 m	54.25 g	43.94 l	1.52 l-o	3.13 l	2.32 m	1.62 no	2.20 j-m	1.91 o-q	2.94 g-k	3.98 i-o	3.46 k-m
		Gemiza-7	38.33 kl	65.67 a-g	52.00 g-j	2.10 e-l	5.32 b-d	3.71 c-g	2.59 a-e	2.72 e-h	2.66 c-f	2.94 g-k	3.10 q	3.02 m-o
		Gemiza-9	51.87 cd	62.17 d-g	57.02 c-i	2.08 e-l	4.15 g-j	3.11 h-k	2.07 hi	2.65 e-i	2.36 h-k	2.51 i-l	3.21 pq	2.86 no

S1 = first season, S2 = second season, Comb. = combined data.

was foliar application of 100 mgl^{-1} ascorbic acid on Sakha-93 variety during the first season and combined data and the same treatment with Gemiza-9 in the second season.

The total photosynthetic pigments

Effect of bio-regulator treatments

Data presented in Table (9) showed that, total photosynthetic pigments (chlorophyll a, b and carotenoids) were significantly increased as affected by bio-regulating substances compared to the untreated plants (control) at the two growth stages.

Tryptophan was more effective in all studied characters, when used at the concentration of 50 mgl^{-1} except the total carotenoids which was more affected by 1000 mgl^{-1} of yeast extract.

Varieties performance

There were no significant differences between Gemiza-7 and Gemiza-9 varieties in chlorophyll (a) content. The highest value was obtained by Gemiza-9 variety during heading stage. While, the last variety was Sakha-93 which recorded the lowest value in this parameter. Gemiza-7 variety exceeded the other two varieties in chlorophyll (b) during heading stage.

Concerning total carotenoids in the leaves Gemiza-7 variety exceeded the other two varieties in this respect during heading stage.

Effect of interaction

Data in Table (10) indicated that, thiamine treatment when used at 100 mgl^{-1} concentration with Gemiza-7 variety resulted in the highest value of chlorophyll (a) at the heading stage.

The highest value of chlorophyll (b) was produced from interaction between 50 mgl^{-1} tryptophan with Sakha-93 variety during heading stage.

As for the effect of the interaction on the carotenoids contents in the leaves of wheat plants, the most effective treatments was 1000 mgl^{-1} of yeast extract with Sakha-93 variety at heading stage.

Table 9. Average values of the photosynthetic pigments content (mg/g fresh weight of wheat leaves) , as affected by some growth bio-regulators treatments and varieties of wheat. (Average of two seasons).

Treatments		Chlorophyll (a) mg/g	Chlorophyll (b) mg/g	Carotenoids mg/g
Control		0.6661 j	0.3028 h	0.2219 h
Tryptophan	50 mgl-1	1.229 a	0.6101 a	0.4390 a
	100 mgl-1	1.021 e	0.5308 c	0.3901 bc
Cystine	100 mgl-1	0.6857 i	0.3446 g	0.2723 g
	150 mgl-1	0.8428 h	0.4350 f	0.3169 ef
Thiamine	50 mgl-1	0.8422 h	0.4370 f	0.3092 f
	100 mgl-1	1.173 b	0.5323 c	0.3977 b
Ascorbic Acid	50 mgl-1	0.8592 g	0.5052 d	0.3218 e
	100 mgl-1	1.092 c	0.5607 b	0.3847 c
Yeast	1000 mgl-1	1.037 d	0.4882 e	0.4434 a
	1500 mgl-1	0.8741 f	0.4360 f	0.3649 d
Sakha-93		0.863 b	0.4673 b	0.3244 b
Gemiza-7		0.971 a	0.4746 a	0.3660 a
Gemiza-9		0.981 a	0.4715 ab	0.3628 a

S1 = first season, S2 = second season, Comb. = combined data.

Table 10. Effect of interaction between wheat varieties and bio-regulator treatments on the photosynthetic pigments content (mg/g fresh weight of wheat leaves) (average of two successive seasons).

Treatments			Chlorophyll (a) mg/g	Chlorophyll (b) mg/g	Carotenoids mg/g
Control		Sakha-93	0.540 n	0.292 o	0.213 q
		Gemiza-7	0.626 m	0.294 o	0.211 q
		Gemiza-9	0.832 jk	0.323 n	0.242 op
Tryptophan	50 mgl-1	Sakha-93	1.263 b	0.638 a	0.437 de
		Gemiza-7	1.261 b	0.624 a	0.479 b
		Gemiza-9	1.162 cd	0.568 cd	0.401 g
	100 mgl-1	Sakha-93	0.993 f	0.526 e	0.365 ij
		Gemiza-7	1.168 c	0.555 d	0.426 ef
		Gemiza-9	0.900 g-i	0.511 e	0.379 hi
Cysteine	50 mgl-1	Sakha-93	0.544 n	0.344 m	0.203 q
		Gemiza-7	0.821 jk	0.379 l	0.370 i
		Gemiza-9	0.692 l	0.311 n	0.244 op
	100 mgl-1	Sakha-93	0.821 jk	0.433 j	0.287 n
		Gemiza-7	0.932 gh	0.435 j	0.382 hi
		Gemiza-9	0.776 k	0.437 j	0.281 n
Thiamine	50 mgl-1	Sakha-93	0.869 ij	0.464 gh	0.319 m
		Gemiza-7	0.939 fg	0.464 gh	0.350 jk
		Gemiza-9	0.718 l	0.384 l	0.259 o
	100 mgl-1	Sakha-93	0.910 g-i	0.492 f	0.324 m
		Gemiza-7	1.473 a	0.526 e	0.456 c
		Gemiza-9	1.135 cd	0.579 c	0.413 fg
Ascorbic Acid	100 mgl-1	Sakha-93	0.778 k	0.481 fg	0.278 n
		Gemiza-7	0.638 m	0.479 fg	0.237 p
		Gemiza-9	11.161 cd	0.556 d	0.451 cd
	100 mgl-1	Sakha-93	0.874 h-j	0.515 e	0.317 m
		Gemiza-7	0.959 fg	0.564 cd	0.342 kl
		Gemiza-9	1.443 a	0.603 b	0.495 ab
Yeast	1000 mgl-1	Sakha-93	1.074 e	0.493 f	0.501 a
		Gemiza-7	0.930 gh	0.458 hi	0.401 g
		Gemiza-9	1.108 de	0.513 e	0.429 ef
	2000 mgl-1	Sakha-93	0.828 jk	0.465 gh	0.326 lm
		Gemiza-7	0.927 gh	0.443 ij	0.373 i
		Gemiza-9	0.867 ij	0.400 k	0.396 gh

S1 = first season, S2 = second season, Comb. = combined data.

4 Discussion

Effect of bio-regulators on growth

According to the present data, the effect of foliar application of the different applied bioregulating substances at different concentrations on plant height, number of tillers and leaves per plant and fresh and dry weight of plant at heading stage were high significantly increased in both season and their combined data of all bio-regulator treatments. These results are in harmony with those obtained by Gamal El-Din and Zaki (2005) who found that, at harvest stage all treatments of (Lysine, phenylalanine

and L-cysteine) led to significant increases in plant height, number of pods and seeds per plant, dry weight of pods of lupine plants. EL-Bassiouny (2005) found that application of nicotinamide or tryptophan resulted in significant increases in plant growth and grain yield, concomitantly with an increase in the level of IAA, GA3, cytokinins, photosynthetic pigments and a decrease in ABA content of wheat plant.

On the other hand, Gadalla, (2009) reported that, application of ascorbic acid increased significantly flag leaf area in wheat plants grown under salinity conditions. These results may be due to the effect of ascorbic acid on osmotic adjustment and maintaining leaf turgor potential as a consequence of increasing leaf water potential and relative water content as compared to control plants. Farouk, (2011).

Yield and yield components

As for the effect of bio-regulating substances on yield and yield components the present data show that, foliar application of all used concentrations of bio-regulating substances significantly increased the yield and yield components of all used wheat varieties. Similarly, El-Bassiouny, (2005) reported that application of tryptophan resulted in significant increase in plant growth and grain yield, concomitantly with an increase in the level of IAA, GA3, cytokinins, photosynthetic pigments and a decrease in ABA content of wheat plant. Also, Abdel-Halim (1995)reported that, ascorbic acid at 100 and 200 ppm led to increased contents of indols (IAA, IAN and IBA) in the shoot of tomato. Amin et al., (2008) reported that ascorbic acid recorded the highest increase in straw yield per plant and per feddan of wheat plant cv. Gemiza10.

These results might emphasize the role of ascorbic acid as scavenge the free radicals which caused increase in the oxidation in plant tissues (Noctor and Foyer, 1998).

Photosynthetic pigments

It is observed from the presented data that, total photosynthetic pigments (chlorophyll a, b and carotenoids) were significantly increased as affected by the different bio-regulating substances compared to untreated plants (control), these results are true for all photosynthetic pigments.

The induced effect of tryptophan on chlorophyll biosynthesis may be due to its role in IAA biosynthesis (Arshad et al., 1995; Barazani and Friedman, 2000). Also, the positive effect of foliar application of amino acids in enhancing all photosynthetic pigments percentage might be attributed to its positive effect on the succnyl COA (Kerb's cycle intermediate) and their effect in initiating the biosynthetic pathway leading to chlorophyll formation.

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A Comparison of the Pre-Competition and Post-Competition Anxiety Levels of Taekwondo Athletes

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ABSTRACT

A total of 468 taekwondo athletes, 231 females and 237 males, in the adult category, who had participated in the 2012 Turkish Taekwondo Championship; with an average age of 20.91 years (Sd=3.66) participated in the present research for the comparison of the pre-competition and post-competition anxiety levels of taekwondo athletes. The research was conducted using the survey technique of data collection and the competitive state anxiety inventory-2 (CSAI-2) (Martens, Burton, Vealey, Bump and Smith 1982) and it was used in the present research to measure the anxiety levels of the sportspeople. The Turkish implementation of the inventory was performed by Koruç (1998). Statistically significant difference cannot be found between the inventory scores of females and males who participated in the research according to their genders (P>0.05). Whether there was a significant difference between the inventory scores of sportspeople according to their ages was tested by one-way analysis of variance, and a statistically significant difference was found between the self-assessment scores of the taekwondo athletes measured 1 day before according to their ages (P<0.05). A significant difference was not found between the selfassessment scores of athletes measured 1 day before according to their sports ages (p>0.05). Consequently, it was observed that the cognitive and somatic anxiety scores of taekwondo athletes increased and their self-confidence scores decreased as the competition drew closer. After the competition, it was detected that the cognitive and somatic anxiety scores decreased and self-confidence scores increased. The anxiety levels of the taekwondo athletes increased just before the competition, but a decrease in anxiety was observed after the competition. Pre-competition and post-competition anxiety levels were found to have an effect on the success of the athletes. All in all, it was observed that the state anxiety measured by CSAI-2 showed pre-competition and post-competition changes and there was a change in anxiety cognition.

