

Effect Of Supplemented Diet With Curcumin, Anthocyanin And Foods Rich In Them On Immune System In Experimental Rats

Asmaa Mahmoud Ahmed Mohamed

Home Economics Department - Faculty of Specific Education -Aswan University-Egypt

Email: drasmaaec2014@gmail.com

asmaa.mahmoud82@aswu.edu.eg

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Abstract

The function of the immune system is to protect individuals against pathogenic microorganisms. Nutrition plays a crucial role for individuals to achieve normal systemic homeostasis and support the immune system. The present study was performed to evaluate the effect of supplemented diet with Curcumin, Anthocyanin and foods rich in them against Cadmium chloride (CdCl₂) - induced immunosuppression in rats. Thirty-six male rats were distributed into 6 groups, of 6 rats each. The first group was kept a negative control, fed on basal diet and fresh tap water was freely provided. The remaining rats were divided into 5 equal groups and allowed to drink water to which 40 mg/L of CdCl₂ was dissolved to inhibit their immune system. Rats of group 2 remained a positive control and the other rats were fed on supplemented diets with Curcumin, Anthocyanin and foods rich in them via the stomach tube for 6 weeks. At end of the experiment, blood biochemical parameters and biomarkers of hepato-renal functions were estimated. Serum levels of antioxidant enzymes and immunoglobulin G (IgG) were determined. Results revealed that there were significant improvements in complete blood count (CBC) and biomarkers of hepato-renal functions, decreases in serum levels of triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and IgG. Antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) significantly increased after feeding rats on the above mentioned supplemented diets. In conclusion, diet supplementation with Curcumin and Anthocyanin stimulates the immune system and may serve as promising and cheaply available immunostimulant ingredients. Therefore, intake of natural and synthetic Curcumin and Anthocyanin may be beneficial to patients who suffer from immunosuppression.

Keywords: Curcumin; Anthocyanin; Biochemical parameters; Lipid profile; Hepato-renal biomarkers; Immunoglobulin G; Antioxidant.

INTRODUCTION

The primary function of the immune system is to protect the body from outside invaders such as bacteria, viruses, fungi, and toxins and maintain normal structural integrity of the body. A fully functioning immune system can distinguish healthy tissues from unwanted substances (John, 2007). Weakened immune system often results in infections, cancers, and autoimmune diseases which affect more than 5% of people globally. The autoimmune diseases include multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease (IBD), myocarditis, thyroiditis, systemic lupus erythematosus, and myasthenia gravis (Bright, 2007).

Curcumin is one of the main ingredients of turmeric which is a member of the ginger family (*Zingiberaceae*). It is a yellow pigment of rhizomes of *Curcuma longa* herbaceous plant (Kocaadam and Şanlıer, 2017). Chemically, Curcumin is a di-arylheptanoid, belonging to the group of curcuminoids, which are phenolic pigments responsible for the yellow color of turmeric. Curcumin has numerous physiological and pharmacological effects, including antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-proliferative, pro-apoptotic, anti-atherosclerotic and immunostimulant activities (Araujo and Leon, 2001). Curcumin is used for the treatment of nephrotoxicity, arthritis, allergy, asthma, inflammatory bowel disease, psoriasis, diabetes type1, Alzheimer's disease, multiple sclerosis, cancers, and cardiovascular diseases (Bright, 2007 and Si-yu et al., 2022).

Anthocyanins are a group of widely distributed water-soluble natural pigments with many biological effects (**Eng Khoo *et al.*, 2017**). They are well-known to have potent anti-oxidant and anti-inflammatory, antidiabetic and anticancer activities. They have an important role in the prevention of cardiovascular and neurodegenerative diseases. Anthocyanins are mostly found in a variety of colored foods, including red cabbage, grapes, berries, plums, and apples. Cyanidin, delphinidin, malvidin, peonidin, petunidin, and pelargonidin are the six common active ingredients of Anthocyanidins (**Mattioli *et al.*, 2020**). One of the richest sources of anthocyanins, particularly acylated anthocyanins, is red cabbage (**Wiczowski *et al.*, 2013**). It acts by scavenging free radicals, activating antioxidant enzymes, and protecting against oxidative stress, Anthocyanins are acknowledged as one of the most potent antioxidants and have a protective activity against lipid peroxidation (**Tang and Zhou, 2009 and Hogan *et al.* 2010**).

