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EFFECT OF LYCOPENE AND ANTHOCYANIN ON LIVER AND KIDNEY FUNCTIONS IN MALE AND FEMALE ALBINO RATS TREATED WITH DEXAMETHASONE

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Influence of lycopene and anthocyanin as protective and curative agents on male and female albino rats treated with dexamethasone for seven weeks were studied on liver function (AST & ALT), kidney function (s. creatinine) and body weights. Results reveal that dexamethasone induce bodyweight loss. However, higher levels of AST & ALT and s. creatinine were measured after dexamethasone treatment. On the other hand, the application of lycopene and anthocyanin as natural antioxidant extracted from peel wastes of Tomato and Pomegranate resulted in revised effect of higher levels of AST, ALT, s. creatinine compared to positive and normal control groups. Meanwhile body weights loss that happen after dexamethasone treatment show slight improve compared to dexamethasone control positive group. These results reveal that both lycopene and anthocyanin could be applied either protective or curative valuable powerful available cheap substances to face undesirable side effects of oxidative stress induced as consequences dexamethasone treatment

Keywords: *Oxidative stress, lycopene, anthocyanin, dexamethasone, liver and kidney function, male and female albino rats*

INTRODUCTION

The concept of oxidative stress can be explained the relation between free radicals and disease[1]. Oxidative stress plays a key role in causing various human diseases[2]. In a normal healthy human body, the generation of pro-oxidants in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are effectively kept in check by the various levels of antioxidant defense. Oxidative stress could also be happened by drugs. If the drug's effect on the body is positive, the drug is termed as medicine, whereas, if it leads to detrimental effects, the drug is categorized as poison[3]**.** The toxicity induced in numerous tissues and organ systems including liver, kidney, ear, cardiovascular and nervous systems on exposure to certain drugs after a certain period is often termed as drug-induced oxidative stress. The drugs that lead to oxidative stress (OS) by excessive generation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and/or by disruption of the endogenous antioxidant system are collectively known as "oxidative drugs"[4,5]. Dexamethasone (Dex) is a non-selective glucocorticoid (GC) drug that is widely used for immunological, allergic, and inflammatory diseases treatment via the activation of the nuclear glucocorticoid receptors (GRs). GRs are widely expressed in the body, and they promote the expression of several genes that regulate multiple metabolic pathways, such as inflammation, and glucose, lipid, and bone metabolism[6,7]. Dex administration can cause several side effects, either at high doses or after long-term use. Insulin resistance and

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hyperglycemia, weight change, and hyperlipidemia are considered the primary adverse metabolic changes strongly associated with Dex administration[8]. Glucocorticoid overuse is strongly associated with steroid-induced osteonecrosis of the femoral head. Zhang *et al.*[9] investigate the effect of dexamethasone (Dex)-induced oxidative stress on osteocyte apoptosis and the underlying mechanisms[10].

Lycopene is the major sources of carotenoid in tomato peel and this pigment represents more than 85% of all carotenoids[11,12]. Lycopene is one of the most extensively studied natural carotenoids and is a fat-soluble carotenoid molecule with 11 conjugated double bonds[13]. Chemically, lycopene are polyunsaturated hydrocarbons containing 40 carbon atoms per molecule, variable numbers of hydrogen atoms and no other elements[14]. Good amounts of lycopene are contained in many natural products, such as tomato (*Lycopersicon esculentum* Mill.), watermelon, red pepper and papaya, this molecule is a red-colored, which gives tomatoes and several other fruits their deep red color, also being responsible for the intense red color of these vegetables[15]. Lycopene, as shown in **(Fig. 1)**[16] is a highly prized antioxidant with associated health benefits and is abundant in natural sources. The molecular structure of lycopene belongs to the carotenoids and occurs widely in nature[17,18].

Anthocyanin is the most important group of pigments, after chlorophyll that is visible to the human eye[19]. The molecular structure of Anthocyanin is subclass of flavonoids that is an important group of water-soluble plant pigments and commonly found in various fruits, vegetables, and tea, as shown in **(Fig. 2)**[20]. Anthocyanidins include mainly cyanidin, pelargonidin, and delphinidin as well as flavonoids such as luteolin, kaempferol, and quercetin[21]. Anthocyanin supplementation may potentially improve markers of liver function and may play key roles in the development of liver disorders because anthocyanin may improve oxidative stress[22].

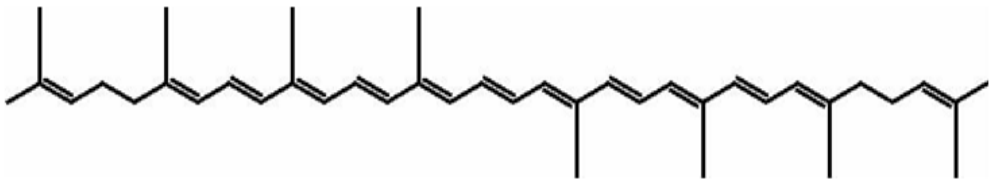


Fig. 1: Molecular structure of lycopene[16].

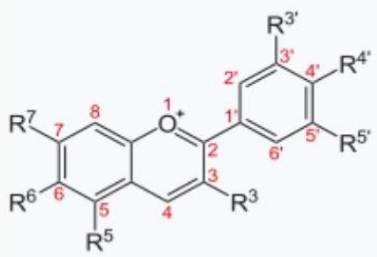
Basic structure	Anthocyanidin	R_3'	R_4'	R_5'	R_3	R_5	R_6	R_7
	Aurantinidin	−H	−OH	−H	−OH	−OH	−OH	−OH
	Cyanidin	−OH	−OH	−H	−OH	−OH	−H	−OH
	Delphinidin	−OH	−OH	−OH	−OH	−OH	−H	−OH
	Europinidin	$-OCH_3$	−OH	−OH	−OH	$-OCH_3$	−H	−OH
	Pelargonidin	−H	−OH	−H	−OH	−OH	−H	−OH
	Malvidin	$-OCH_3$	−OH	$-OCH_3$	−OH	−OH	−H	−OH
	Peonidin	$-OCH_3$	−OH	−H	−OH	−OH	−H	−OH
	Petunidin	−OH	−OH	$-OCH_3$	−OH	−OH	−H	−OH
	Rosinidin	$-OCH_3$	−OH	−H	−OH	−OH	−H	$-OCH_3$

Fig. 2: Molecular structure of anthocyanin[20].

So, the current study is aimed to extract both Lycopene from tomato processing waste and anthocyanin from pomegranate industrial by-products and using these natural antioxidants Lycopene and Anthocyanin extract from agricultural waste, to evaluate nutritional activity as antioxidants and if it possible to use lycopene of tomato (*Lycopersicum Esculentum*) and anthocyanin from Pomegranate (*Punica granatum* L.) peel as protective and curative substances as food supplements. In addition, study their effect on the liver function and kidney function of male and female albino rats to eliminate the harmful effects of dexamethasone long run treatments which induce oxidative stress on the liver and kidney functions in male and female albino rats.

MATERIALS AND METHODS

Materials

Lycopene and anthocyanin were extracted from tomatoes and pomegranate peels that purchased from local market, respectively. Dexamethasone was purchased from Arab Company for Medical Product, Obour City, Industrial Area, Cairo, Egypt. Male and female albino rats were obtained from the Animal House of Agriculture Research Center, Cairo, Egypt.

Lycopene and Anthocyanin extraction and quantification

Lycopene was extracted according to the method of Shahzad *et al*.[23] and determined according to the method of Nagata and Yamashita[24]. Anthocyanin was extracted according to the method of Harborne[25] and determined according to the method of Martinez and Favret[26]. The extracts were measured spectrophotometrically at 527 nm, then readings absorbance was converted to total amount of anthocyanin as a cyanidin-3-glucoside equivalent using a molar extinction coefficient (ε) 2.96×10^4 mentioned by Cheng and Breen[27]. Results were expressed as gram of cyanidin-3-glucoside equivalents per 100 grams of dry weight.

HPLC lycopene analysis

Instrumentation and chromatographic conditions was applied according to[28] as follow: The analysis was performed by using Inertsil ODS-3V, C-18, 150 X 4.6mm internal diameter with 5 micron particle size column and PDA detector set at 472 nm, in conjunction with a mobile phase of methanol, tetrahydrofuran and water in the ratio of 66:30:4 % v/v at a flow rate of 1.5 ml/min. The retention time of lycopene was found to be 6.805 minute. The injection volume was 10µl.

Biological experiment design

Rats, housing and diets

Sixty albino rats (30) male and (30) female (age 8 weeks and about 160±10 g female to 190±10 g male body weight). Indeed this study included male and female, as there are a significant difference between testosterone and estrogens hormones, respectively. Rats were classified into six main groups (5/group) for both male and females. Rats were housed in the Animal Lab under controlled conditions (12-hour light:12-hour darkness) with room temperature (20°C ± 2) and had free access food and tap water. Rats were kept under normal healthy conditions and fed on the commercial diet without any treatment for one week for acclimatization. Diet composition as follow Ground corn meal (60%), Ground beans (15%), Bran (10%), Fat (10%), Casein (3%), minerals (1%), and vitamins (1%) according[29]**.** Diet and water were offered ad libitum all over the experimental period. Rats groups were as follow: the first negative control group (Cont-Neg), the second dexamethasone control group (Cont-Dexa) treated with dexa 5 mg/kg bw, the third protective group (Lyco-Dexa) (treated with Lycopene at a dose of 100 mg/kg bw, then after an hour treated with Dexamethasone at a dose of 5 mg/kg bw), the fourth protective group (Antho-Dexa), treated with Anthocyanin at a dose of 100 mg / kg bw, then an hour later Dexamethasone at a dose of 5 mg / kg bw), the fifth curative group (Dexa-Lyco) were injected with Dexamethasone at a dose of 5 mg/kg bw three time aweek for three weeks to cause drug stress, after that treated with lycopene at a dose of 100 mg/kg bw), the sixth curative group (Dexa-Antho) were injected with Dexamethasone at a dose of 5 mg/kg bw three times a week for three weeks to cause stress occurred, after that treated with Anthocyanin at a dose of 100 mg/kg bw). Rats were weighed

weekly, and the blood samples were taken after 15, 30 & 45 days. Blood samples were collected from conscious animals (blood drops from plexus) into heparinized tubes until analysis.