Key Words

Taekwondo, Competition, State Anxiety, Cognitive anxiety, Somatic anxiety, Self-confidence.

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Introduction

One of the most interesting aspects of sport psychology literature is competition anxiety, which is also one of the important psychological factors in respect of sports performance, and it is accepted that anxiety has a strong influence on performance (Gould, Horn and Spreeman, 1993).

It is clearly accepted nowadays that good physical training for a sportsperson alone cannot increase the performance level. Not reaching the appropriate level of motivation leads the athlete to experience problems in locomotive coordination, difficulty in action, and to give a poor performance (Harris and Williams, 1993).

Sport scientists work hard to improve sportive performance. They search for new training principles and continue to seek ways to make provisions for athlete to reach high performance levels. All such research revealed that the perfection of the physical capacity alone was not sufficient for sport performance, and that psychological capacity was an important factor (Akarçeşme, Koruç and Yılmaz, 2004).

Performance is not only a physical quality but also a psychological process. An athlete knows that if he/she loses a competition, he/she will experience an economic loss as well as a loss in fame. As a result, he/she has to display performance under the pressure of feeling anxious in each competition (Akarçeşme, Koruç and Yılmaz, 2004).

Anshell (1994) defines anxiety as a

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multilateral internal process consisting of physiological and behavioral reactions as well as a cognitive or emotional impact of subjective fear, tension, nervousness, excitement, the perceived threat and inducement felt following the increase in activity of the sympathetic nervous system of an individual in various periods of time. Spielberger (1989) examines anxiety in their state and continuity dimensions, and defines this as a temporary fear and tension feeling experienced under certain circumstances and emphasizes that it is effective in a sports environment. Continuous anxiety is dealt with as a personality trait and characterized as a permanent emotional state.

The determination of the cognitive and somatic anxiety and self-confidence change in the pre-competition closing timeframe which was experienced by the sportsperson in the sportive environment, and what kind of differences occur in the post-competition period, is considered to be an important subject by both researchers and practitioners.

Anxiety may affect the right decision taking skills of athlete in behaviors negatively. The more the anxiety level increases, the less the athlete makes the right decision and performs to his/her abilities. Athlete under high pressure may take some wrong actions. Extreme anxiety may cause an athlete to forget some well-known actions that have been repeated many times in training, and in addition may lead to some negative actions by causing emotional confusion.

Considering the severe anxiety and stress felt by athlete before or during the competition, uncontrollable anxiety states can negatively affect the performances of athlete and lead to failure. Therefore, knowing the anxiety levels of athlete and the causes of the anxiety is clearly very important for athlete and the trainers who will attempt to overcome it.

The purpose of the present study is to determine what kinds of changes are experienced by taekwondo athletes in their competition anxiety and cognition (cognitive anxiety, somatic anxiety and self-confidence levels) 1 day, 1 hour before and 1 hours after the competition.

MATERIAL AND METHODS

A total of 468 taekwondo athletes (Table 1), 231 females and 237 males from the adult category, who had participated in the 2011-2012 Turkish Teakwondo Championship, with an average age of 20.91 years, (Sd=3.66) participated in the research.

FINDINGS

In the research, the competitive state anxiety inventory-2 (CSAI-2) which was developed by Martens, Burton, Vealey, Bump and Smith (1982) was used for determining anxiety levels and a personal information form was used for collecting information from the participants. The Turkish implementation of the inventory was conducted by Koruç (1998). An anxiety inventory was developed for measuring the feelings of people 1 day, 1 hour before and 1 hour after the competition. The internal consistency of measures taken 1 day, 1 hour before, and 1 hour after the competition with the 27-items ILLNIOS Self-Assessment Inventory was used by sportspeople for defining feelings before the competitions, and this was evaluated by Cronbach's Alpha. It is a Likert type scale comprising 27 items and 1-4 points. The state of anxiety inventory is comprised by 13 positive and 14 negative questions (reverse statements). The total score values are between 27 and 108. A high score means a high anxiety level while a low score means a low anxiety level. It is stated that the Cronbach's Alpha is between 0.60-0.70 and this result is an indicator that the inventory is highly valid.

For the assessment of the data and finding calculated values (Table 1), the SPSS 16.0 statistical package program was used. Data were summarized by giving average and standard deviation. T test and One-Way ANOVA tests which were matched from parametric tests according to test of normality were used. Also, Tamhane and Tukey tests from Post Hoc Multiple Comparisons tests were used according to the variance homogeneity. The error level was detected as 0.05 in the study.

Whether there was a significant difference between the scale scores of taekwondo athletes according to their genders was tested by t test for independent samples, and the self-assessment score (x=2.30) of athlete measured 1 day before was found to be higher than that of sportsmen (Table 1). The self-assessment score which was measured 1 day before became different statistically according to the genders of athlete ($p<0.05$).

The self-assessment score (x=2.24) of female taekwondo athletes which was measured 1 hour before was found to be a little higher than that of the male taekwondo athletes (Table 1). However, the self-assessment score which was measured 1 hour before became different statistically according to the genders of the athlete ($p>0.05$).

The self-assessment score (x=2.27) of female taekwondo athletes which was measured 1 hour after was found to be relatively higher than that of the male taekwondo athletes (Table 1). However, the self-assessment score which was measured 1 hour after became statistically different according to the genders of the athlete ($p>0.05$).

Whether there was a significant difference between the scale scores of the taekwondo athletes according to their ages was tested by one-way variance analysis and the self-assessment score

Table 1. An examination of the differences between the scale scores of taekwondo athletes according to their genders.

	Sex	N	Average	Std. Deviation	t	Sd	P
1 Day Before	Male	236	2.22	0.34	-2.082	465	0.038*
	Female	231	2.30	0.40			
1 Hour Before	Male	236	2.24	0.32	0.109	465	0.913
	Female	231	2.24	0.34			
1 Hour After	Male	236	2.25	0.31	-0.570	465	0.569
	Female	231	2.27	0.32			

(x=2.27) of athlete between 19-25 ages measured 1 day before was found to be higher in respect of the athlete at different ages (Table 1). A significant statistical difference was found between the self-assessment scores of taekwondo athletes measured 1 day before, according to ages ($p<0.05$). The difference found was between athlete between the ages 19-25 and athlete between the ages 26-45.

The self-assessment score (x=2.27) of taekwondo athletes between the ages of 19-25 measured 1 hour before was found to be higher in respect of the athlete at different ages (Table 2). However, a statistically significant difference was not found between the self-assessment scores which were measured 1 hour before in relation to the ages of the taekwondo athletes ($p>0.05$).

The self-assessment score (x=2.27) of taekwondo athletes between the ages 19-25 measured 1 hour after was found to be higher in respect of the athlete at different ages (Table 2). However, a statistically significant difference was not found between the selfassessment scores which were measured 1 hour before in relation to the ages of the athlete ($p>0.05$).

Whether there was a significant difference between the scale scores of taekwondo athletes according to sports ages was tested by one-way variance analysis and the self-assessment score (x=2.25) of athlete between the sport ages of 6-10 measured 1 day before was found to be higher in respect of the athlete at different ages (Table 2). However, a statistically significant difference was not found between the self-assessment scores which were measured 1 hour before according to the sport ages of taekwondo athletes ($p>0.05$).

The self-assessment score (x=2.27) of taekwondo athletes between the sport ages 6-10 measured 1 hour before was found to be higher in respect of the sportspeople at different ages (Table 3). However, a statistically significant difference was not found between the self-assessment scores which were measured 1 hour before in relation to the ages of the taekwondo athletes ($p>0.05$).

The self-assessment score (x=2.255) of taekwondo athletes between the sport ages 11-22 measured 1 hour after was found to be relatively higher in respect of the athlete at different ages (Table 3). However, a statistically significant difference was not found between the self-assessment scores which were measured 1 hour before according to the ages of the athlete ($p>0.05$).

Considering the cognitive anxiety levels of the taekwondo athletes, while the levels were just above average 1 day before the competition, the levels were

Table 2. An examination of the differences between the scale scores of taekwondo athletes according to their ages.

	Age	N	Average	Std. Deviation	F	P
	14-18	104	2.23	0.42		
1 Day Before	19-25	308	2.27	0.34	3.703	0.025*
	26-37	55	2.13	0.29		
	14-18	104	2.19	0.31		
1 Day Before	19-25	308	2.27	0.32	2.242	0.107
	26-37	55	2.21	0.38		
	14-18	104	2.24	2.27		
1 Hour After	19-25	308	2.27	0.33	1.334	0.264
	26-37	55	2.19	0.27		

Table 3. An examination of the differences between the scale scores of taekwondo athletes according to sports ages.

	Age	N	Average	Std. Deviation	F	P
	1-5	98	2.24	0.34		
1 Day Before	6-10	214	2.24	0.37	0.063	0.939
	11-12	106	2.23	0.30		
	1-5	98	2.21	0.28		
1 Day Before	6-10	214	2.27	0.33	1.151	0.317
	11-12	106	2.24	0.32		
	1-5	98	2.25	0.34		
1 Hour After	6-10	214	2.24	0.30	0.056	0.946
	11-12	106	2.25	0.32		

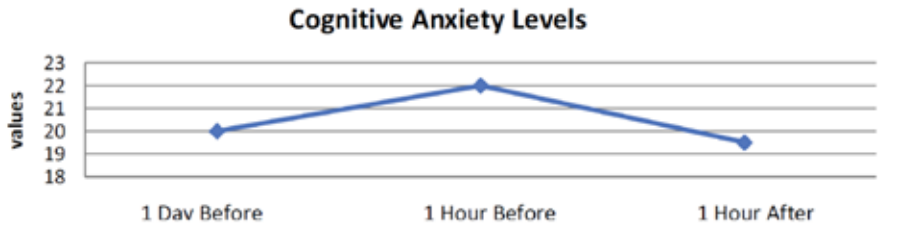

Fig.1. Cognitive Anxiety Values of Taekwondo Athletes in the Adult Turkish Championship Competitions.

Fig.2. Somatic Anxiety Values of Taekwondo Athletes in the Adults Turkish Championship Comp.

Fig.3. Self-Confidence Levels of Taekwondo Athletes in the Adult Turkish Championship Competitions.

seen to increase when the competition got closer, and they decreased to the lowest value 1 hour after the competition (Figure 1).

Considering the somatic anxiety values of the taekwondo athletes, somatic anxiety, which was at the lowest point in the profile 1 day before the competition, started to increase 1 hour before and reached the highest point in the profile (Figure 2). The values were seen to decrease 1 hour after the competition.

Considering the self-confidence values of taekwondo athletes in the Adults Turkish championship competitions, their self-confidence scores were seen to be under the average (Figure 3). The values started to decrease 1 day before the competition and continued to decrease until 1 hour before the competition; then they were at the highest value in the profile 1 hour after the competition.