Cadmium is a toxic metal harmful to the environment and to humans, affecting mainly kidney and the skeleton and it is also carcinogen and causes severe risk to human health (**Genchi *et al.*, 2020**). In humans, cadmium exposure results in a variety of toxic effects, such as immunosuppression, renal and hepatic dysfunction, pulmonary edema, testicular damage, and hemopoietic system damage (**Tinkov *et al.*, 2018**).

The purpose of this study was to investigate the effect of supplemented diet with Curcumin, Anthocyanin and foods rich in them against Cadmium chloride (CdCl₂) induced - immunosuppression in experimental rats.

Materials and Methods

Rats

The present study was performed on thirty-six male albino rats weighting from 180 ±10 g and 8 months age. Rats were purchased from Animals House of National Research Centre, Cairo, Egypt. Rats were kept in metal wire cages under controlled environmental conditions of light/dark cycle (12hr/12hr), temperature (22±4°C) and relative humidity (45% to 50%) during the experimental period. The rats were left for one week for acclimatization prior to start of the experiment.

Preparation of Basal Diet

The components of purified diet AIN M-93 (basal diet) as approved by **Reeves *et al.*, (1993)** were purchased from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Corn starch was obtained from the Egyptian Starch and Glucose manufacturing company, Cairo, Egypt. Sucrose and soybean oil were purchased from a local market. All ingredients were formulated to meet the nutritional requirements of the rat.

Chemicals and Kits

Cadmium Chloride (CdCl₂) as an anhydrous powder was purchased from Middle East Company, Cairo, Egypt. The vitamin mixture was purchased from Araminovit Company as a fine powder and salt mixture was supplied from Vigora Pharmaceutical Industries, Sadat City, Egypt. The other chemicals used in this study were obtained from the El-Gomhoriya Company for Trading Drugs and Chemicals, Cairo, Egypt. Kits for biochemical assays were purchased from the Gamma Trade Company for Pharmaceutical and Chemical, Dokki, Egypt.

Natural Plants and Synthetic Active Ingredients

Curcumin which is a polyphenol derived from turmeric plant. It has a wide variety of therapeutic benefits including anti-inflammatory, anti-oxidative, and chemopreventive effects. Turmeric plant contains a high amount of Curcumin. Synthetic purified Curcumin powder was obtained from a local market, Cairo, Egypt as a powder. The natural turmeric (*curcuma longa*) rhizomes were freeze dried and grinded in a mill into a dry powder before mixing with the basal diet. Synthetic powder of Anthocyanin was purchased from a local market Cairo, Egypt. The fresh red cabbage plant which contains high amount of Anthocyanin was obtained from a green grocery market, cleaned, freeze dried and grinded in a mill into a dry powder before mixing with the basal diet.

Grouping of Rats

Thirty-six male rats were distributed into six equal groups. The first group was a negative control, fed on basal diet and fresh tap water was freely provided. The remaining rats were allocated into five groups of six rats each, fed on basal diet and allowed to drink water dissolved in it 40 mg/L of Cadmium chloride (CdCl₂) for 6 weeks to inhibit immune system. Group 2 was a positive control and groups 3 and 4 were fed on the basal diet supplemented with Curcumin at 200 mg /kg (equal to 1100 mg/ kg commercial Curcumin) and freeze dried turmeric as a natural source of Curcumin, respectively, given orally by gavage for 6 weeks according to **Hocking *et al.*, (2018)**. Groups 5 and 6 were fed on basal diet supplemented diet with commercial Anthocyanin at 75µg/kg and dried powder of red cabbage as a natural source of Anthocyanin at 0.028 mg kg (equal 75 µg/kg Anthocyanin), respectively, given orally by stomach tube for 6 weeks as described by **El-Boshy *et al.*, (2015)**.

Blood sampling

Blood samples were withdrawn from orbital plexuses of veins of the eye into capillary tubes which contain sodium ethylene diamine tetra acetic acid (EDTA) as an anticoagulant (1 mg /mL blood) and used for Hemogram parameters. The other blood samples were left to clot and centrifuged at 9000 r.p.m for 15 minutes using cooling centrifuge for serum separation. Serum samples were kept in deep freezer at - 18°C till used for biochemical analysis.