Dexamethasone dosage

Dexamethasone dosages were calculated 5 mg/Kg bw according to Yang *et al*.[30], for curative groups were injected seven times through three weeks, to cause osteoporosis disease, while positive and protective groups injected intraperitoneal fifteen times (three times a week) for seven weeks.

Liver function measurement

Assess hepatic function in male and female albino rats, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were conducted according[31].

Kidney functions measurement

Creatinine in serum is measured using an alkaline picrate colorimetric (Jaffé) method[32].

Statistical analysis

Data statistically were analyzed using computer software according to IBM SPSS Statistics software[33]. The results are expressed by one-way ANOVA with a completely randomized design. Duncan's multiple range tests were used to differentiate between means; a p-value of 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results

Lycopene extraction and quantification

Results in **(Table 1 and Fig. 3a)**, **s**how lycopene content extracted from industrial tomatoes processing wastes was 27.78 mg/100g tomatoes waste peel (on dry weight basis). These results are coincided with Markovic *et al*.[34] reported that lycopene content in peel of five fresh tomato cultivars were ranged between (4.14-8.24) mg/100g fresh weight and (50.6-72.3) mg/100g dry weight as measured spectrophotometrically. Meanwhile lycopene content was lower to (3.28-7.17) mg/100g fresh weight and (40-62.8) mg/100g dry weight as determined by HPLC technique. As previously study[35], found that lycopene content was approximately 12 mg/100g fresh tomatoes. Also, Ho *et al*.[36] reported that lycopene content in dried tomato skins was about ~13.0 mg/100g when extracted with hexane overnight. Meanwhile, lycopene content in the tomato peels dried at 50 and 80 °C by hot air and with fluidized bed dryer was within the range 15–414 mg/kg/dw[37]. In the same connection[38-41], mentioned that the total lycopene content of tomato processing waste was 5.32 ± 0.15 mg/100 g.

The variation between lycopene contents between various authors may be due to that lycopene extracted from tomato wastes should be done at the optimum standard conditions, whereas the extraction process for all samples were 40ºC and 45 min[41]. This indicates that the operation used in industrial scale such as high temperature used in hot-break (79°C) and the finishing steps resulted in extraction of high concentration of lycopene into the juices. For wet samples, lycopene extract resulted from laboratory tomato waste and that obtained from industrial waste had 89.21 and 81.75% lycopene recovery, respectively. Several researchers have obtained different quantity of lycopene by using different types of starting material and different extraction conditions. Tan and Soderstrom[42] were recovered 25 mg/kg of lycopene from tomato paste with 95% ethanol and low boiling petroleum ether (40–60ºC). Extracted lycopene from tomato skin with hexane: acetone: ethanol in 2:1:1 ratio and reported a maximum lycopene yield of 19.8 mg/kg with a 30:1 v/w solvent/meal ratio, four extractions, 50ºC temperature and 8 min extraction time[37]. Lavecchia and Zuorro[43] were extracted lycopene from tomato peels with hexane, ethyl acetate and hexane: acetone: ethanol (2:1:1, v/v). They found that total lycopene content was 450±21 mg/100 g of dry material and the lycopene yield ranged between 136 and 1044 mg/kg, on a dry weigh basis, however, they showed that the lycopene yield could be significantly enhanced by using samples treated by cell-wall degrading enzymes. This indicates that the extraction yield of lycopene greatly depends upon the extraction conditions employed (solvent composition, solvent/meal ratio, temperature and cycles of extractions) as well as nature of the starting material used (tomato variety, composition of the waste, portion of the fruit, and measurement technique etc.). The

operating parameters of lycopene extraction were temperature, time and the initial moisture content of raw materials.

Anthocyanin extraction and quantification

Anthocyanin is considered as the major sources of antioxidant in pomegranate peel[44]. The results in **(Table 1 and Fig. 3b)**, show that anthocyanin content was (64.56 mg/100g dw) of pomegranate waste peel on dry weight basis. This result agrees with Zhu *et al.*[45] reported that in three different varieties of pomegranate peel total ACS concentration were ranged between (45.16 - 118.65 & 344.12) mg/100g fresh peel, and there is a significant difference in the ACS concentration of the same part among different cultivars, . In addition, More and Arya[46] stated that anthocyanin content in pomegranate waste peels was 21.65 mg cyn-3-glc/100 g in dry pomegranate peel. In the same connection, Sami *et al.*[47] confirmed that the peel extract showed the highest anthocyanin content (3.8 mg/g fw) followed by whole fruit (1.9 mg/g fw), seeds (1.7 mg/g fw), and flesh (0.14 mg/g fw) respectively. Also, found that pomegranate peel methanolic extract exhibited the highest amount of total anthocyanin as compared to the other parts (peel, flesh, seeds and whole fruit) compared to ethyl acetate extract. And reported that methanol as solvent was more effective in extraction of anthocyanin from pomegranate than ethyl acetate from peel, flesh, seeds and overall whole fruit. These results agree with[48] reported that total anthocyanin in pomegranate peel were 105±15, & 236±75 100 ml pomegranate juice in aqueous extract of pomegranate peel and organic extract of pomegranate peel respectively. Also, Azarpazhooh *et al.*[44] reported that pomegranate peel represents total anthocyanin content (TAC, 40.2 mg c3g/kg dmp dry matter powder. On the other hand, Zahed *et al.*[49] show the value of TAC in different extracts, then add the highest amount of TAC measured by differential pH by spectrophotometric colorimetry related to microwave extraction (ME) extract is equivalent to 4.00 mg/g PPP (pomegranate peel powder), and the lowest amount of anthocyanin composition with a large difference from other methods for ultrasound extraction (UE) extract is 0.35 mg/g PPP It is clear that anthocyanin extracts prepared from pomegranate waste had higher anthocyanin than those prepared from wet wastes. Also, that total anthocyanine content (TAC) in pomegranate peel was 40.2 mg c3g/kg dmp (dry matter powder)[44].

Table 1: Lycopene & Anthocyanins content (mg/100g) peel dry weight.

Fruit Parts	Lycopene Tomatoes	Anthocyanin Pomegranate
Peel	**27.78 mg/100g**	**64.56 mg/100g**

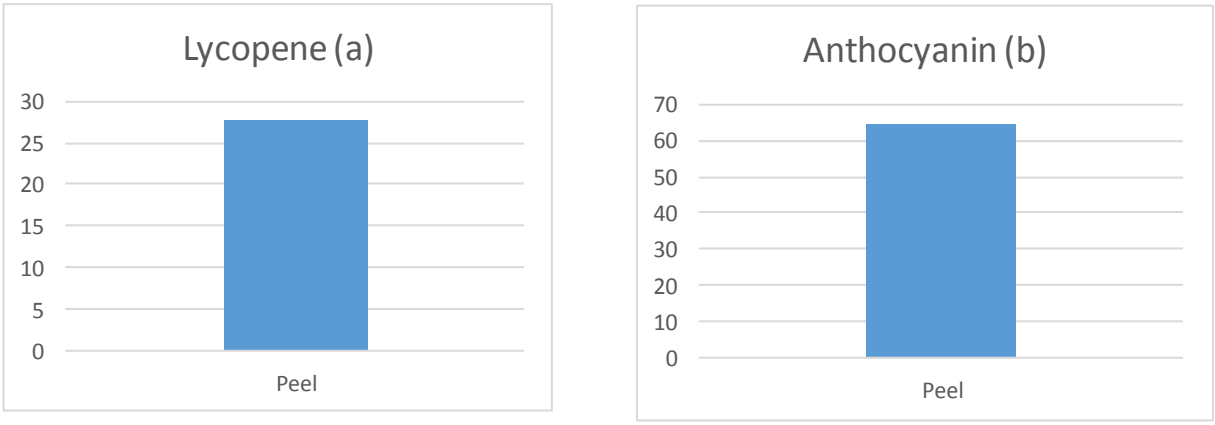


Fig. 3: Total lycopene (a), total anthocyanin (b) (mg/100g dw) from tomatoes and pomegranate waste peels.

HPLC Lycopene purity analysis

Purity of lycopene extracted from tomato peel wastes was confirmed by HPLC analysis. RP-HPLC method was applied for the estimation of lycopene in industrial tomatoes peel. The chromatogram was illustrated in **(Fig. 4)**. The retention time of lycopene was found to be 2.51 minute. As shown HPLC chromatogram of extracted lycopene reveal that lycopene purity was 94.4% that reflect the higher percentage peak area at 2.51 min which depicted in HPLC analysis chromatogram.

Biological experiment (Liver and kidney functions)

Liver functions

AST in serum male albino rats

Results in **(Table 2 and Fig. 5 a,b)** show serum AST values as affected by lycopene and anthocyanin as protective and curative agents on male albino rats treated by dexamethasone through seven weeks. AST level show higher values in male albino rats after dexamethasone treatment where the values were 173.5, 208.3 & 137 compared to 119.25, 154, & 125.75 U/L in normal control group through phase I, II & III.

However, in protective group both lycopene and anthocyanin could not lower these higher values through phase I, 160.5 & 165, phase II 206 & 190.8 and phase III 139.25 & 138.5 compared to 119.25, 154 & 125.75 in normal control group. Despite both lycopene and anthocyanin at third phase could not decrease AST value to be close to 125.75 in normal control group, and the value 139.8 & 138.5 were high as compared to positive control level 137 U/L.