DISCUSSION AND CONCLUSION

The present study was conducted to compare the pre-competition and post-competition anxiety levels of female and male taekwondo athletes participating in the Turkish championship for adults.

The obtained data revealed the relationships among cognitive anxiety, somatic anxiety and selfconfidence 1 day before the competition. While there was a positive relationship in the cognitive and somatic anxiety scores between the data obtained 1 hour and 1 day before the competition, a negative relationship was observed between somatic anxiety and self-confidence.

In a study, it is revealed that the precompetition stress levels of winners of competitions increase the state of anxiety. It was concluded that athlete delivering an outstanding performance were stress tolerant people who knew how to act in stressful environments, could turn the disadvantages of stressful environments into advantages and that they resistant to stress of competition (Özbekçi, 1989).

Jones, Swain and Cale (1991) stated that the cognitive anxieties of athlete increased when the competition drew closer. The somatic anxieties of both women and male athletes were seen to decrease on the day of the competition. But the decrease in women was higher than it was fore the men.

In a study conducted with female gymnasts, it was stated that cognitive anxiety increased and selfconfidence decreased 1as the competition drew nearer (Krane, 1994).

Hanton, Thomas and Maynard (2003) stated in their study that the intensity of cognitive and somatic anxiety increased and the score for selfconfidence decreased in the measures made 2 hours and 30 minutes before the competition. The intensity of the cognitive anxiety showed an increase from 1 week towards 30 minutes, and the intensity of somatic anxiety showed an increase from 1 week towards 30 minutes. The intensity of self-confidence also increased from 1 week towards 2 days.

Similar results were obtained in studies conducted in Turkey. The study of Koruç, Altay and Yılmaz (2004) revealed that the cognitive and somatic anxiety scores of a young women's national team increased but the self-confidence scores decreased. After the competition, while the cognitive and somatic anxiety scores decreased, the self-confidence scores increased.

It was stated in the present research that the anxiety levels of athlete regularly increased as the competition drew near. Accordingly, it can be said that conducting exercises for overcoming stress and anxiety to help athlete will be beneficial in increasing self-confidence and overcoming increasing anxiety.

The obtained data revealed that the cognitive and somatic anxiety scores of taekwondo athletes increased and their self-confidence decreased as the competition drew near. After the competition, it was determined that the cognitive and somatic anxiety scores were decreased and self-confidence scores were increased. Consequently, the state of anxiety which was measured by CSAI-2 showed precompetition and post-competition changes, and there was a change in anxiety cognition.

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Nutraceuticals from Bitter Leaf (*Vernonia amygdalina* Del.) Protects against Cadmium Chloride Induced Hypertension in Albino Rats

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ABSTRACT

In recent years, the consumption of nutraceuticals, natural plant foods, and the use of nutritional therapy and phytotherapy have become progressively popular to improve health, and to prevent and treat diseases. This study investigated the cardioprotective and hepatoprotective effects of some nutraceuticals from *Vernonia amygdalina* namely, β-sitosterol (BSS), β-sitosterol glucoside (BSSG) and BSS: BSSG mixture on certain parameters in hypertensive wistar albino rats. Hypertension was induced with Cadmium Chloride and the biochemical analyses of serum were carried out following treatment with BSS, BSSG, BSS:BSSG mixture and lisinopril. Serum urea, creatinine, calcium and electrolytes levels were assayed using appropriate standard methods as tests for renal function, while alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine aminotransferase (ALT) served as enzyme indices of the liver function. The effect on the serum lipid profile was also assessed. Data collected were expressed as mean ± SEM and analysed using one-way ANOVA. The sodium levels had a significant ($p<0.05$) reduction in BSS and BSS:BSSG mixture treated rats. BSS treatment also gave a significant ($p<0.05$) decrease in triglyceride and total cholesterol levels while BSSG treatment gave 60.4% increase in HDL-Cholesterol levels and increased HDL-Cholesterol: LDL-Cholesterol ratio. Generally, treatments with the phytosterols reduced the levels of serum AST, ALT, ALP and tends to maintain urea and creatinine basal levels while lisinopril significantly increased ($p<0.05$) serum urea and creatinine levels. Tissue sections from phytosteroltreated groups show no visible lesion as against those from hypertensive rats that show areas of extensive necrosis. This study revealed that these nutraceuticals possess cardioprotective and hepatoprotective properties, with possible practical application in the management of cardiovascular diseases (CVDs).

Key Words

Nutraceuticals, *Vernonia amygdalina*, bioactivity, lisinopril, hypertension.

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1 Introduction

Plants contain a cornucopia of micronutrients and other compounds that promote health and reduce the risk of heart disease. Vernonia amygdalina, commonly called bitter leaf, is a small shrub that is native to tropical Africa. It is a member of the Asteraceae family whose leaves have been consumed for many years, either as a vegetable (macerated leaves in soup) or aqueous extracts for the maintenance of good health. One family of plant compounds, phytosterols, found naturally, mainly as β-sitosterol, campesterol and stigmasterol in Vernonia amygdalina and many food substances of plant origin with vegetable oils (especially unrefined oils), nuts, seeds and grains as the major dietary sources (Piironen et al,.2000) have been investigated for their biochemical roles in chronic diseases. β-sitosterol had earlier been reported in the literature to be effective as nutraceuticals and in combination therapy for the treatment of hypercholesterolemia, benign prostatic hyperplasia and breast cancer. It is also suggested to be immunomodulatory (Bouic, 2001), anti-artherogenic and antioxidative (Awad et al, 2001). Thus there is substantial evidence that the incidence of cardiovascular diseases can be reduced by phytosterols mainly via serum cholesterol levels lowering actions. There are extensive studies and research

Olaiya CO, Choudhary MI, Ogunyemi OM, Nwauzoma AB. Nutraceuticals from bitter leaf (Vernonia amygdalina Del.) protects against cadmium chloride induced hypertension in albino rats. Science and Nature (2013) 2(3): 88-94. (ISSN 2324-7290) © ZolCat Academic House. www.zolcat.com

findings as to the use of phytosterols through diet or through combination therapy with other lipid lowering agents such as statin and fibrates. However there are still insufficient findings as to the roles they play in hypertensive conditions.

The phytosterols are biochemically heterogeneous in plant derived food matrices and the form of the phytosterols might be important in bioactivity. For instance esterified phytosterol solubilized in the triglyceride phase of margarines tends to produce a more desirable bioactivity than the crystalline form (Katan et al,.2003; Spilburg et al,.2003). A portion of β-sitosterol is glycosylated with little known effects on biological activity. However, the amphipathic structure of phytosterol glycosides raises questions about the degree of solubility in intestinal bile salt micelles and reactivity with pancreatic enzymes. Although literature on the physiology of phytosterol glycosides is sparse, previous workers have shown that fatty acids are cleaved from glycosylated phytosterols in vitro by pancreatin, but the sugar moiety itself is not removed (Moreau, 2004).

Dyslipidemia and hypertension, the two major risk factors of CVDs have been shown to contribute individually and synergistically to the pathogenesis and progression of cardiovascular diseases (Edward, 1994). The kidney also plays a primary role in the genesis and maintenance of essential hypertension. The work of Curtis et al. (2000) demonstrated that the remission of essential hypertension and monogenic hypertension with hypokalemia (Liddle's syndrome) was possible after successful renal transplantation with kidneys from normotensive donors. Renal capacity for the handling of sodium, potassium, chloride, and other electrolytes is important in the context of extracellular volume expansion and hypertension. Also important are the epidemiologic observations that there is an inverse continuous relationship between renal function and cardiovascular events (Samak et al., 2003). Modulation of various parameters associated with dyslipidemia and hypertension with natural products could be valuable in the prevention and treatment of cardiovascular diseases.

With the current incidence of cardiovascular diseases, there is a need for population-based, costeffective, adverse-effect free hypertension control strategies to be developed. The development of a safe and effective way to manage hypertension which has challenged medical researchers for centuries has prompted a lot of researchers to shift focus to the therapeutic uses of medicinal plants and their natural products as other means for the populace, especially low-risk individuals and patients experiencing adverse effects of drugs (Kharb and Singh, 2004). While the literature is replete with findings about hyperlipidemia and the associated therapeutic agents in relation to cardiovascular diseases, there are continuous needs to better investigate the therapeutic options of hypertension. This study was therefore designed to investigate the cardioprotective and hepatoprotective effects of bitter leaf nutraceuticals, β-sitosterol and its glucoside form on some selected parameters in hypertensive rats and to compare the biological activities of the two forms.

2 Materials and Methods

Plant material, extraction and chemical analysis

Fresh leaves of Vernonia amygdalina were collected from the premises of the University of Ibadan, Ibadan, Nigeria. They were authenticated at the Department of Botany, University of Ibadan where a specimen voucher was deposited. The leaves were air-dried, finely powdered and further processed at the H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan. The finely powdered leaves, weighing 1.4Kg were extracted three times consecutively with ethyl acetate and 80% ethanol. The extracted solutions were concentrated in vacuo (Buchi Rotavapor R-200, Tokyo Rikakikai Co. Ltd.) to obtain crude extracts. Thin layer chromatography (TLC), column chromatography, and high-performance liquid chromatography (HPLC) were used to fractionate the bioactive extracts and to isolate the active compounds. Spectroscopic analyses (1H-NMR, 13CNMR, LC-MS, EI-MS, IR, and UV) were employed to determine their chemical structures. All work were carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 2000; Trease and Evans, 2002; Reuben, et al., 2012).

Animal Treatments

Thirty albino rats of Wistar strain, weighing between 75-100g were procured from the Central Animal House, University of Ibadan, Nigeria. They were housed at room temperature with a 12-hour light and dark cycle, acclimatized for a week, allowed free access to clean drinking water and fed on standard feed throughout the period of study. They were grouped into six different groups according to their weight, with five animals in each group. The first group served as negative control and were fed on standard feed with distilled water throughout the study, while the animals in the other five groups were given Cadmium Chloride (CdCl2) orally for four weeks at 1mg/kgbw/day to induce hypertension (Puri, 1999). Using the second group as a 'positive control' the animals in the last four groups

were placed on treatments of standard antihypertensive drug (lisinopril), the nutraceuticals (BSS, BSSG and BSS:BSSG (1:1) mixture), respectively at 2.3mg/kg/day.

Histopathological Studies

Small pieces of heart and kidney tissues were fixed in 10% formalin solution, followed by embedding in melted paraffin wax. Histopathological assessment and photomicrography of the prepared slides was done by using an Olympus light Microscope with attached Kodak digital camera.