Biochemical Analysis

Determination of Complete Blood Count (CBC).

Red and white blood cell counts and hemoglobin concentration, were measured using Hemogram according to the methods described in **Schalm, (1986)**. Leukocytes were counted in the four corner squares of the haemocytometer counting chamber. The value obtained was multiplied by the factor 50. Hemoglobin concentration was conducted spectrophotometrically according the manufacturer. Blood was withdrawn by hematocrit (HCT) capillary tubes, centrifuged for 10 minutes using micro hematocrit centrifuge at 3000 r.p.m. HCT was read and recorded directly.

Differential Leukocyte Counts and Platelets Indices

Mean cell volume, mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC), Lymphocytes (Lym), Neutrophil (Neut), Mean corpuscular hemoglobin concentration (MCHC) and platelets (PLTs) were measured by automated blood cell counting with electronic blood cell analyzers including those manufactured by Beckman coulter and system and differential of leukocyte counts were examined according to **Weitzman (1975)**.

Determination of Lipid Profile

Serum total cholesterol (TC) (**Richmond, 1978**), triglycerides (TG) and high-density lipoprotein cholesterol (HDLc) (**Friedewald et al., 1972**) were chemically determined by specific biochemical kits and measured using a spectrophotometer. Low density lipoprotein cholesterol (LDL-c) was calculated using Friedewald formula: where $LDL-c = (TC) - (HDL-c) - (TG/5)$.

Assessment of Antioxidant Enzymes

Assays of the activity of antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were estimated according to methods described by **Paglia and Valentaine (1979)**, **Spitz and Oberley (1989)** and **Sinha (1972)**, respectively.

Determination of Liver and Kidney FUNCTIONS

At the end of the experimental period, the rats were fasted overnight before sacrificing. Blood samples were collected from the hepatic portal vein then centrifuged 3000 r.p.m for 15 min. to obtain serum then stored at -20oc until biochemical analysis of liver biomarkers. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Young (2001)**. The activity of alkaline phosphatase (ALP) enzyme was determined using spectrophotometer DU 7400 adjusted at 510 nm according to the described method by **Roy (1970)**. Quantitative ELISA-based colorimetric kits assay were used for the measurements of serum levels of kidney function parameters: urea nitrogen (UN), creatinine (Cr) and uric acid (UA) based on colorimetric assay. The absorbance of the colored solutions was recorded using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 540, 530 and 750 nm, respectively, according to kits procedures (**Friedman and Young, 1997**).

Determination of Immunoglobulin G (Ig G) Concentration

Serum levels of total Ig G were measured using enzyme linked immunosorbent assay (ELISA) which is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples according to the method of **Engvall (2010)**.

Statistical Analysis

The obtained data were presented as means \pm SD and statistically analyzed using computerized SPSS (Statistic program sigma stat, statistical software, SAS institute, Cary, North Carolina, USA). Effects of different treatments were analyzed by one were ANOVA (Analysis of variance) test using Duncan's multiple range test (**Snedecor and Cochran, 1980**).

Results and Discussion

The results presented in Table (1) indicated that complete blood count (CBC) had highly significant ($P \leq 0.01$) differences between all the tested groups. The RBCs count decreased in the positive control group as compared to the negative control group. The other rats fed on commercial Curcumin had significantly increased RBCs count, Hb concentration and HCT as compared to the positive control group. These findings were partially similar to those of **Abdel- Tawwab et al., (2022)** who found that the counts of red blood cells, hemoglobin concentration and hematocrit levels of Nile tilapia fed with 30–60 mg Curcumin nanoparticulates /kg diet were statistically ($P < 0.05$) higher than fish

in the control group. On the other side, Curcumin has been shown to possess an iron-cheating effect in mice model maintained on iron sufficient diet. This property may contribute to the development of mild microcytic anemia. The results of our study disagreed with those of **Macha et al., (2019)** who found that Curcumin feeding caused mild anemia in mice model. The discrepancy between the two studies could be attributed to species differences (rats and mice models). In one of earlier studies, reported changes in erythrocyte sedimentation rate and hemoglobin concentration as a result of Curcumin feeding **Taty Anak (2011)**. Rats fed on natural and commercial Anthocyanin had an increase in red blood cells count and hemoglobin concentration. These results were consistent with those of **Mpiana et al., (2010)** who found that feeding Anthocyanin extracts plays a role in both stabilizing the red blood cell membrane and increasing hemoglobin concentration.