In curative group, in first phase both lycopene and anthocyanin can't lower AST value 137 compared to 119.25 in negative control, but this value was lowered compared to positive control 173.5 U/L. On one side, lycopene and anthocyanin have not effect in lowering AST values during first phase but on the other side, they decrease this value to be 150.3 & 144.3 U/L lower than positive and negative control groups 208.3 & 154.0 U/L through phases II, meanwhile during third period lycopene and anthocyanin could not lower AST value to be close to normal group, but they laying in the range of positive control group.

Regarding general mean, AST in male albino rats was increased after dexa treatment from 133 to 172.9 U/L. However, this higher value does not decrease significantly after using lycopene and anthocyanin in protective group 168.58 & 164.8 U/L, but anthocyanin treatment could maintain this value around 164.8 in protective group which reflect that anthocyanin was more potent than lycopene in this protective effect. On the same trend, both lycopene and anthocyanin slightly lower this value to be 143.11 & 141.3 in curative group after using lycopene and anthocyanin respectively compared to 133.0 & 172.9 in NCG & dexa group. This result reflects that both lycopene and anthocyanin have protective and curative influence on AST parameter during short duration, but anthocyanin reveal more potent effect than lycopene.

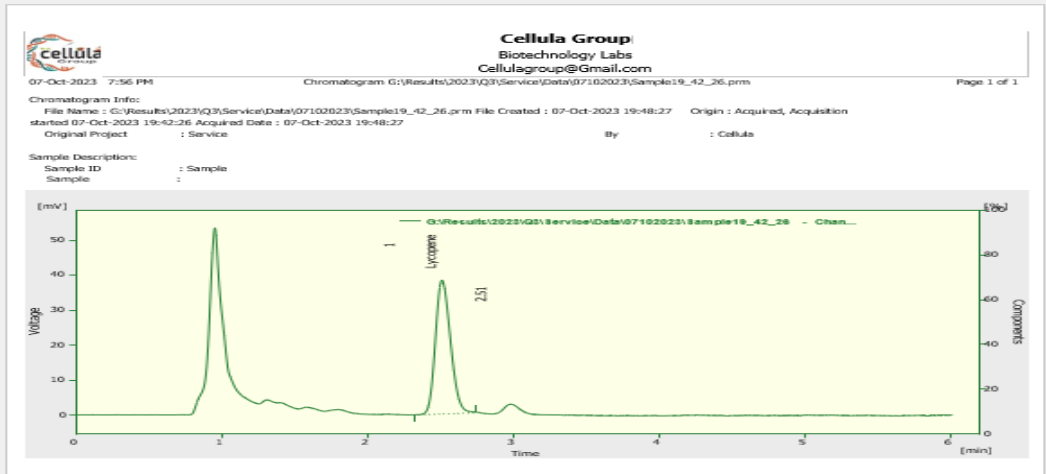


Fig. 4: HPLC chromatogram of lycopene identification and purification.

Table 2: AST units (U/L) as influenced by lycopene and anthocyanin in serum male albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	119.25[a]	173.5[d]	160.5[c]	165[c]	137[b]	137[b]
II	154[c]	208.3[f]	206[f]	190.8[d]	150.33[c]	144.3[b,c]
III	125.75[a]	137[b]	139.25[b]	138.5[b]	142[b,c]	142.5[b,c]
General Mean	133[b]	172.9[d]	168.58[c]	164.8[c]	143.11[b,c]	141.3[b,c]

*Values represent as means of 4 replicates.
* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.
*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

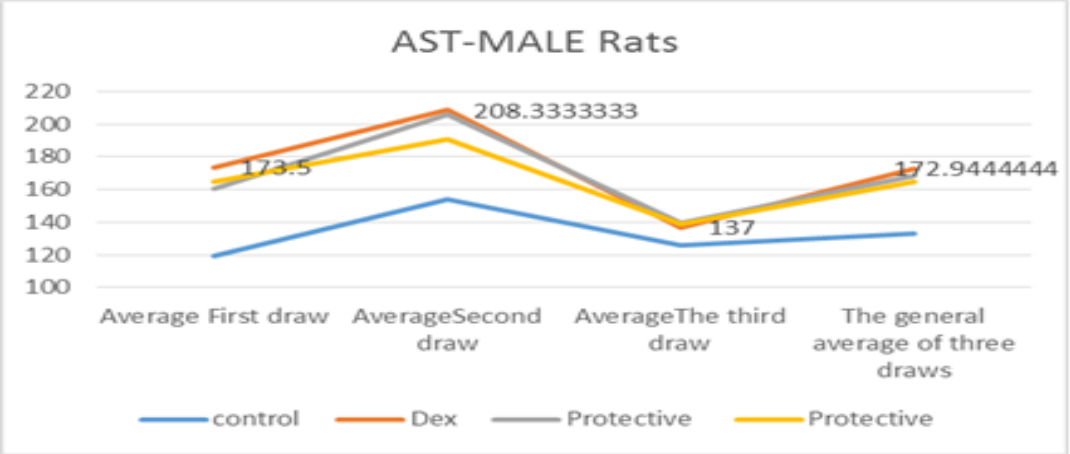


Fig. 5a: AST levels (U/L) as influenced by lycopene and anthocyanin as protective agents in male albino rats treated with dexamethasone through seven weeks.

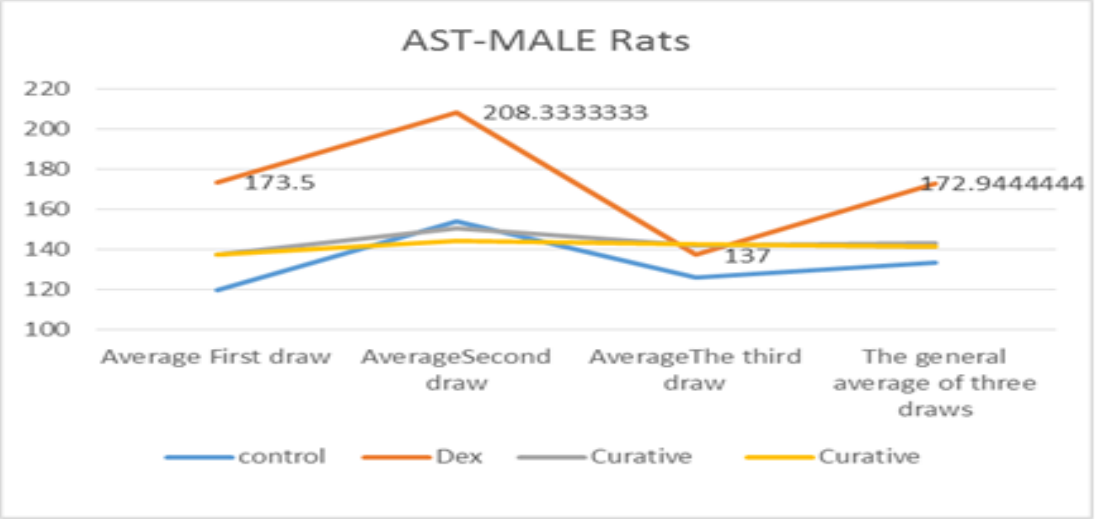


Fig. 5b: AST (U/L) as influenced by lycopene and anthocyanin as curative agents in male albino rats treated with dexamethasone through seven weeks.

ALT in serum male albino rats

Data in **(Table 3 and Fig. 6 a,b)** show ALT levels as influenced by lycopene and anthocyanin in male albino rats treated with dexamethasone through seven weeks. Results show that dexa treatment led to increase ALT level from 87.75, 65.25 & 61.25 to 111, 80 & 87 U/L in normal control male rats and dexa groups through phase I, II & III respectively. However, in protective group lycopene and anthocyanin lower ALT parameter from 111, 80 & 87 in dexa group to be 97.5, 63.5 & 69 and 103.3, 65.75 & 63.33 U/L through phase I, II & III respectively. These results obviously appear the protective role of lycopene and anthocyanin against dexamethasone side effect stress. Respecting curative groups, lycopene and anthocyanin decrease ALT level at first phase to 103 & 103, second phase to 82.50,79.50 and third phase to 82.66 ,82 compared to 111, 80,87 U/L in dexamethasone group. These results show the influence of those antioxidants have protective and curative effect depending on time duration.

Showing general means, ALT values in male albino rats was increased after dexa treatment from 71.41 to 92.67 U/L. While in both protective and curative groups these values were lowered to 76.66 and 77.44 U/L in protective group and 89.38 & 88.17 in curative group compared to 92.67 in dexamethasone group. Results reveal that, these values were slightly lower after lycopene and anthocyanin treatment either protective or curative treatments. These results reflect that both lycopene and anthocyanin not only have lowering influence on ALT parameter but also could lower ALT value to be close to normal control group especially in protective treatment. These results reveal that both lycopene and anthocyanin could maintain ALT value near normal control group and protect liver from rising of ALT value induced by long term of dexamethasone.

Table 3: ALT units (U/L) as influenced by lycopene and anthocyanin in serum male albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	87.75^{c}	111^{f}	97.5^{d}	103.3^{d}	103^{d}	103^{d}
II	65.25^{a}	80^{c}	63.5^{a}	65.75^{a}	82.5^{c}	79.5^{b,c}
III	61.25^{a}	87^{c}	69^{a}	63.33^{a}	82.66^{c}	82^{c}
General Mean	71.41^{b}	92.6^{d}	76.66^{b}	77.44^{b}	89.38^{c}	88.17^{c}

*Values represent as means of 4 replicates.

* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.