Biochemical Analysis

Indices used in hypertension diagnosis were selected as recommended in Harrison's Principles of Internal Medicine.(Loscalzo et al., 2008). Serum urea, creatinine and electrolytes were assayed as kidney and endocrine function tests while lipid profile was assessed as a metabolic system function test. In addition, serum Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotranferase (AST) served as liver function tests. Estimation of serum calcium was done using colorimetry. Serum sodium and potassium ions were estimated by Flame Emission Spectrophotometry using SEAC FP 20. The method of 'back titration' described by Meites and Faulkner, (1962) was used for the determination of serum bicarbonate levels. Estimation of Serum Chloride was assessed by the method described by Mather et al., (1982). The reaction mixture in the tubes were mixed and incubated for 5mins in the dark and absorbance of sample and standard were measured at 590nm against the reagent blank. Estimation of serum triglycerides, Total Cholesterol, HDL-Cholesterol and LDLCholesterol levels were done by standard kit methods based on CHOD-PAP colorimetric method, and the analyses carried out following the standard protocols. Serum creatinine levels were determined according to the standard kit method of Bartels et al, (1972). Serum urea levels, AST, ALT and ALP enzyme activities were measured using standard kit methods.

Statistical analysis

Data collected were expressed as mean ± SEM and subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1990). Least significant differences (LSD) were used as a test of significance within treatments. A p-value of < 0.05 was considered statistically significant in statistical comparison.

3 Results

Serum Electrolyte, Urea and Creatinine Levels

Table 1 shows that there was a significant ($p<0.05$) increase in sodium levels in rats with untreated hypertensive conditions (Group 2) in comparison to that of the control (normal rats), while there was no significant difference ($p>0.05$) on potassium, chloride, bicarbonate and calcium levels. Treatments with phytosterols have no significant effect ($p>0.05$) on the urea and creatinine levels, while the lisinopril treatment shows significant increase ($p<0.05$) in both urea and creatinine levels (Table 2).

Effects on Lipid Profile and Liver function

Figures 1 and 2 shows that induction of hypertension gave no significant difference ($p>0.05$) in serum lipids except for triglycerides which was significantly increased ($p<0.05$) relative to control. However, the phytosterol treatment shows modulation of lipid profile: the groups treated with lisinopril, BSS, BSSG and BSS:BSSG mixture show slight modulation of triglyceride levels while the total cholesterol levels were significantly reduced ($p>0.05$) in comparison to the hypertensive untreated group. LDL-Cholesterol levels were not obviously modulated but HDL-Cholesterol levels were increased in the groups treated with BSS and BSSG. Most importantly, the HDL-Cholesterol: LDLCholesterol ratio increased in the group treated with BSSG. Figure 3 shows an increase in AST, ALT and ALP

Table 1. Effects of BSS, BSSG, BSS:BSSG mixture and lisinopril on serum electrolytes of normal and hypertensive rats.

	SODIUM*	POTASSIUM*	CHLORIDE*	BICARBONATE*	CALCIUM*
Group 1	134.25±3.02	3.90±0.32	99.25±2.01	26.50±1.26	0.90±0.19
Group 2	149.00±2.41[a]	3.98±0.43	101.50±2.40	25.00±1.58	0.94±0.15
Group 3	161.25±2.48[f]	4.33±0.39	104.25±1.44	22.25±1.25	0.90±0.16
Group 4	137.25±3.98	4.40±0.34	102.25±3.52	22.75±2.18	1.05±0.13
Group 5	138.50±3.38	4.45±0.45	104.50±2.50	25.50±1.50	0.57±0.05
Group 6	132.33±2.33[e]	4.22±0.48	98.67±4.49	22.33±2.33	0.98±0.25

*Values expressed in mmol/l are means ± SEM, n = 5
a = statistical significance for comparison between the group 1 and 2 ($p < 0.05$).
f = statistical significance for comparison between the group 1 and 3 ($p < 0.05$).
e = statistical significance for comparison between the group 2 and 6 ($p < 0.05$)..

Table 2. Effects of BSS, BSSG, BSS:BSSG mixture and lisinopril on serum urea and creatinine levels of normal and hypertensive rats.

	Urea (mmol/l)	Creatinine(µmol/l)
Group 1	134.25±3.02	3.90±0.32
Group 2	149.00±2.41[a]	3.98±0.43
Group 3	161.25±2.48[f]	4.33±0.39
Group 4	137.25±3.98	4.40±0.34
Group 5	138.50±3.38	4.45±0.45
Group 6	132.33±2.33[e]	4.22±0.48

*Values are expressed as means ± SEM, n = 5
b = statistical significance for comparison between the group 2 and 3 within treatments ($p < 0.05$).
h = statistical significance for comparison between the group 1 and3 within treatments ($p < 0.05$).

activities under hypertensive conditions. Treatments with the phytosterols and lisinopril shows reduction in the levels of these liver function markers, particularly, the significant reduction ($p<0.05$) in serum ALT in group 6 (treated with BSS:BSSG mixture).

Effects on Kidney and Heart Tissues

Figures 4 to 15 show the effects of treatments on the kidney and heart tissues. While there are some foci of tubular necrosis and interstitial cellular infiltration by mononuclear cells (mild) in the kidney section of the normal rats (figure 4), there was an extensive area of tubular necrosis at the cortex in the kidney section of group 2 (treated with cadmium chloride). There appear to be no visible lesions seen in the kidney tissue sections of rats in groups 4, 5 and 6 (treated with BSS, BSSG and BSS: BSSG mixture, respectively).

The heart tissue section shows foci of myofibre necrosis with cellular infiltration by mononuclear cells in the hypertensive group. There appear to be no visible lesions seen in the heart tissue sections of groups of rats treated with BSS, BSSG and BSS:BSSG mixture.

4 Discussion

Balance of electrolytes is essential for normal function of cells and organs. Electrolyte tests are commonly used to monitor treatment of certain health problems, including high blood pressure (hypertension), heart failure, liver and kidney disease. Common electrolytes that are measured with blood testing include sodium, potassium, chloride, and bicarbonate. The observed significant increase ($p<0.05$) in sodium levels in the untreated hypertensive rats is expected, and could have resulted from retention and volume overload, culminating in the expression of hypertensive phenotype

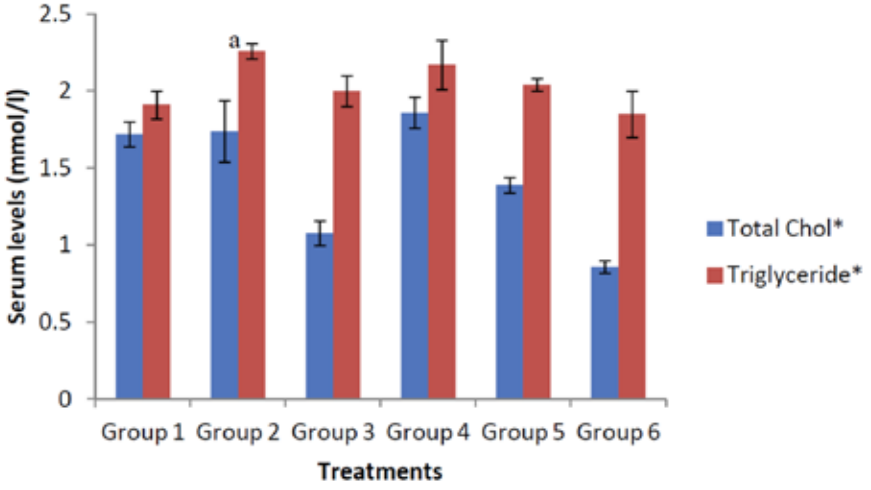

Fig.1. Effects of BSS, BSSG, BSS:BSSG mixture and lisinopril on serum levels of Total-Cholesterol and Triglyceride of normal and hypertensive rats
a = statistical significance for comparison between the group 1 and 2 ($p < 0.05$).

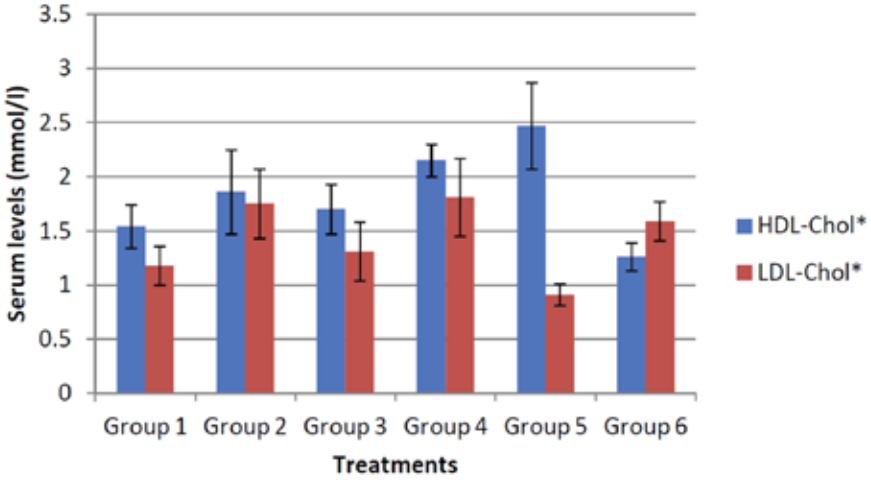

Fig.2. Effects of BSS, BSSG, BSS:BSSG mixture and lisinopril on serum levels of HDL-Cholesterol and LDL-Cholesterol of normal and hypertensive rats
HDL-Chol = High Density Lipoprotein Cholesterol
LDL-Chol = Low Density Lipoprotein Cholesterol

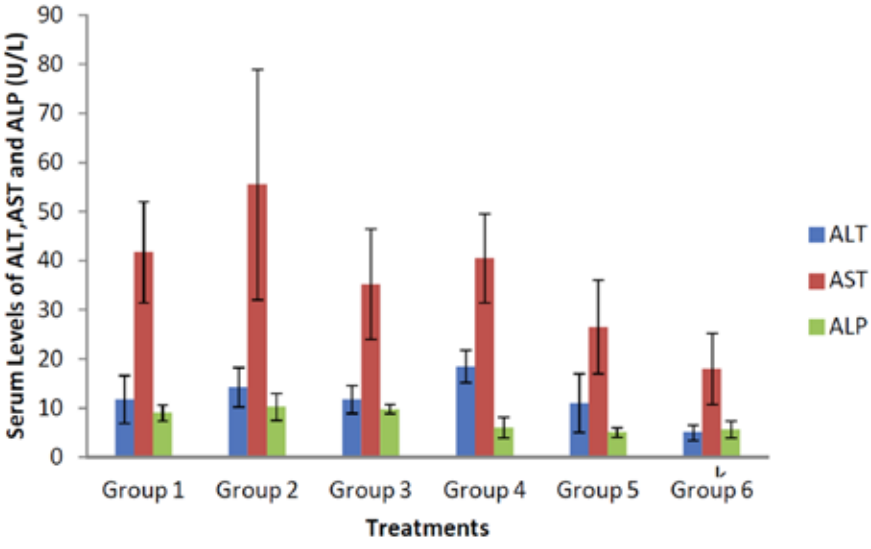

Fig.3. Effects of BSS, BSSG, BSS:BSSG mixture and lisinopril on ALT, AST and ALP levels in the normal and treatment groups