The count of white blood cells decreased in the positive control group as compared to the negative control group. Rats fed on commercial Curcumin showed an increase as compared to the negative control group. The best results were in rats fed on natural Curcumin. These findings were in agreement with an earlier research which mentioned that feeding of Curcumin suppressed the proliferation of lymphocytes (**Ranjan et al., 2004**). In addition, **Gautam et al., (2007)** reported that turmeric produces an immunomodulatory effects including its effect on lymphoid cells, antigen presentation, humoral and cell-mediated immunity, and cytokine production.

Table (1): Effect of different sources of Curcumin and Anthocyanin on complete blood count (CBC)

	Groups	RBCs (ml/U)	HB (ml/U)	HCT (%)	PLTs(ml/UL)	WBCs(ml/UL)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	Negative control group (1)	7.67 ^a ± 0.61	14.98 ^a ± 1.46	54.42 ^a ± 0.50	117.53 ^a ± 2.86	10.22 ^{bc} ± 2.16
	Positive control group(2)	4.00 ^d ± 0.28	10.17 ^d ± 0.30	33.86 ^e ± 0.52	90.40 ^e ± 1.98	12.60 ^a ± 1.02
Curcumin	Commercial Curcumin group(3)	5.12 ^c ± 0.69	15.28 ^a ± 0.37	52.63 ^{ab} ± 0.67	114.03 ^b ± 2.49	11.22 ^{ab} ± 1.82
	Natural Curcumin group(4)	6.03 ^b ± 0.26	11.11 ^c ± 0.57	42.65 ^c ± 1.32	100.88 ^d ± 1.80	10.22 ^{bc} ± 1.86
Anthocyanin	Commercial Anthocyanin group(5)	7.13 ^a ± 0.49	13.22 ^b ± 1.36	38.62 ^d ± 3.71	110.48 ^c ± 2.10	9.86 ^{bc} ± 2.99
	Natural Anthocyanin group(6)	7.13 ^a ± 0.39	14.19 ^a ± 0.53	50.55 ^b ± 4.50	108.13 ^c ± 3.55	8.08 ^c ± 1.90
	F	51.81	43.60	65.00	100.74	4.77
	Significance	**	**	**	**	**

Data are presented as mean ± SD (n=6).

(**) =highly significant at p<0.01, (*) = significant at p<0.05,

a, b, c and d means different letters among treatments in the same columns are significantly different at p≤0.05.

The results given in Table (2) revealed that serum total cholesterol (TC) showed no significant differences among all studied groups. The TC, TG, LDL-c, increased significantly in the positive control group as compared with the negative control group.

The rats fed on Curcumin had the best results these results were in agreement with previous trials of **Mohammadi et al. (2013)** and **Di Pierro et al. (2015)** who found that that curcuminoids supplementation (1 g/day for 30 days) caused a significant reduction in serum triglycerides. However, **Kaur, (2012)** reported that oral administration of combinatorial extract of Curcumin with piperine and quercetin over 28 days reduced plasma levels TG, LDL-c, and total cholesterol in diabetic rats.

In Anthocyanin -fed rats, the concentration of TG, LDL-c and VLDL-c decreased as compared to the positive control group and HDL-c significantly increased. The results in this study were in agreement with earlier studies of **Changfeng et al., (2016)** and **Maria et al., (2020)** who found that Anthocyanin supplementation significantly reduced serum TG and LDL-c levels in patients with dyslipidemia and increased HDL-c levels.

Table (2) Effect of different sources of Curcumin and Anthocyanin on serum lipid profile