*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

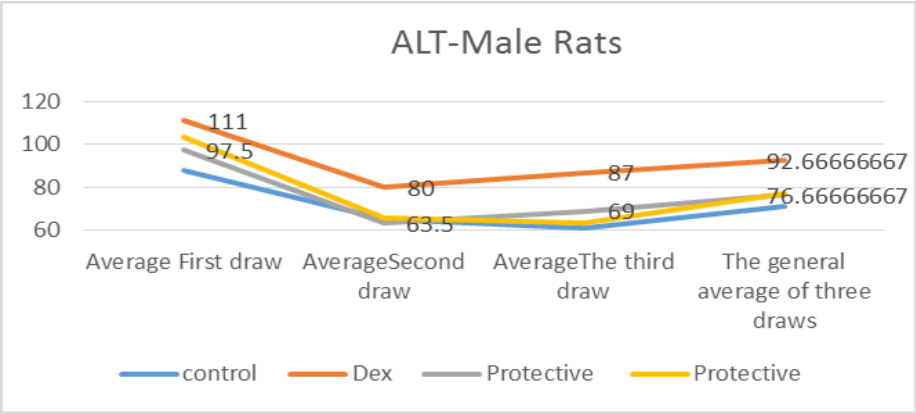


Fig. 6a: Protective effect of lycopene and anthocyanin on ALT (U/L) in male albino rats treated with dexamethasone through seven weeks.

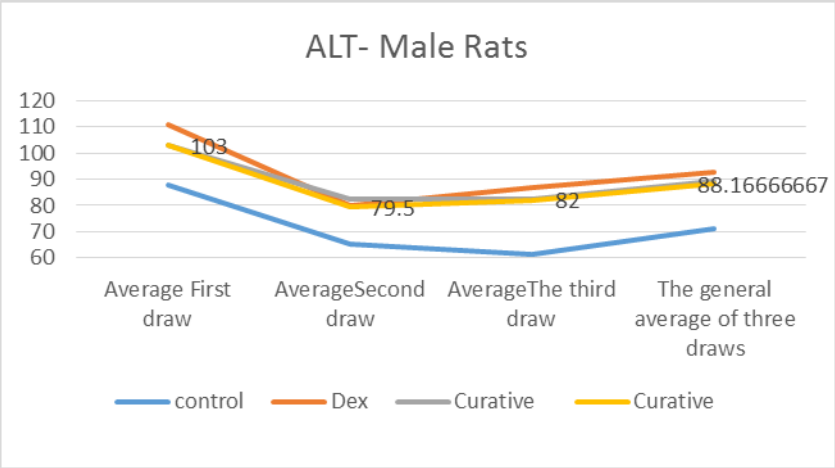


Fig. 6b: Curative effect of lycopene and anthocyanin on ALT (U/L) in male albino rats treated with dexamethasone through seven weeks.

AST in serum female albino rats

Results in **(Table 4 and Fig. 7 a, b)** show serum AST values as affected by using lycopene and anthocyanin as protective and curative agents on female albino rats treated by dexamethasone through seven weeks. AST level show higher values in female albino rats after dexamethasone treatment where the values were 178.3, 123.5 & 213.3 compared to 155.25, 115.75 & 161 U/L in normal control group through phase I, II & III. Regard to protective groups anthocyanin resalted in AST value 143 U/L while the value was 177 in lycopene compared to 178.3 & 155.25 in dex and normal control groups at phase I, which reflect that anthocyanin was more effective than lycopene in phase I. The same trend of lowering of AST value was observed in phase II & III for anthocyanin compared to dex and normal control groups. Meanwhile lycopene show lowering in phase II & III compared to dex group and slightly increase was found compared to normal control group during the two phases. These results indicate that anthocyanin was more influence in lowering activity of AST in normal albino rats compared to lycopene treatment. In curative group treatment with lycopene and anthocyanin reveal significant decrease in AST level compared to dex group during phase III and AST value was 160.3 in Antho group compared to 161 in NCG and 213.3 in positive control group.

On the other hand, general mean either in protective or curative groups show lowering in AST level compared to dex group. Also, antho in protective and curative groups recorded AST level 138 & 153.3 compared to 144 U/L in NCG which reflect the potential activity of anthocyanin as protective and curative agent compared to NCG.

Table 4: General average of AST (U/L) as influenced by lycopene and anthocyanin in serum female albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	155.25^{c}	178.3^{d}	177^{d}	143^{b}	178.3^{d}	178.3^{d}
II	115.75^{a}	123.5^{b}	124^{b}	107^{a}	123.5^{b}	121.3^{b}
III	161^{c}	213.3^{f}	179^{d}	163^{c}	175.3^{d}	160.3^{c}
General Mean	144^{b}	171.7^{d}	160^{c}	138^{b}	159^{c}	153.3^{c}

*Values represent as means of 4 replicates.
* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.
*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

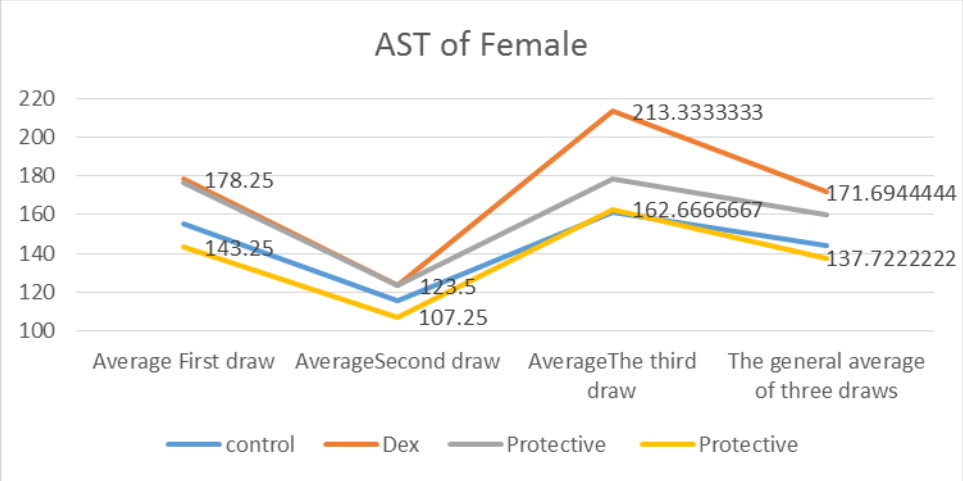


Fig. 7a: AST (U/L) as influenced by lycopene and anthocyanin as protective agents in female albino rats treated with dexamethasone through seven weeks.

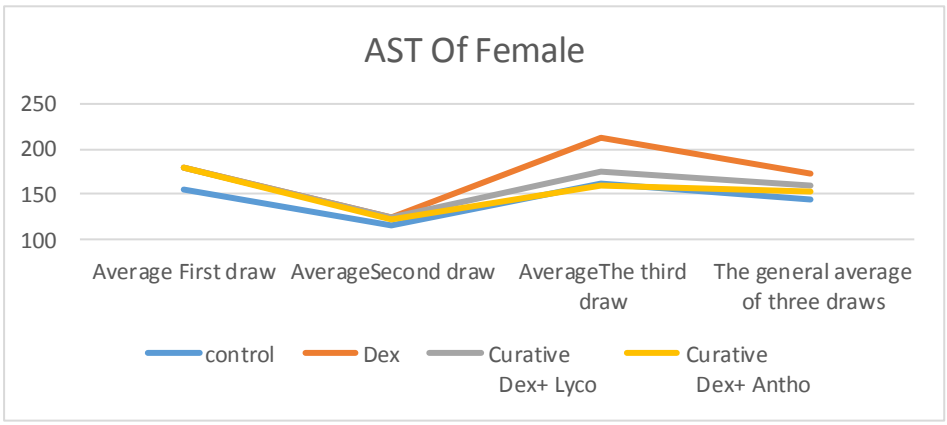


Fig. 7b: AST (U/L) as influenced by lycopene and anthocyanin as curative agents in female albino rats treated with dexamethasone through seven weeks.

ALT in serum of female rats

According to **(Table 5 and Fig. 8 a,b)** show ALT levels as influenced by lycopene and anthocyanin in female albino rats treated with dexamethasone through seven weeks. Results show that dexa treatment increases ALT activity from 51.25, 59.25 & 64 to 79.25, 89.5 & 93.33 U/L in NCG female rats and dexa positive control groups through phase I, II & III respectively.

However, in protective groups lycopene and anthocyanin treatment in first, second & third phases decrease these higher values to be 62.8, 81, 79.3 & 66.5, 70.8, 72 compared to 79.25, 89.5. 93.33 in dexa positive group. Meanwhile, lycopene and anthocyanin did not show any lowering change and ALT activity still higher compared to normal control group.

Showing to curative groups at first phase neither lycopene nor anthocyanin showing lowering effect on ALT activity. while at second phase lycopene and anthocyanin showing lowering effect on ALT activity. However, at the third phase both lycopene and anthocyanin appear lowering influence where ALT activity were 61 & 62 compared to NCG 64 and Dexa positive group 93.33. Respecting general mean dexamethasone treatment induced higher ALT activity from 58.16 NCG to 87.36 DCG. These higher levels were not return to the levels of control normal group but decrease compared to positive control group which indicate that both lycopene and anthocyanin have possess protective and curative effect on ALT level.