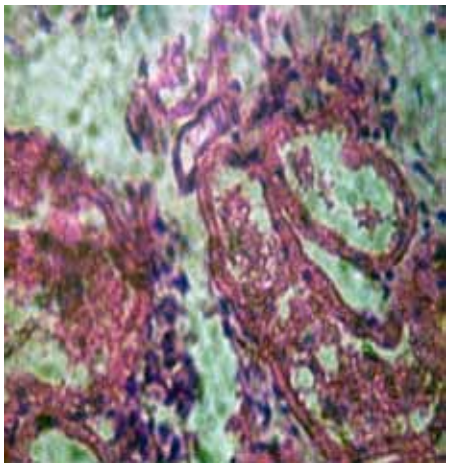
Fig.4. Kidney- normal rats

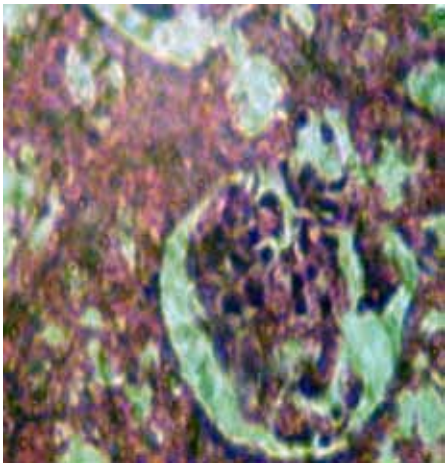
Fig.8. Kidney- BSS treated rats

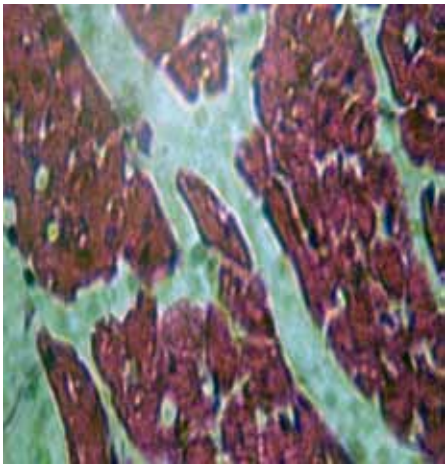
Fig.12. Heart- lisinopril treated rats

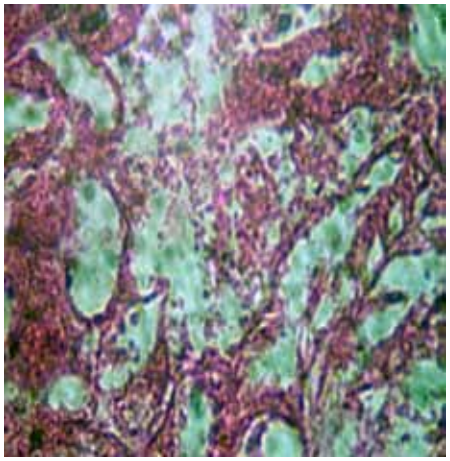
Fig.5. Kidney- hypertensive rats

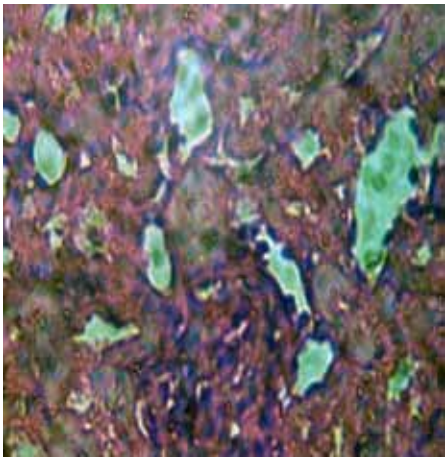
Fig.9. Kidney – mix*

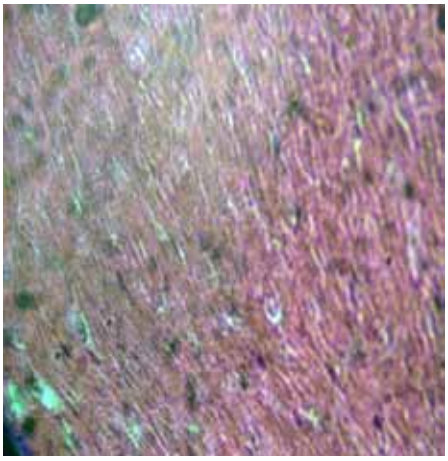
Fig.13. Heart – BSSG treated rats

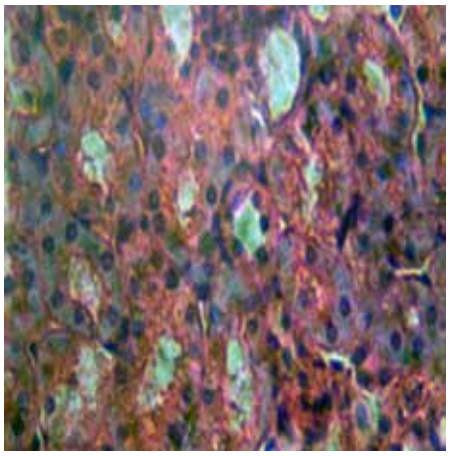
Fig.6. Kidney – lisinopril treated rats

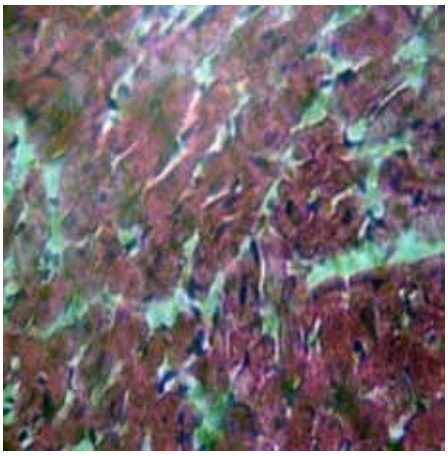
Fig.10. Heart – normal rats

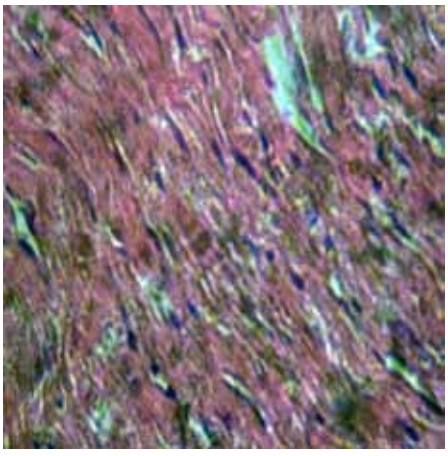
Fig.14. Heart – BSS treated rats

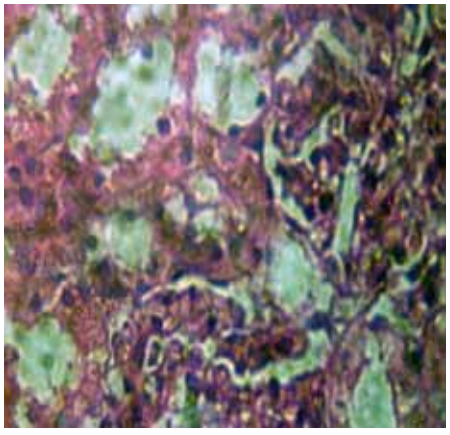
Fig.7. Kidney – BSSG treated rats

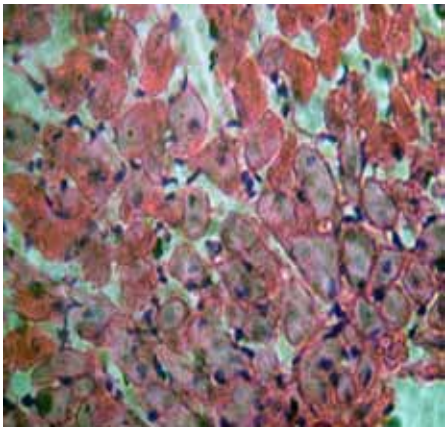
Fig.11. Heart – hypertensive rats

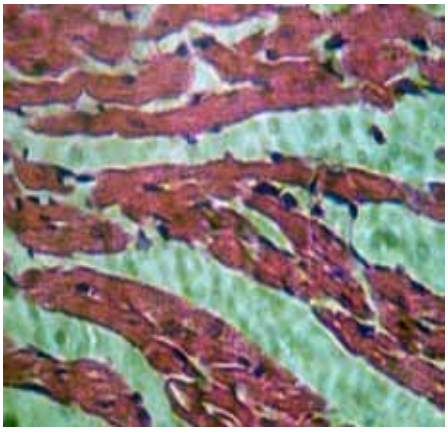
Fig.15. Heart – mix*

*BSS:BSSG mixture treated rats.

(Blaustein et. al., 2006). Other electrolytes such as chloride, potassium and calcium also tend to show slight modulations in hypertensive conditions; however, no significant change ($p>0.05$) in their levels was observed in this study.

Significant independent relationships have been found to exist between blood pressure and several serum cations (Agada and Braide, 2009). The mechanism of hypertension induction might occur through the tubular injury pathway and the current study showed that the tissue section of the kidneys in the hypertensive group (Figure 5) exhibits an extensive area of tubular necrosis at the cortex and one total whole kidney damage; however, it is not clear why the urea and creatinine levels were not significantly affected.

There was reduction of sodium in the groups treated with BSS and BSSG when compared with hypertensive rats (Table 1). The BSS:BSSG mixture significantly reduced ($p<0.05$) the sodium level when compared with the hypertensive group and also with the normal rats. These results suggest that BSS and BSSG may have the potential to correct a condition of sodium retention and volume overload which may be associated with salt induced hypertension. The repair and toning effects of these compounds on tissue damage may contribute to this biological action as shown by appearance of no observable lesions in all the kidney sections from treatment groups in comparison with the extensive necrosis observed in the hypertensive group (Figures 4 to 9). The significant reduction shown by the BSS:BSSG mixture may predict an additive interactions in their biological activities (Chobanian, 2009; Xiaobo et. al., 2009). It is noteworthy that the lisinopril treatment gave a significant increase ($p<0.05$) in sodium levels (Table 1). Thus the beneficial role played by the phytosterols in this study on electrolyte levels versus the toxic role played by the standard drug at 2.3mg/kg/day suggests that BSS and BSSG may be more efficacious and less toxic than the standard drug.

There appears to be slight effects of BSS and BSSG on urea and creatinine. They tend to maintain the basal levels of these parameters while lisinopril treatment led to significant increase in both urea and creatinine levels (Table 2), suggesting that lisinopril tends to be nephrotoxic. It is therefore obvious that BSS and BSSG could maintain basal kidney function. Moreover, the observation from the hispathological studies of the kidney section with no visible lesion gave credence to this fact.