	Groups	TC (Mmol/L)	TG (Mmol/L)	LDL-c (Mmol/L)	HDL-c (Mmol/L)	VLDL-c (Mmol/L)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	Negative control group(1)	173.39 ^a ± 18.18	160.60 ^c ± 14.23	68.59 ^d ± 9.92	72.68 ^a ± 2.68	32.12 ^c ± 3.41
	Positive control group(2)	182.55 ^a ± 8.25	217.77 ^a ± 14.34	94.08 ^a ± 8.18	44.92 ^c ± 4.63	43.55 ^a ± 2.87
Curcumin	Commercial Curcumin group(3)	184.77 ^a ± 10.93	188.78 ^b ± 9.89	78.63 ^c ± 7.54	68.38 ^a ± 3.74	37.76 ^b ± 1.99
	Natural Curcumin group(4)	173.13 ^a ± 8.09	165.00 ^c ± 10.27	68.45 ^d ± 5.65	71.68 ^a ± 4.77	33.00 ^c ± 2.05
Anthocyanin	Commercial Anthocyanin group(5)	180.86 ^a ± 10.77	192.32 ^b ± 9.51	83.63 ^{ab} ± 3.93	58.77 ^b ± 6.62	38.46 ^b ± 1.90
	Natural Anthocyanin group(6)	184.83 ^a ± 3.17	202.42 ^b ± 5.67	81.50 ^{bc} ± 2.57	62.85 ^b ± 2.67	40.48 ^b ± 1.17
	F	1.46	23.46	15.90	33.63	18.65
	Significance	-	**	**	**	**

Data are presented as mean ± SD (n=6).

(**) =highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non-significant.

a, b, c and d means different letter among treatments in the same columns are significantly different at p≤0.05.

The results recorded in Table (3) revealed that there were increases in the levels of AST, ALT and ALP in the positive control group as compared to the control group. Serum levels of AST, ALT and ALP decreased in the groups fed on Curcumin as compared to the positive control group. These results are in agreement with the study of **Saeede et al., (2019)** who found that Curcumin supplementation was associated with significant decrease in hepatic fibrosis (p < 0.001). There are several plausible mechanisms that suggest favorable effect of Curcumin on liver function. Curcumin might ameliorate hepatic steatosis and block fatty liver disease progression through inhibiting fatty acids synthesis and biosynthesis of unsaturated fatty acids such as stearic acid, oleic acid and linoleic acid (**Egashira et al., 2012**). Anthocyanin-fed groups showed a decrease in AST, ALT and ALP enzymes as compared to the positive control group.

Table (3) Effect of different sources of Curcumin and Anthocyanin on liver enzymes

	Groups	AST(g/dl)	ALT(g/dl)	ALP(g/dl)
		Mean ± SD	Mean ± SD	Mean ± SD
Control	Control negative group(1)	22.82 ^d ± 3.32	8.00 ^d ± 1.18	114.88 ^b ± 2.99
	Control positive group(2)	36.18 ^a ± 4.72	14.23 ^a ± 1.09	131.18 ^a ± 6.23
Curcumin	Commercial Curcumin group(3)	25.55 ^{cd} ± 3.72	10.63 ^{bc} ± 1.51	126.10 ^a ± 10.12
	Natural Curcumin group(4)	33.15 ^{ab} ± 3.64	8.43 ^d ± 0.70	113.75 ^b ± 3.22
Anthocyanin	Commercial Anthocyanin group(5)	26.58 ^{cd} ± 1.48	11.23 ^b ± 2.22	113.17 ^b ± 4.65
	Natural Anthocyanin group(6)	29.00 ^{bc} ± 5.53	9.20 ^{cd} ± 0.77	114.00 ^b ± 4.15
	F	9.64	17.52	10.92
	Significance	**	**	**

Data are presented as mean ± SD (n=6).

(**) highly significant at p<0.01, (*) = significant at p<0.05

a, b, c and d means different letter among treatments in the same columns are significantly different at p≤0.05.

The results presented in Table (4) showed an increase in the level of urea, urea acid and creatinine in the positive control group as compared to the negative control group.

While urea, uric acid, and creatinine levels decreased in the groups treated with Curcumin as compared to the positive control group. These results were in agreement with the study (**Chyou et al., 2022**) who concluded that Curcumin has anti-oxidant, anti-inflammatory and anti-apoptotic effects which may be potential used to protect against renal damage. In Anthocyanin-fed groups also showed a decrease in urea levels, and the best results were found in the natural Curcumin-fed group.