Table 5: ALT (U/L) as influenced by lycopene and anthocyanin in serum female albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	51.25[a]	79.25[c]	62.8[b]	66.5[b]	79.25[c]	79.25[c]
II	59.25[a]	89.5[d]	81[d]	70.8[c]	71.75[c]	61[b]
III	64[b]	93.33[f]	79.3[c]	72[c]	61[b]	62[b]
General Mean	58.16[a]	87.36[d]	74.4[c]	69.8[b]	70.67[c]	67.42[b]

*Values represent as means of 4 replicates.
* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.
*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

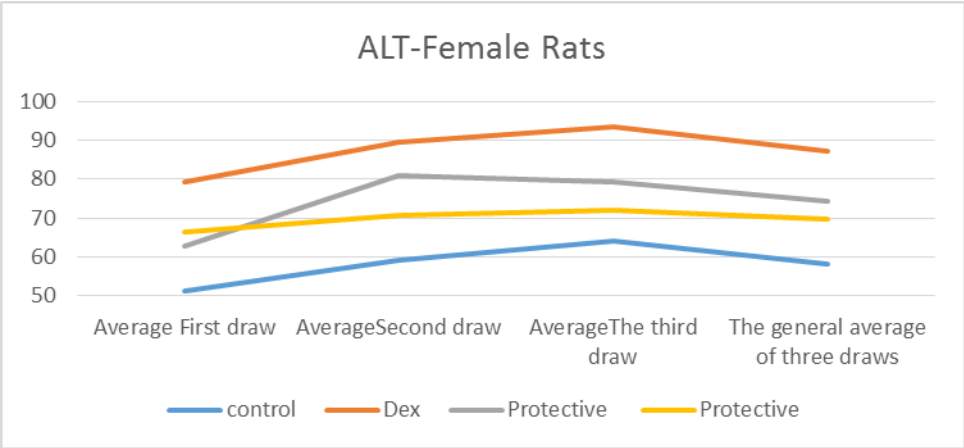


Fig. 8a: ALT (U/L) as influenced by lycopene and anthocyanin as protective agents in female albino rats treated with dexamethasone through seven weeks.

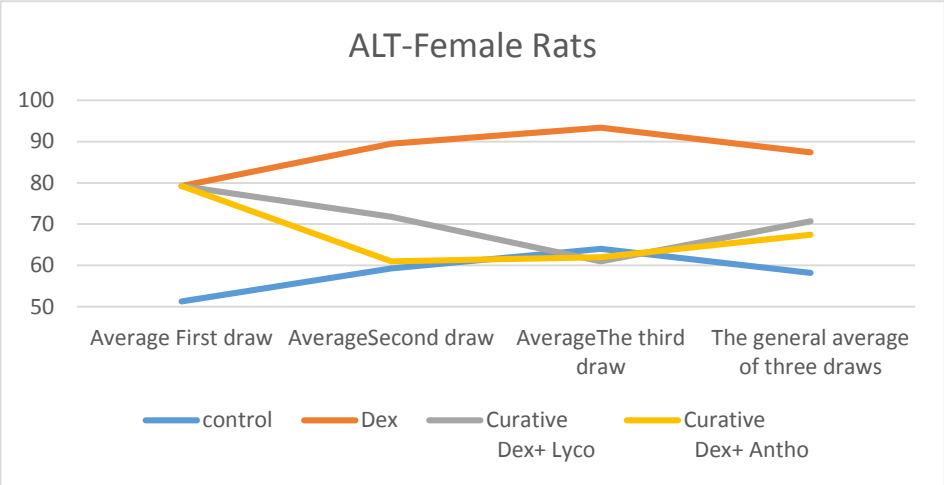


Fig. 8b: ALT (U/L) as influenced by lycopene and anthocyanin as curative agents in female albino rats treated with dexamethasone through seven weeks.

Comparison between lycopene and anthocyanin as protective and curative agents in male and female rats.

AST Levels in serum male and female albino rats

Regarding serum AST levels show higher level in female compared to male at zero time, then the levels was comparable after dexa injection, in **(Fig. 9a)**. However, after that show lowering levels of female compared to male albino rats in protective groups. However, male rats show lower level due to Dex-Lyco and Dex-Antho in curative group. These results show that both Lyco and Antho in protective group show lower in female compared to male rats. These finding reveal that lycopene and anthocyanin were more effective in lowering AST in protective treatment on female rats compared to male rats and vice versa in curative groups.

ALT Levels in serum male and female albino rats

Respecting ALT in serum male and female albino rats in (**Fig. 9 b),** reveal that ALT level in serum female albino rats was lower than male levels in all treatments starting from zero time and passing through protective and curative treatments in normal control group and after dexa treatment, Also, this lowering was still observed through all treatments. In addition, anthocyanin in female was more effective as protective and curative agents.

According to the previous comparison, these current results agree with Razzaq *et al*.[50] they reported that dexamethasone causes significant elevations in aminotransferases enzymes (AST and ALT), in male rat. In addition, in a male albino rat dexamethasone caused a significant elevation of all the liver enzymes (AST, ALT, ALP, and GGT) and serum total and conjugated bilirubin[51,52]. Moreover, Zohreh *et al.*[22] studied anthocyanin supplementation on liver enzymes, and they reported that intake of anthocyanins (ACNs) was significantly associated with the reduced level of ALT and AST in the studies that evaluated liver enzymes as their primary outcomes. Also, anthocyanin supplementation may potentially improve markers of liver function and may play key roles in the development of liver disorders because anthocyanin may improve oxidative stress. Hasona *et al.*[53] found that dexamethasone administration to female albino rats caused elevation of serum levels of glucose, uric acid, creatinine, ALT, AST activities, and a decrease in other parameters such as hepatic glutathione, total protein levels, and catalase enzyme activity. In addition of lycopene to the male goose diets led to significant decrease in AST and ALT activities[54]. Also, lycopene decreases ALT & AST, then add lycopene corrects metabolic syndrome and liver injury induced by high fat diet in obese rats through antioxidant, anti-inflammatory, antifibrotic pathways[55].

However, the intraperitoneal administration of lycopene significantly decreased the serum ALT and AST levels, where lycopene could reduce ALT and AST activity against carbon tetrachloride-induced acute liver injury in rat[56]. Also, Shimizu *et al*.[57] observed the hepatoprotective effect of lycopene on Con A-induced liver injury in mice. Also, lycopene decreased the serum ALT and AST and they reported that lycopene has hepatoprotective and antioxidant effects on non-alcoholic fatty liver disease in rat[58]. On the other hand, Seymour *et al.*[59] reported that anthocyanin increases AST and decreases ALT. Meanwhile, Sangsefidi *et al.*[60] studied the effect of anthocyanins supplementation on liver enzymes and found that anthocyanin decreases both AST and ALT. Moreover, lycopene reduces the levels of ALT and AST compared to the control group.

In general, showing protective and curative groups it could summarized that dexa raise AST and ALT values either in male or female albino rats. In additions in male and female rats both AST & ALT were more affected due to lycopene and anthocyanin treatments as protective or curative agents. Also, in some parameter's anthocyanins show more potential effect compared to lycopene.

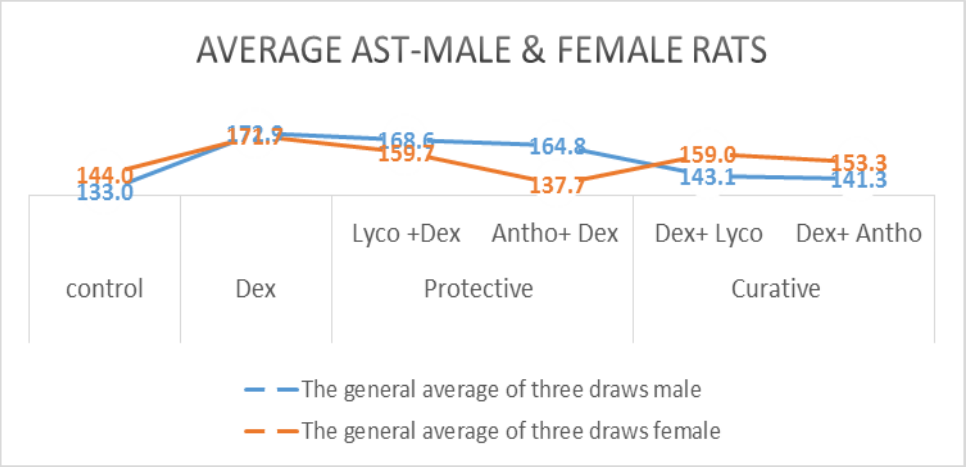


Fig. 9a: Comparison AST Levels in serum male and female albino rats.

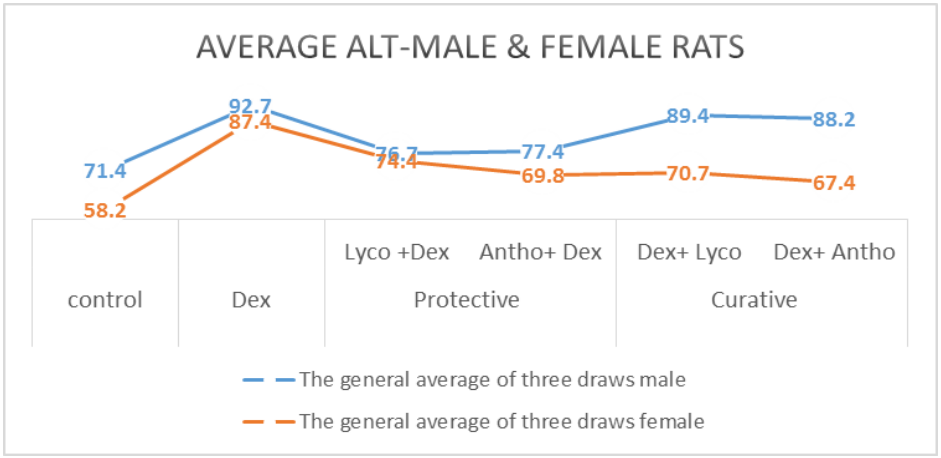


Fig. 9b: Comparison ALT Levels in serum male and female albino rats.

Kidney functions

Creatinine levels in serum male albino rats

Serum creatinine (S. Creatinine) is a more reliable indicator of renal function. Creatinine is a non-protein nitrogenous compound that is produced by the breakdown of creatinine in muscle. Creatinine is found in serum, plasma, and urine and is excreted by glomerular filtration at a constant rate and in the same concentration as in plasma.