A build-up of cholesterol in blood vessel walls leads to atherosclerosis, which increases with age (Shanmuganayagam et. al, 2007). High total cholesterol, triglyceride and LDL-Cholesterol accelerate atherosclerosis while low levels of HDLCholesterols are correlated with risk of CVDs (NCEP, 2002). Atherosclerosis and increased blood pressure have synergistic effects in causing CVDs. Atherosclerosis also reduces the diameter of blood vessels around the body. A lipid profile consists of total cholesterol, LDL, HDL and triglycerides. Each component of the lipid profile is usually evaluated when checking cholesterol. The significant increase ($p<0.05$) in triglyceride levels observed in the hypertensive group is consistent with the reports of Bonaa and Thelle (1991) that established a positive association between triglyceride levels and blood pressure. The rise in the triglyceride level may contribute to the expression of hypertension phenotype. There are slight modulations in the other lipid profile parameters, however total-cholesterol was significantly increased ($p<0.05$) in the hypertensive group.

The group treated with BSS:BSSG mixture significantly decreased ($p<0.05$) triglyceride levels when compared to the untreated hypertensive rats in group 2 (Figure 1). These findings are consistent with those of Gupta et al., 2011 who observed that phytosterols significantly reduced triglyceride levels in the hypertensive state. Lowering serum LDLCholesterol and increasing high-density lipoprotein cholesterol (HDL-C) has been shown to lead to a regression in atherosclerotic lesion progression (Aviram et al., 2000). The significant increase ($p<0.05$) in HDL-Cholesterol observed in the BSSG - treated group (Figure 2) together with the observed activity of BSS suggests that these phytosterols could reduce risk of cardiovascular disease in hypertensive conditions. The cholesterol reducing potential of phytosterols had long been known and used in combination with medicine such as statins or fibrates. The combined effects of statins, which inhibit cholesterol synthesis and phytosterols which act on intestinal absorption of cholesterol has been studied in patients suffering from moderate hypercholesterolaemia, showing 44 to 45% decrease in cholesterol (Nature life, 2005). Also combination with fibrates resulted in a supplementary reduction of total cholesterol and LDL-cholesterol by 8.5 and 11.1% respectively with no side effects of the treatment observed (Nature life, 2005).

The desirable observations on the ALP, AST and ALT levels showed that the phytosterol treatments had ameliorating effects on liver damage that may be associated with hypertensive conditions. While tissue sections from the hypertensive untreated group shows extensive tubular necrosis in the kidney, those from the heart show little or no damage, thus confirming that the kidney rather than the heart, is the direct target for cadmium toxicity as reported by Satarug et al. (2006) who enumerated the multiplicity of cadmium targets and toxicities with kidneys and livers as specific targets through accumulation, and renal hepatic necrosis as outcomes. The heart and kidney sections from

treatment groups had no visible lesion. This suggests that tissue repair occurred upon treatment with the phytosterols. Thus, the BSS, BSSG and BSS:BSSG mixture are capable of ameliorating tissue damage such as endothelial injury, left ventricular injury, and renal injury that may occur in association with hypertension.

The results in the present study indicate that BSS and BSSG have some biological activities comparable to that of the standard drug, lisinopril at 2.3mg/kg/day in hypertensive conditions. The phytosterols portrayed little or no associated toxic effects in comparison with lisinopril which could be nephrotoxic at high doses. However, further studies are needed to fully understand the impact of the chemical structure of these compounds on their biological activities, especially their interactions, in mediating key biochemical processes in hypertensive conditions. This is relevant in the therapeutic and nutraceutical applications of these plant natural products.

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Can Ultrasound Be an Effective Imaging Tool in Life Threatening Complications of Pregnancy?

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ABSTRACT
Acute life threatening complications can arise during pregnancy and early postpartum period and result in maternal and fetal morbidity and mortality. Common clinical signs are vaginal bleeding and pelvic or abdominal pain. The aim of this work was to improve the role of Ultrasound in identifying the causes, differential diagnosis and management of major acute life threatening complications of pregnancy Sixty eight pregnant women complaining of vaginal bleeding with pelvic or abdominal pain were followed up in the US out patients, US emergency unit at 6th October hospital over a period of one year. Ten cases were in the postpartum period and the remaining cases were at different ages of gestation. They were examined by trans abdominal, using 3.5 MHz sector probe or transvaginal technique according to the suspected clinical examination. Our results could depict, according to US findings, five groups of pregnant complications: ectopic pregnancy, placenta previa, placenta morbid adhesion, placenta abruption and retained product of conception. ***Conclusion*** It was proved that US is an important and helpful tool in investigation and diagnosis of threatening life complications of pregnancy, but should not override the clinical obstetric judgment.

Key Words
US : Ultrasound, MHz : Megahertiz.

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1 Introduction

Vaginal bleeding associated pelvic or abdominal pains are the most common complains of pregnant women presenting to the emergency department. In addition to clinical history, physical examination and laboratory data, ultrasound imaging is very essential for evaluating these patients. Ultrasound technique in the last few decades has provided ultrasound machines of high quality and resolution with the option of compact and mobile unit. Grey scale and Doppler facilities have made ultrasound a useful tool for obstetrical assessment and diagnosis of life threatening complications in pregnancy. Ectopic pregnancy, morbid adhesion of placenta, placental abruption, placenta previa and retained product of conception are some of the most common emergency conditions during pregnancy and the immediate postpartum period (Kadasine A.R and MerghaniH.M, 2011).

Shaw et al. (2010) stated that ultrasound examination is an important and helpful tool in investigation and diagnosis of threatening complications of pregnancy, but should not override the clinical obstetric judgment.

Tikkanen M. (2011) stated that, the presenting complains in these cases are usually vaginal bleeding with pelvic and abdominal pain and he added that ultrasound proved to be an important tool in diagnosis and management of emergency conditions of pregnancy and early post partum period.

2 Subjects and Methods

Sixty eight pregnant women presenting with clinical pictures of threatening life complications of pregnancy (severe vaginal bleeding, pelvic and abdominal pain and shock) were collecting over period of one year. Their diagnosis and differential diagnosis were of essential need for rapid management and saving life of both mother and fetus. They were examined by high resolution US machine using 3.5 MHz sector probe for transabdomenal scanning or transvaginal probe for endovaginal scan, according to the suspected diagnosis. The study was carried at outpatient US emergency Unit of 6 October University Hospital. Our work could depict 5 groups of life threatening pregnant emergencies.

- Placenta previa was detected in 10 cases.
- Morbid adhesion of the placenta was detected in 2 cases.
- Placenta abruption was detected in 3 cases.
- Retained products of conception were in 50 cases.
- Ectopic pregnancy was detected in 3 cases.

Technique of placental scanning:

As a general rule, the highest frequency transducer which provides adequate penetration was used. In transabdomenal imaging, we used 3.5 - 5MHz. It was essential for ensuring optimum image quality. We applied

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coupling Gel to the patients skin to ensure an air free contact between the patient and the transducer face. Types of transducers used were sector, curvilinear or straight linear probe according to the case examined. Most scans were performed with the woman supine. Trendlenberg position scanning was needed to detect the inferior placental margin. Optimal bladder distention was requested in those of less than 1 week gestation or in women whom a low lying placenta was suspected. Scanning started from the umbilicus to the symphysis pubis longitudinally in the midline and then more laterally on both sides. Transverse scanning was done only over the lower pelvis.

Transvaginal scanning was done for proper visualization of the placenta using 5 MHz edovaginal probe with the patients in a supine lithotomy position. Bladder should be empty, the probe should be disinfected before use and be covered by a latex condom and lubricated by GK Gell.

The umbilical artery was sampled at the middle of the umbilical cord. This was achieved by placing the probe in the middle of the abdomen just below the umbilicus. Change the gain setting was done till the wave form fills about two thirds of the screen. Doppler scanning of the umbilical vein was also assessted.

Utero- Placental vascular wave forms was also assessted through transabdominal imaging.

3 Results

Sonographic and color duplex findings for the sixty eight women included in this study revealed five groups of life threatening pregnant emergencies. Each of them showed characteristic ultrasound findings. Placenta previa were diagnosed by US in 10 cases. Transabdominal ultrasonography done allowed adequate visualization of the area of the internal os. Longitudinal and transverse scans were performed to determine the relation between the lower borders of the placenta to the internal os. Transabdominal US with half filled urinary bladder could properly depict the low position of placenta. Endovaginal ultrasonography was more accurate in detecting and specified the types of placenta previa. It was in five cases 2cm or less, in three cases the placenta was covering completely the internal os. In the remaining two cases, the placenta incompletely covered the internal os. The first group was known as low-lying placenta, the second group as complete placenta previa or central complete placenta previa, and the third group was eccentric placenta..

Morbid adhesion of the placenta was detected in two cases. In this condition, there was an abnormal penetration of the chorionic villi into uterine wall. US showed thinning of the retroplacental hypoechoic zone, presence of multiple placental lacunae and elevation of tissue beyond the uterine outer layer. Doppler sonography revealed turbulent blood flow at the uteroplacental interface. Both cases gave history of multiple CS Delivery.

Placenta abruption: Only three cases were seen along the period of the study denoting premature separation of the placenta implanted in the upper segment of the uterus. External trauma was the preexisting factor in those cases. US showed sonolucent areas between the placenta and uterus and thickened placenta.

Retained Product of conception was detected in 50 cases. All of them were presented by severe vaginal bleeding. Ten cases had, in addition, signs of infection. Two cases were in shock.

The products were resulting from spontaneous pregnant loss in 35 cases

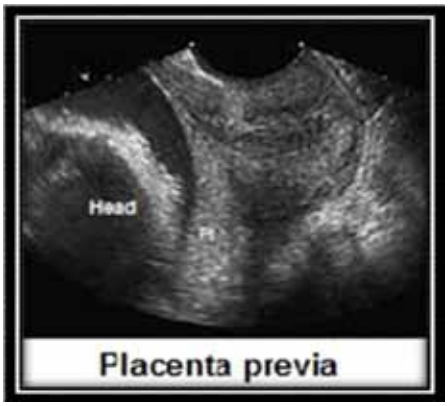

Fig.1. Placenta previa.

Fig.2. Morbid adhesion of placenta.

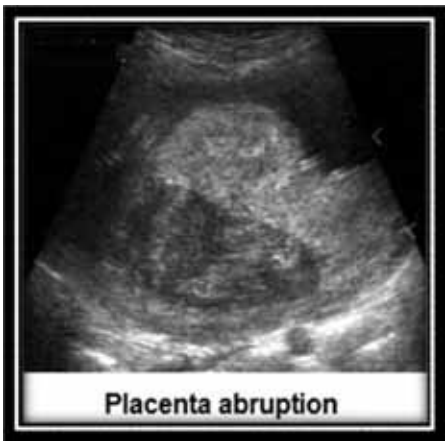

Fig.3. Placenta abruption.

Fig.4. Retained products of conception.