Table (4) Effect of different sources of Curcumin and Anthocyanin on kidney function

	Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
		Mean \pm SD	Mean \pm SD	Mean \pm SD
Control	Negative control group(1)	33.32 ^{cd} \pm 1.19	2.75 ^e \pm 0.46	0.50 ^e \pm 0.13
	Positive control group(2)	58.63 ^a \pm 4.64	4.67 ^a \pm 0.29	1.13 ^a \pm 0.24
Curcumin	Commercial Curcumin group(3)	34.48 ^c \pm 5.00	3.28 ^{cd} \pm 0.17	1.00 ^{ab} \pm 0.17
	Natural Curcumin group(4)	29.02 ^d \pm 5.56	4.03 ^b \pm 0.59	0.62 ^{de} \pm 0.15
Anthocyanin	Commercial Anthocyanin group(5)	40.67 ^b \pm 2.98	3.73 ^{bc} \pm 0.51	0.90 ^{bc} \pm 0.16
	Natural Anthocyanin group(6)	35.68 ^{bc} \pm 4.67	2.88 ^{de} \pm 0.29	0.72 ^{cd} \pm 0.17
	F	36.18	18.73	11.47
	Significance	**	**	**

Data are presented as mean \pm SD (n=6).

(**) =highly significant at p<0.01, (*) = significant at p<0.05

a, b, c and d means with different letter among treatments in the same columns are significantly different at p \leq 0.05.

The results found in Table (5) showed an decrease in the level of Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the three major enzymatic antioxidant defense systems responsible for scavenging free radicals and nascent oxygen. Serum concentration of IgG significantly increased in the positive control group as compared to the control group. In the groups fed on Curcumin, the activity of SOD, GPx and CAT significantly increased, and decreased the level of IgG as compared to the positive control group. In Anthocyanin-fed rats, there were increases in the activity of SOD, GPX and CAT as compared to the positive control group. These results were in agreement with the previous findings of **Intuyod et al., (2014)** and **Bendokas et al., (2020)** who reported that Curcumin and Anthocyanin caused an antioxidant activity via scavenging free oxygen radicles (ROS). Immunoglobulin G (IgG) is the most common type. Curcumin supplementation significantly decreased serum level of IgG in rats. This finding was partially similar to those of **Hafez et al., (2022)** who reported that supplemental Curcumin improved birds' growth, behaviors, and immunity by lowering oxidative stress, enhancing humoral immune response. However, feeding Nile tilapia fish with anthocyanin-containing diets caused increases in the innate immune parameters (**Yilmaz, (2019)**).

Table (5) Effect of different sources of Curcumin and Anthocyanin on antioxidant enzymes (SOD, GPX, and CAT) and immunoglobulin G (IgG)

	Variables groups	SOD(g/dL)	GPX(g/dL)	CAT(g/dL)	IgG(g/dL)
		Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Control	Group(1) Negative control	66.98 ^a \pm 4.65	45.54 ^a \pm 2.91	37.92 ^{ab} \pm 4.83	682.70 ^c \pm 17.90
	Group(2) Positive control	33.73 ^e \pm 2.78	15.58 ^d \pm 1.04	25.55 ^d \pm 1.43	857.40 ^a \pm 25.44
Curcumin	Group(3) Curcumin commercial	55.42 ^c \pm 2.79	29.60 ^c \pm 2.61	28.95 ^{cd} \pm 3.03	755.18 ^b \pm 36.45
	Group(4) Curcumin natural	65.53 ^{ab} \pm 6.31	40.50 ^b \pm 5.96	40.27 ^a \pm 5.31	681.33 ^c \pm 12.20
Anthocyanin	Group(5) Anthocyanin commercial	43.58 ^d \pm 5.77	32.02 ^c \pm 3.63	30.50 ^{cd} \pm 2.03	773.70 ^b \pm 33.36
	Group(6) Anthocyanin natural	58.97 ^{bc} \pm 9.80	37.82 ^b \pm 1.78	34.17 ^{bc} \pm 7.03	705.68 ^c \pm 21.40
	F	29.57	58.15	9.68	41.25
	Significance	**	**	**	**

Data are presented as mean \pm SD (n=6).

(**) =highly significant at p<0.01, (*) = significant at p<0.05

a, b, c and d means with different letters among treatments in the same columns are significant at p \leq 0.05.

Conclusion

Diet supplementation with natural and synthetic Curcumin and Anthocyanin produces immunostimulant, hepato-renal productive, anti-oxidant activities and improves blood picture in rats with weakened immune system. Therefore, intake of Curcumin and Anthocyanin ingredients may be beneficial to patients who suffer from immunosuppression.

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