Results in **(Table 6 and Fig. 10 a,b)** show serum creatinine values as influenced by applied lycopene and anthocyanin as protective and curative agents on male albino rats treated with dexamethasone through seven weeks. S. creatinine show higher values in serum of male albino rats after dexa treatment where the values were 1.437, 0.773 & 1.075 compared to 0.96, 0.57 & 0.63 mg/dl in dexa group and normal control group NCG through phase I, II & III in male albino rats.

Regard to protective groups despite lycopene and anthocyanin lower s. creatinine compared to dexa group through all three phases. Also, both lyco and antho lower s. creatinine level during second phase to be 0.29 & 0.58 close to NCG 0.57 mg/dl respectively but both lycopene and anthocyanin could not lower these higher values through phase I, and phase III compared to NCG.

In curative groups both lycopene and anthocyanin decrease s. creatinine value 0.89 & 0.89 to be close to normal control group 0.96 mg/dl and lower than Dexa group 1.437 mg/dl as shown in **Table (6)** during first phase but neither lycopene nor anthocyanin could decrease this level through phase II & III. This result reflect that both lycopene and anthocyanin have protective effect after long run but has not curative influence through long duration on s. creatinine parameter in male albino rats. Generally, it is obvious clear that s.

creatinine in male albino rats was raised from 0.72 to 0.88 mg/dl in NCG and dexa groups, this value was still constant raised 0.82, 0.92 & 1.13, 1.06 after rats treated with lycopene and anthocyanin in protective and curative groups and can't return to normal control group, but lycopene and anthocyanin in protective group show slightly lower levels than curative group.

Table 6: S. creatinine (mg/dl) as influenced by lycopene and anthocyanin in Serum male albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	0.96^{c,d}	1.43^{f}	1.34^{d}	1.36^{d}	0.89^{c}	0.89^{c}
II	0.57^{b}	0.77^{c}	0.29^{a}	0.58^{b}	1.23^{d}	1.15^{d}
III	0.63^{b}	1.07^{c,d}	0.83^{c}	0.83^{c}	1.27^{d}	1.15^{d}
General Mean	0.72^{c}	1.09^{c,d}	0.82^{c}	0.92^{c,d}	1.13^{d}	1.06^{c,d}

*Values represent as means of 4 replicates.
* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.
*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

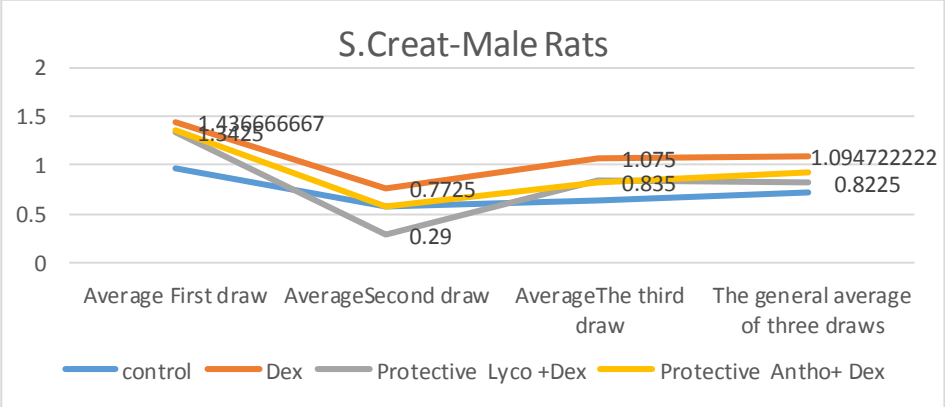


Fig. 10a: S. creatinine value (mg/dl) as influenced by lycopene and anthocyanin as protective agents in male albino rats treated with dexamethasone through three phases.

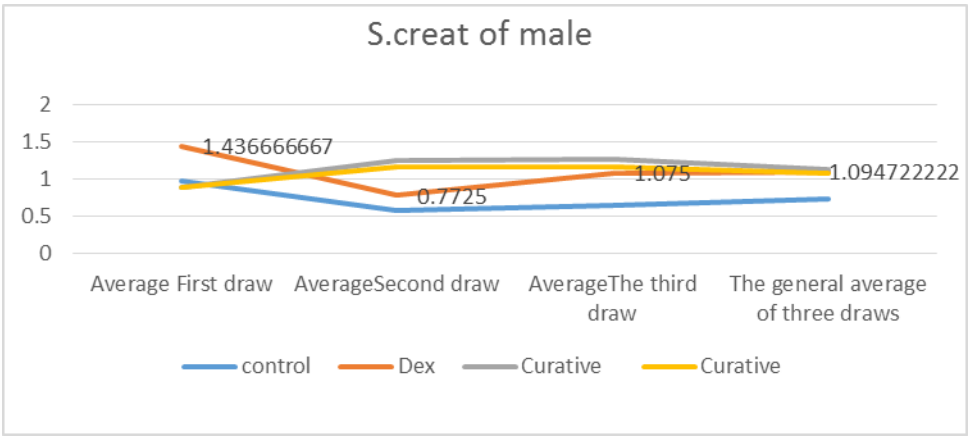


Fig. 10b: S. creatinine (mg/dl) as influenced by lycopene and anthocyanin as curative agents in male albino rats treated with dexamethasone through seven weeks.

Serum creatinine levels in female rats

Results in **(Table 7 and Fig. 11 a,b)** show serum creatinine values as influenced by applied lycopene and anthocyanin as protective and curative agents on female albino rats treated with dexamethasone through seven weeks. S. creatinine show higher values in serum of female albino rats after dexa treatment where the values were 0.48, 0.58, 0.83 compared to 0.66, 0.94 & 1.8 mg/dl in normal control group and dexa group through phase I, II & III, respectively. However, in protective group lycopene could not lower higher values through phase I and phase II, but anthocyanin show marked decrease 0.55 & 0.82 compared to dexa group 0.66 & 0.94 in first and second phase, while third phase both lycopene and anthocyanin lower s. creatinine to 1.37 and 1.18 compared to 1.8 in dexa group. On contrary, in curative group both lycopene and anthocyanin decrease this value 0.48, 0.60, 1.18 & 0.48 & 0.64, 1.3 to be lower than dexa group 0.66 & 0.94, 1.8 mg/dl during phases I, II & III. This result reflect that both lycopene and anthocyanin have curative effect on s. creatinine parameter. According to general means of s. creatinine show that dexamethasone increases s. creatinine from 0.63 to 1.13 mg/dl. On the other hand, in protective group this value recorded lowering to be 1.06 & 0.85 in lycopene and anthocyanin groups, these levels were lowered after applied lycopene and anthocyanin on female albino rats. However, both lycopene and anthocyanin could decrease these higher values to 0.75 & 0.81 mg/dl in curative groups compared to 1.13 in dexa group.

Table 7: S. creatinine (mg/dl) as influenced by lycopene and anthocyanin in female albino rats treated with dexamethasone through seven weeks.

Phases	Treatments					
	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho+ Dex	Dex+ Lyco	Dex+ Antho
I	0.48[a]	0.66[b]	0.74[c]	0.55[a]	0.48[a]	0.48[a]
II	0.58[a]	0.94[c]	1.08[d]	0.82[c]	0.60[b]	0.64[b]
III	0.83[c]	1.8[f]	1.37[d]	1.18[d]	1.18[d]	1.3[d]
General Means	0.63[b]	1.13[d]	1.06[d]	0.85[c]	0.75[c]	0.81[c]

*Values represent as means of 4 replicates.

* Phases I, II, III refers to the blood samples were taken after 15, 30 & 45 days, respectively.

*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

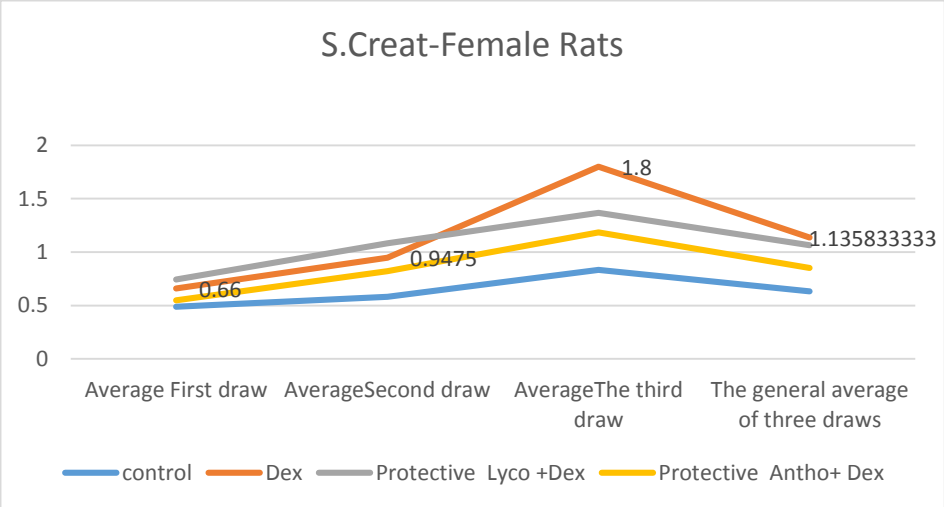


Fig. 11a: Serum creatinine (mg/dl) as influenced by lycopene and anthocyanin as protective agents in female albino rats treated with dexamethasone through three phases.