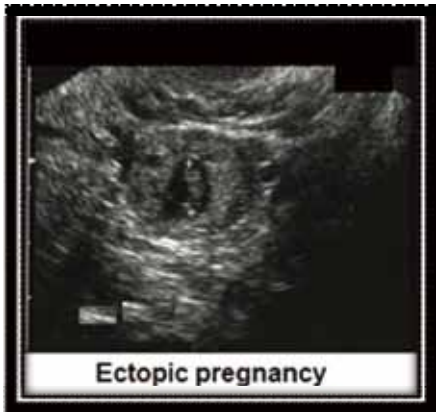

Fig.5. Ectopic pregnancy.

and planning pregnant termination in 5 cases suffering from other medical disorders. The remaining ten cases were in preterm and term delivery state US findings revealed heterogenous hyper echogenic material filler the utrine cavity with thickening of the endometrium.

4 Discussion

Marrian G.et al., (2011) stated that placenta previa is a condition, in which the placental tissue implanted, abnormally in the lower uterus. It is of two types: low lying placenta or marginal placenta previa and major placenta previa or central type. Cesarean section and uterus curettage are most of the predisposing factors. The possibility of one of these diagnosis complicating placenta previa must be excluded.

Yeo and Ananth (2008) stated that the neonatal mortality and massive postpartum hemorrhage and maternal near miss cases are of high possibility with placenta previa. The sensitivity of ultrasound examination in detecting placenta previa is more than 95%. Transvaginal or perineal evaluation is adequate with 1`% false +ve and 2% false –ve rate. Digital vaginal examination is contraindicated.

Masselli G.et al. (2008) stated that transabdominal US with mildly filled urinary bladder is the best approach to confirm placenta previa implanted in the anterior wall.

Marrian G.et al.(2011) stated that any of the following US critera exclude placenta previa:

Direct apposition of the presenting part of the fetus and cervix without space for interposed tissue, the present of amniotic fluid between the presenting part of the fetus and the cervix. And a placental distance of greater than 2cm between the inferior aspect of the placenta and the internal os. He also recorded that sonography is more than 95% accurate in diagnosis of placenta previa while transvaginal evaluation of placenta previa has 1% false +ve and 2% false _ve rate and concluded that transabdomenal US is the best test of choice to confirm placenta previa. He also stated that, care should be taken not to mistake a more serious situation such as placenta abruption or placenta accerta for placenta previa , because the management of these conditions is different. The possibility of one of these diagnoses, complicating placenta previa must be excluded.

Our findings are exactly agree with the previous results and we add that the transabdomina US was quite enough to detect placenta previa with high accurancy using 3.5 MHz probe.

Yeo and Ananth (2008) stated that morbid adhesion of the placenta occurs when placenta attached to the superfacial layer of myometrium instead of endometrium. He further categorized it into two subtypes , placenta increta when the chorionic villi penetrate the myomertium and placenta percreta when the placenta penetrates the whole utrine wall reaching peritoneal layer. In pregnancy complicated by morbid attached placenta, the placenta does not easily separated from the utrine wall during the 3rd stage of delivery causing severe postpartum hemorrhage which needs blood transfusion. Arterial embolization or an emergency hysterectomy may be highly required.

Cristine H.et al., (2003) stated that the main ultrasound features are thinning of the retroplacental hypoechoic zone with the presence of multiple placental lakes. Sebire. and Sepulveda in 2008 stated that the incidence of placenta accrete increases due to repeated C.S. delivery. Women with placenta previa have a substantially increase risk of placenta accreta. MRI and US are best imaging modalities used to confirm the diagnosis.

Kadasine and Merghani (2011) Stated that ultrasound findings of morbid adhesion of the placenta are thinning of the retroplacental hypoechoic zone, present of multiple placental lakes, thinning of the uterine serosa-bladder wall complex, elevation of the tissue beyond the uterine serosa , turbulent or complicated blood flow at placental interface and irregular blood flow underlying the maternal urinary bladder.

Alsereh et al. (2007) stated that transvaginal and transabdominal ultrasonography are complementary diagnostic technique and should be used as needed. Abnormal placental attachment site is characterized by a hypoecoic boundary between placenta and the bladder. The ultrasound findings suggest of placental morbid adhesion include irregularly shaped placenta lacunae (vascular spaces) within the placenta, thinning of myometrium overlying the placenta , loss of retroplacenta clear space , protrusion of the placenta to the urinay bladder , increased vascularity of the utero-serosa interface and turbid blood flow through the lacunae on Doppler ultrasound.

GREY SCALE ultrasound is sufficient to diagnose placenta with sensitivity of 77% -89% and specivity of 96% -98% .The use of Doppler US or 3D imaging does not significantly improve the diagnostic sensitivity compared with that achieved by grey scasle US alone.

ULTRASOUND findings include obliteration of normal anaechoic space behind the placenta , and abnormal placental vasularity by color Doppler show hypervacularity of dilated lacunar spaces.

We only depict in our result two cases of morbid adhesion of placenta. The main US findings were thinning of retro placental hypoechoic zone with multiple placental lacuene. In both cases turbid blood flow was detected by Doppler US at utero placental interface.

Cristine H. et al., (2003) stated that antenatal US is the technique of choice to establish the diagnose and guide to clinical management. Signs of accretion may be seen as early as in the first trimester .All had low lying gestation sacs which are clearly attached to the uterine scar. The myomerium was thin in the area of the scar to which the sac is attached.

Placenta abruption is a condition in which the placenta, implanted in the upper segment, peels from the utrine wall , partially or almost completely before birth , in mild cases cause few problem ,

and in severe cases can deprive the baby of oxygen and nutrients .It also causes bleeding which may be dangerous to both mother and the fetus . It cause 10% of premature birth of fetus of high risk of health problems (Eller et al. 2009).

External trauma, eclampsia, hypertension, vascular diseases are among the most common risk factors. Ultrasound findings in these cases may be one or more of these findings: preplacental collection (under chorionic plate) . jell - like movement of chorionic plate with fetal movement , retroplacental collection (between placenta and myometrium), marginal collection (at placental margen) , increase placental thickness or echogenicity more than 4 - 5 cm throughout pregnancy and intra amniotic hematoma (Yeo and Ananth ,2008).

On the other hand Tikkanen in 2011 stated that the sensitivity of an ultrasound examination in detecting placenta abruption is as low as 25%, therefore physicians should rely on the clinical presentation for diagnosis as vaginal bleeding, abdominal pain, tachycardia, drop in blood pressure hard abdomen and signs of fetal distress and so requesting US examination in such patients is of little significance and might waste valuable time.

Eller et al. (2009) stated that the size and location of abruption identified by US may be important prognostically. Retroplacental collections have a worse prognosis for fetal survival than subchorionic collection. In addition a large retroplacental hemorrhage has been associated with 50% fetal mortality.

Yeo and Ananth (2008) stated that the US features of placenta abruption are preplacental collection under the chorionic plate, jell- like movement of chorionic plate with fetal movement. retroplacental collection , collection at placental margin and lastely placental thickness or echogenicity more than 5cm perpendicular to the plane of placenta throughout pregnancy. Eller et al. (2009) described placenta abruption as partially or completely peeling of the placenta from the uterine wall before birth, severe cases can deprive the baby of O2 and nutrients and it also cause bleeding which may be dangerous to both mother and fetus, it can appeared as an ill defined echogenic collection either hyperechoic or isoechoic with respect to the placenta seen.

Our results of the 3 cases of placental abruption were presenting at immediate post partum period by severe vaginal hemorrhage, US findings revealed retroplacental collection with thickened placenta. We agree with the opinion of Soisson in that the size and location of abruption identified by US has very important prognostic markers where our two cases showed worse prognosis for fetal survive.

Retained Product of Conception refers to placenta and / or fetal tissues that remains in the uterus after spontaneous pregnant loss (miscarage), planned pregnant termination, or preterm / term delivery .Women presenting with vaginal bleeding and / or signs of infection. Transvaginal sonography and clinical data are complematory for more accurate diagnosis. Karimpour M. et al. (2010).

Shaw J.I et al, (2012) Stated that between several diagnostic findings, only endometrial thickening significantly correlate with the presence of remain product of conception where endometrial thickness greater than 12mm has the best value for confirming presence of retained product of conception.

Tikkanen (2011) stated that ectopic pregnancy is still remains to be a common cause of maternal morbility and mortality, the major causes of death are excessive hemorrhage, shock, and renal failure, therefore ectopic pregnancy must be excluded in every women in childbearing age. Ultrasound finding shows a normal intrauterine gestation sac is eccentrically placed in the endometrial cavity and surrounding by a double dicidual layer, the presence of the yolk sac confirm the diagnosis.

Ectopic pregnancies are classified into tubal pregnant (flobian tube) , non tubal (in adenxia or ovary), heterolopic two fertilized eggs ,one outside uterus and other inside uterus)and persisted ectopic continuation of trophplastic growth after a surgical intervention to remove an ectopic pregnancy, as concluded by Yeo and Ananth (2008).

Conclusion: It was proved that US was an important and helpful tool in investigation and diagnosis of threatening life complications of pregnancy, but should not override the clinical obstetric judgment.

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Antioxidant and Trace Metals Levels in Anemia

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ABSTRACT

Anemia is a disorder characterized by reduction in hemoglobin concentration of blood below the normal level. It is a global problem, mainly affecting poor people in developing countries. For the determination of these metals fifty intravenous blood samples anemic patients and normal subjects were collected and immediately centrifuged to obtain the supernatant liquid, serum of both the groups for analysis. The mean serum levels of magnesium, zinc, copper, and iron and antioxidant activity in anemic patients were 16.05±1.14ppm, 4.33±0.28 ppm, 2.5±0.2ppm, 1.50±0.28ppm and 1.27±0.05ppm respectively. Serum magnesium, zinc, iron and antioxidant levels were significantly lower whereas, the higher level of Copper was observed in anemic patients as compared to the controls. Supplementation with deficient trace elements may reduce the severity of symptoms and complications associated with anemia.

Key Words

Anemia, Iron, Zinc, Copper, Magnesium, Antioxidant activity.