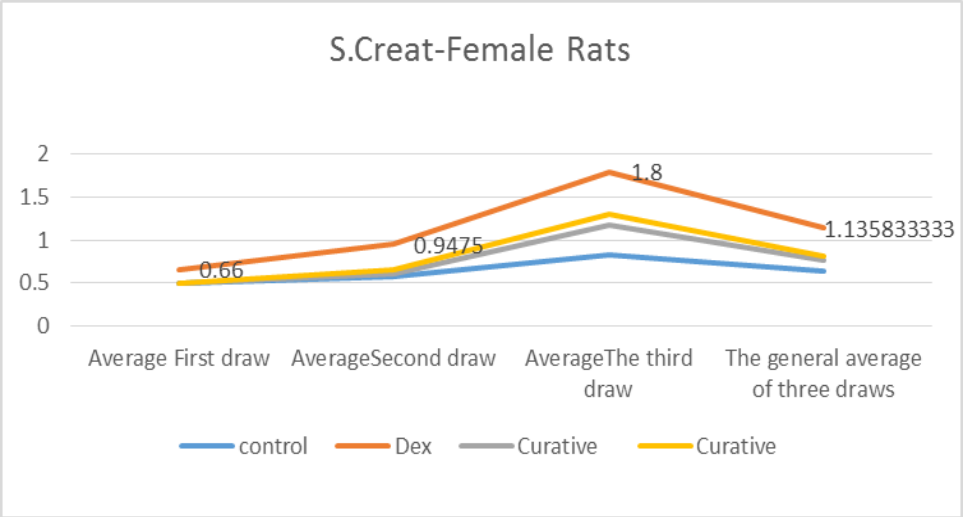


Fig. 11b: Serum creatinine (mg/dl) as influenced by lycopene and anthocyanin as curative agents in female albino rats treated with dexamethasone through three phases.

Comparison average of s. creatinine in male and female

Regarding S. creatinine show lowering level of female albino rats compared to male rats, in **(Fig. 12)**. Meanwhile dexa show higher comparable levels in both male and female albino rats. However, male rats show lower level due to lyco-dex group, while antho in protective group and both lyco and antho in curative group show lower in s. creatinine in female compared to male albino rats. These results reflect that curative groups were more affected with lycopene and anthocyanin than protective group on s. creatinine in female rats. Meanwhile in male rat's protective groups were more affect with lycopene rather than anthocyanin treatment compared to curative groups.

According to the previous comparison, these current results agree with El-Wakf *et al*.[29] in their study on plum extract against dexamethasone-induced osteoporosis in male rats showed significant elevation in serum creatinine (CR) level as compared to control group. Also, Pereira *et al.*[61] found High serum creatinine after intravenous dexamethasone administration, that's mean Dexamethasone increases creatinine. Moreover, treatment of hyperlipidemic rats with lycopene produced significantly decreased creatinine levels ($P<0.05$) suggesting its nephroprotective effect against hyperlipidemia[62]. In addition, lycopene decreases creatinine levels in rat serum[63]. Meanwhile, Shiyan *et al.*[64] reported that anthocyanin pigment from red cabbage extract can decrease the levels of creatinine on their study on nephroprotective of anthocyanin pigments extract from red cabbage against gentamicin-captopril-induced nephrotoxicity in rats. At the same trend, lycopene reduces s. creatinine[65], this finding in their study in title: Impact of Celecoxib on serum creatinine along with beneficial effects of lycopene on albino rats; an observational study. On the other hand, Pre-administration, post-administration or co-administration of tomato extract (30 mg/kg) with injection of gentamycin (100 mg/kg, i.p) significantly decreased creatinine when compared to the affected group[66]. On their study on the effect of tomato extract (*Lycopersicon esculentum*) on gentamycin-induced acute kidney injury in albino Wistar rat. In addition, lycopene supplementation significantly decreased creatinine as[62] on their study on efficacy of lycopene on modulation of renal antioxidant enzymes, ACE and ACE gene expression in hyperlipidaemic rats. Besides, these current results agree with[50] they reported that dexamethasone causes significant elevations in aminotransferases enzymes (AST and ALT), and creatinine in male rat.

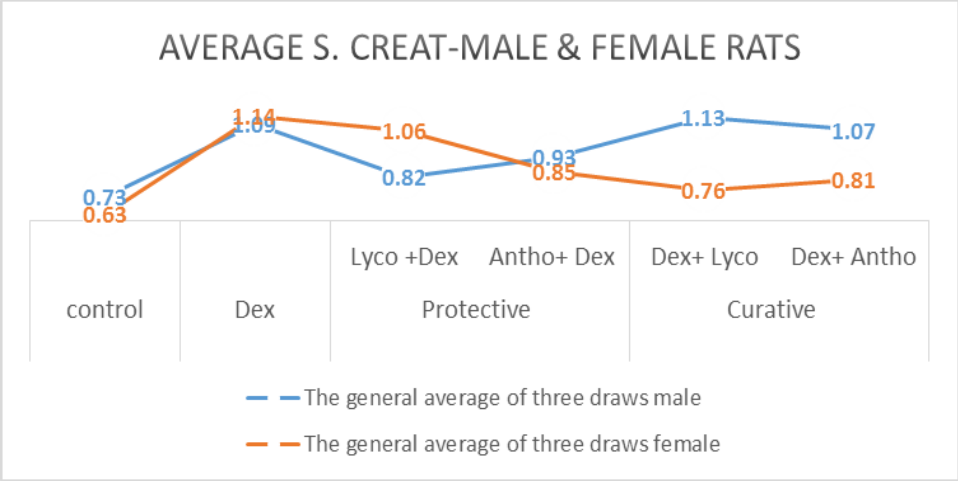


Fig. 12: Serum creatinine (mg/dl) as influenced by lycopene and anthocyanin in male and female albino rats treated with dexamethasone through three phases.

Body weight of male and female albino rats

Male albino rats weight

Results of body weight influenced with lycopene and anthocyanin of male albino rats treated with dexamethasone presented in **(Table 8 and Fig. 13 a,b)**. Results reveal that dexamethasone treatment induced body wight loss compared to normal control group through all seven weeks and in average mean from 231.3 to 174.8 and the weight loss was markedly in third week where body weight was 143.5 compared to 239.2 gram. However, in protective group lycopene and anthocyanin treatment slightly body weight was enhanced but still lower than normal control group, except anthocyanin show little improvement in body weight at fifth week.

Meanwhile, in curative group lycopene and anthocyanin treatment show enhanced body weight through first, second, third and sixth weeks, but after that body weight still lower than dexamethasone group until the seventh week.

Table 8: Body weight (g) as affected by lycopene & anthocyanin as protective & curative agents on male albino rats treated with dexamethasone for 7 weeks.

Groups / Weeks	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho +Dex	Dex+ Lyco	Dex+ Antho
Zero Time	194.6^{b}	178.4^{c}	195.4^{b}	180.2^{b,c}	198.6^{b}	187.8^{b,c}
1	226.6^{a}	164.2^{d}	168^{d}	163^{d}	166.6^{d}	165.6^{d}
2	237.8^{a}	157.8^{d}	161.6^{d}	165.5^{d}	188.3^{b,c}	167.2^{d}
3	239.2^{a}	143.5^{f}	148.5^{f}	151.3^{d}	203.7^{a,b}	206^{a,b}
4	220.6^{a}	165.8^{d}	154.3^{d}	157^{d}	165^{d}	165^{d}
5	228.6^{a}	189.5^{c}	183^{c}	195.7^{b}	165.7^{d}	166^{d}
6	247.8^{a}	210.5^{a,b}	191.3^{b}	200.7^{a,b}	206.3^{a,b}	207^{a,b}
7	255^{a}	188.8^{c}	182^{c}	194^{b}	191^{b}	177.7^{c}
Average body weight	231.3^{a}	174.8^{c}	173^{c}	175.9^{c}	185.6^{b,c}	180.3^{b,c}

*Values represent as means of 4 replicates.

*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

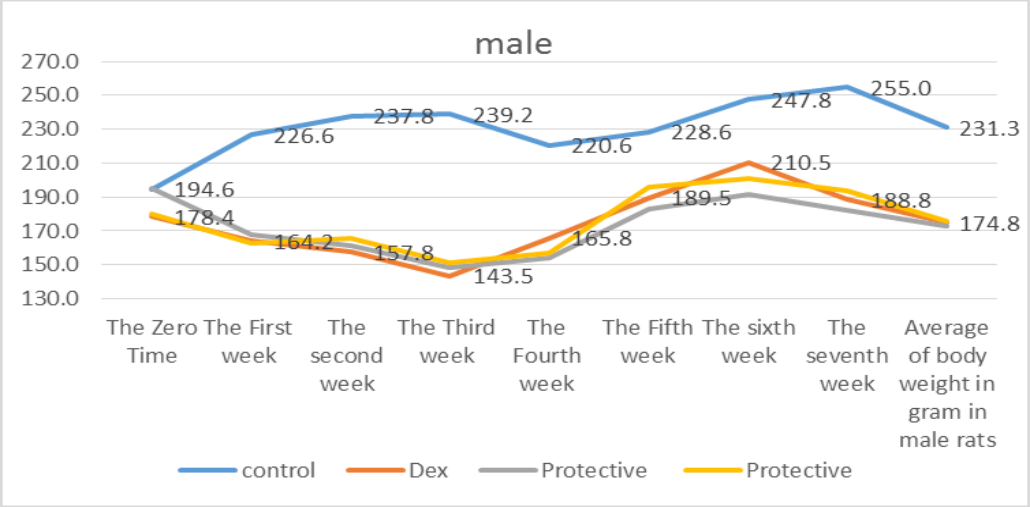


Fig. 13a: Average body weight in gram of male albino rats treated with dexamethasone and give lycopene & anthocyanin as protective agent for seven weeks.