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1 Introduction

Anemia is one of the world's most prevalent health problems, especially among adult and pregnant females approximately 35% to 75% (56% on average) are anemic in developing countries (Hall A and Miguel E (2001). "The World Health Organization has identified that iron and folic acid deficiency is a severe problem in Pakistan and it has devastating impacts on health and survival. It has been estimated that iron deficiency leads to 22,000 maternal deaths for the last 10 years (ADB 2006).Whereas there are various other nutritional and communicable causes of anemia, iron (Fe) deficiency is often a causative aspect in many of these cases (Lanzkowsky P 1995). Fe deficiency anemia leads to weakness (Haas JD, 2001) , poor physical growth (Grantham-McGregor S, 2001), and delayed psychomotor development (Sherriff A, 2001). A recent study on the prevalence and etiology of nutritional anemia in urban areas of Hyderabad, Pakistan indicated a high prevalence of anemia and Fe deficiency in 60% of adults. Zinc (Zn) deficiency in humans has been found in infants, schoolchildren and adults (Castillo-Duran C, 1994). Zinc deficiency associates with Fe deficiency anemia in areas where Fe deficiency is a problem (Singh S, 2003). It was investigated that anemia is one of the major manifestations of copper (Cu) deficiency both in animals and human beings (Percival SS, 1995). It is essential to maintain Fe homeostasis in the human body; its deficiency leads to anemia and neutropenia (Tammura H, 1994). Cuassisted enzymes are necessary for the utilization of Fe to make hemoglobin, a main component of red blood cells (Tammura H, 1994). Recent attention has been directed to the element chromium (Cr); it can improve insulin sensitivity and therefore may be involved in carbohydrate and lipid metabolism Lukaski HC,1999). The determination of trace quantities of metals in biological samples requires the use of sensitive and selective techniques such as atomic absorption spectrometry.

In this study, we evaluate whether Fe, Cu, Mg and Zn deficiency is related to increased level of toxic metals in blood samples of anemic and controls of both gender with age range 16– 50 years. The samples were prepared method. Fe, Cu, Mg and Zn concentrations in samples under study were evaluate by atomic absorption spectrometer, while antioxidant activity was analyzed by spectrophotometer.

In this study, we aimed to evaluate the levels of Cu, Mg, Zn, and Fe and antioxidant activity in serum of anemic patients.

2 Materials and Methods

This investigation has been

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conducted among three medical units of Liaquat University of Medical &Health Sciences (LUMHS) Jamshoro. The group of patients were been selected within the age range of 16-50years of both gender. The metals copper, iron, magnesium, and zinc in the blood serum were determined by Atomic Absorption Spectrometry (AAS) (Model, A-20 Varian). Whereas anti oxidative activity was measured as a ratio of Fe (II)/Fe (III present in the blood serum. Iron (II) was determined spectrophotometrically as Fe (II)-TPTZ (2, 4, 6-tris (2-pyridyl)-s-triazine) colored complex formed at pH 4-5 and measured at 595nm wavelength. Whereas, for the determination of total iron, iron (III) was first reduced to Fe++ using vitamin C as reducing agent and then complexed with TPTZ .The complex so produces is directly proportional to the concentration of Fe (II) produced and Fe (II) already present i.e. total iron in the blood sample in presence of antioxidant. The difference of the total iron and the iron (II) present in the serum is the measure of iron (III).

Trace metals were determined using airacetylene flame. The standards from 1 to 5 ppm for each of the metal separately were run on the spectrometer and the calibration curves were obtained prior to run the samples for the determination of metals in the blood serum of normal subjects and the malarial patients. Blood samples were collected from 50 healthy controls in fasting conditions and a similar condition was maintained while taking blood samples of anemic patients. Each blood sample was centrifuged at 5000 rpm for 20 minutes. The supernatant blood serum was used for the analysis of metals copper, iron, magnesium, and zinc using Atomic Absorption Spectrometer inserting appropriate hollow cathode lamp in it. All standards used were of analytical grade.

Chemicals and reagents

Sulphosalicylic acid was obtained from Merck,Damstadt, Germany and other chemicals to prepare standards were purchased from Sigma Chemical Co. All chemicals were of analytical grade.

Stock Solutions and working Metal standards

Stock solution of 1000 ppm Cu, Fe, Mg, and Zn for each were prepared for corresponding sulphate salts of analytical grade (Sigma Chem.). Working standards were prepared from the stock solutions by diluting with appropriate volume of deionized water and addition of few drops of corresponding concentrated acid.

Statistical Analysis

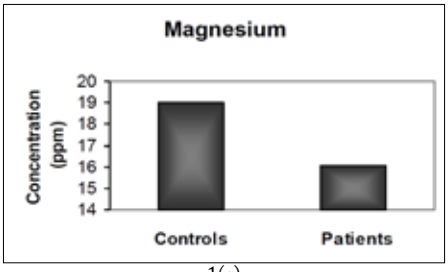
All statistical analyses were performed using computer program Excel X State (Microsoft Corp., Redmond, WA) Student's t test was used to assess the significance. Results were expressed as mean ± SD.

3 Results

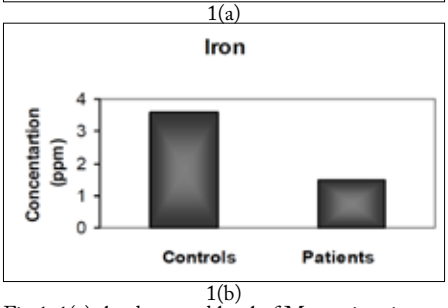
Results and Discussion

Table 1 shows the blood serum levels of trace metal and antioxidant levels in anemic patients with compare to control subjects. The results show significant increase in serum copper levels, where as serum iron, magnesium, zinc and antioxidant levels were decreased in anemic patients as compared to the controls.

The Table 1 show the levels of trace metals (Fe,Cu, Mg and Zn) and antioxidant activity were evaluated in blood samples of anemic patients as compared to controls. Fe deficiency is probably the most common nutritional disorder in the world. Presently estimate based on the WHO criteria specify that around 600–700 million people worldwide have a marked Fe deficiency anemia and about half the adults in developing countries are affected (Oski FA,1993). The data of the trace metals under study in blood samples of control and anemic patients are shown in Table 1 and Figure 1(a) to 3(a). The results are given as mean values with standard deviation (±SD)

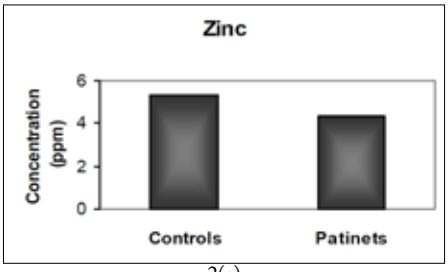


1(a)

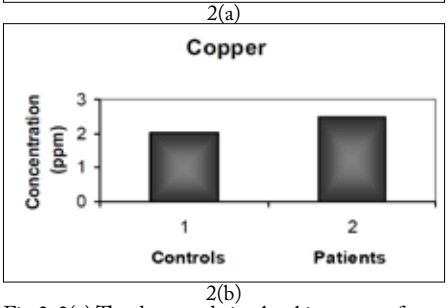


1(b)

Fig.1. 1(a) the decreased level of Magnesium in serum of anemic patients whereas figure 1(b) the decreased level of Iron in serum of anemic patients as compared to control subjects.

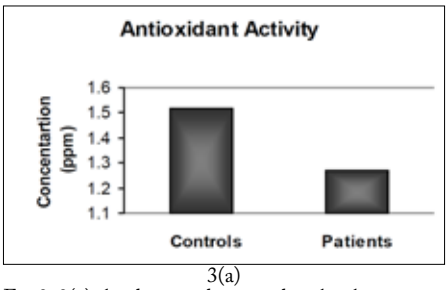


2(a)



2(b)

Fig.2. 2(a) The decreased zinc level in serum of anemic patients and in figure 2(b)the elevated level of copper in serum of anemic patients as compared to control subjects.



3(a)

Fig.3. 3(a) the decreased antioxidant level in serum of anemic patients as compared to control subjects.

Table 1. Trace metals and antioxidant levels in Healthy Controls and Anemic.

Variables	Controls	Anemic
Mg	19.01±0.68	16.05±1.14
Iron	3.60±0.23	1.50±0.28
Zinc	5.34±0.39	4.33±0.28
Copper	2.01±0.11	2.5±0.2
Anti Oxidant Activity	1.52±0.05	1.27±0.05

for each metal and antioxidant activity. The mean values of iron in the anemic patients were significantly lower as compared to healthy controls(Fig: 1(b). The results indicate the prevalence of Fe deficiency anemia in both genders. In the population of developing countries, the amount of Fe absorbed from the diet is not sufficient to meet many individuals' requirements. The consumption of a predominantly cereal-based diet, rich in phytate, oxalate, phosphate, fiber, and other inhibitors of Fe absorption, was the main cause of Fe deficiency diseases. Phytates strongly inhibit Fe absorption in a dose-dependent fashion, and even small amounts of phytates have a marked effect (Hallberg L, 1989). If the amount of absorbable Fe in the diet cannot be immediately improved, Fe supplement must be included in the diet to control Fe deficiency anemia (Lozoff B, 1991). The concentrations of Zn in anemic patients were found to be significantly lower as compared to healthy controls (Fig: 2(a). Zn deficiency may be a contributing factor in anemia (Sondstrom B,1990). Zn deficiency in developing countries is due to low consumption of meat and fish along with food rich in phytate. Food rich in phytate significantly reduce the absorption of Zn, increasing the chance of Zn deficiency. The higher level of Cu was observed in anemic patients as compared to the healthy controls (Fig: 2(b) Copper is required for normal infant development, red and white blood cell maturation, Fe transport, bone strength, cholesterol metabolism, myocardial contractility, glucose metabolism, brain development, and immune function (L'Abbe MR, 1992). A deficiency of either Fe or Cu will result in anemia, namely, Fe deficiency anemia or Cu deficiency anemia. Copper is essential for the functioning of many Cu-dependent enzymes (Larsson S, 1995) such as ceruloplasmin (responsible for antioxidant protection, Fe metabolism, and Cu transport), and it was established that the anemia appears to be related to defects in Fe mobilization due to the combined defect of both red ceruloplasmin ferroxidase activity and intracellular utilization (Tapiero H, 2003). Cu is a major component of catalytic centers of different redox enzymes, and thus, its presence is essential for normal physiologic function such as cellular respiration, free radical defense, synthesis of melanin pigment, connective tissue biosynthesis, and cellular Fe metabolism (Gacheru N, (1990). About 95% of the Cu in the blood is bound to ceruloplasmin. These enzymes play a role in the regulation of Fe metabolism.

The mean values of magnesium in the anemic patients were significantly lower as compared to healthy controls (Fig: 1(a). Low serum magnesium causes potassium, calcium and neuromuscular disturbances, central nervous system and cardiovascular alterations, like arrhythmias (Swaminathan R, 2003). Furthermore, it can alter glucose homeostasis, increase atherosclerosis, hypertension, myocardial infarction, osteoporosis, migraine, asthma, chronic fatigue syndrome, among others (Swaminathan R, 2003) and Barbagallo M, 2007). More research on the effects of magnesium deficiency on the health of people is needed to warrant interventions to prevent it. The mean values of Anti-oxidant activity in the anemic patients were significantly lower as compared to healthy controls (Fig: 3(a). Anti-oxidant activity which shows decreasing tendency in patients and this parameter could be used as a biomarker for obtains the status of these patients under medical therapy and its affectivity.

Conclusion

It suggests that use of magnesium as a supplementary diet and use antioxidant activity as the status of drug response to greater number of patients. The decreased levels of iron and zinc can be maintained by given supplement of these metals as therapy.

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