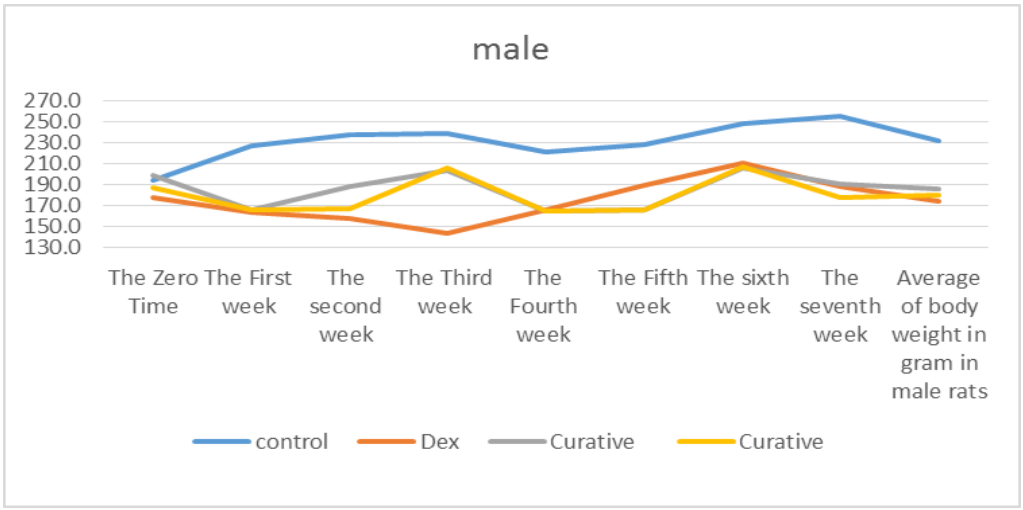


Fig. 13b: Average body weight in gram of male albino rats treated with dexamethasone and give lycopene & anthocyanin as curative agent for seven weeks.

Body weight Female albino rats

Results of body weight influenced with lycopene and anthocyanin of female albino rats treated with dexamethasone illustrated in (**Table 9 and Fig. 14 a,b)**. Results depict that dexamethasone treatment induced body wight loss compared to normal control group and the highest loss was observed in fifth week where body weight was 142 in dexa group compared to 202.4 in NCG. However, as protective agents' lycopene and anthocyanin treatment enhanced body weight starting from the first, second, third, fourth, fifth and sixth weeks compared to dexa group. In addition, lycopene and anthocyanin show similar improvement in body weight. Meanwhile, lycopene and anthocyanin as curative agents treatment show enhancement in body weight starting from the first week till the end of seventh week and markedly at fourth, fifth and the sixth weeks, also at the seventh week body weight show little increase than dexamethasone group.

Comparison between body weight in male and female rats as influenced by lycopene and anthocyanin as protective and curative agents and treated with dexamethasone.

Regarding general means for male and female groups **(Fig. 15)**, reveal that body weight of male albino rats was higher in body weight in all treatments compared to female's albino rats. On one side dexa induce body loss in both male and female rats. On the other

hand, lycopene and anthocyanin either protective or curative agent's treatment show enhancement in body weight and markedly at curative groups compared to protective groups. As these comparison, body weight results showed marked body weight reduction in DEX-treated rats as compared to normal control[29]. Filippopoulou *et al*.[67] reported that dexamethasone treatment led to less weight gain during the treatment period without affecting food consumption these effects of dexamethasone were similar between male and female mice. In addition, anthocyanins even in normal circumstances have the capability to reduce body weight and food[68]. El-Gerbed[69] reported that lycopene reduces body weight, not only that but also, lycopene reduces kidney weight.

Table 9: Body weight (g) of female albino rats treated with dexamethasone and give lycopene & anthocyanin as protective & curative for 7 weeks.

Groups / Weeks	Control	Dex	Protective		Curative	
			Lyco +Dex	Antho +Dex	Dex+ Lyco	Dex+ Antho
Zero Time	**159.2^{c}**	**155.2^{d}**	**160.2^{c}**	**168.8^{c}**	**159.8^{c}**	**150.6^{d}**
1	**188.4^{b}**	**178.8^{c}**	**181^{b}**	**158.8^{d}**	**182.6^{b}**	**186.6^{b}**
2	**179.4^{c}**	**149.8^{f}**	**166.2^{c}**	**167.2^{c}**	**156^{d}**	**149.6^{f}**
3	**189.2^{b}**	**163^{c}**	**168.4^{c}**	**182.4^{b}**	**167.8^{c}**	**163^{c}**
4	**210.6^{a}**	**148.4^{f}**	**177.8^{c}**	**178.2^{c}**	**194.4^{b}**	**193.6^{b}**
5	**202.4^{a}**	**142^{f}**	**157^{d}**	**162.2^{c}**	**166.2^{c}**	**168.8^{c}**
6	**198.4^{b}**	**153^{d}**	**154.6^{d}**	**163^{c}**	**173.8^{c}**	**167.6^{c}**
7	**219.8^{a}**	**172^{c}**	**171.6^{c}**	**171.8^{c}**	**175.2^{c}**	**176.2^{c}**
Average body weight	**193.42^{b}**	**157.78^{d}**	**167.1^{c}**	**169.05^{c}**	**172^{c}**	**169.5^{c}**

*Values represent as means of 4 replicates.

*Superscript letters (a, b, c, d and f) indicate significant differences ($p<0.05$).

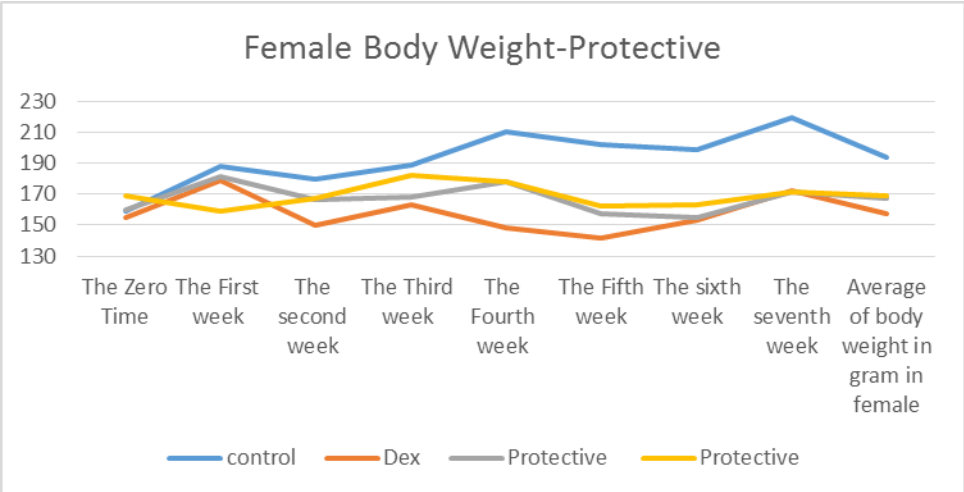


Fig. 14a: Influence of lycopene and anthocyanin as protective substances on body weights of female albino rats treated with dexamethasone for seven weeks.

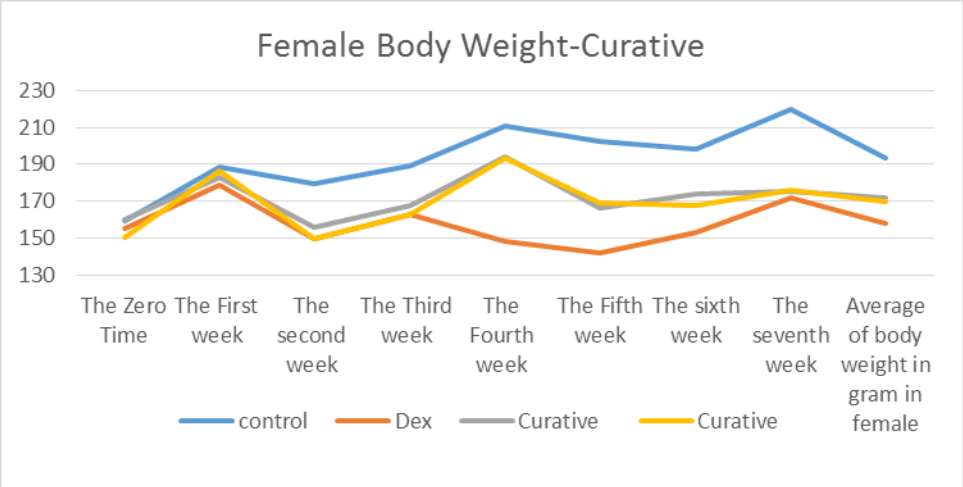


Fig. 14b: Influence of lycopene and anthocyanin as curative substances on body weights in female albino rats treated with dexamethasone for seven weeks.

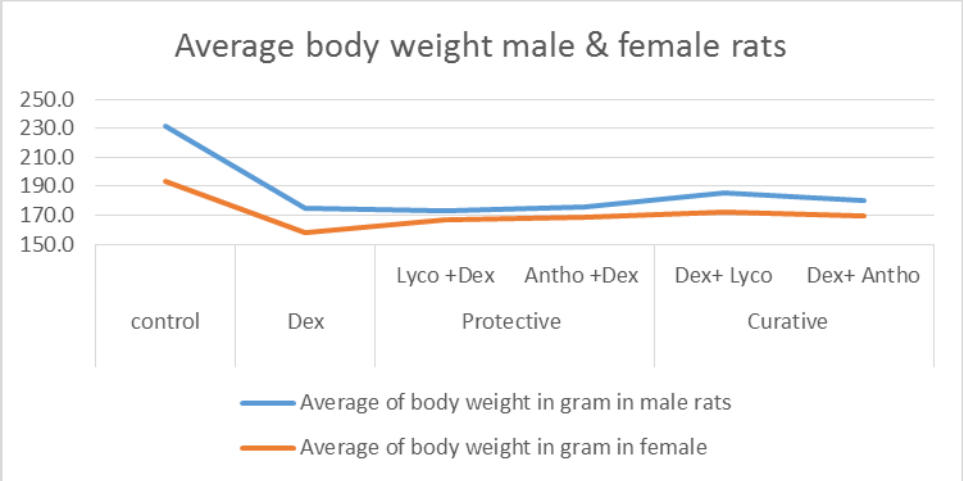


Fig. 15: Comparison between body weight in male and female rats as influenced by lycopene and anthocyanin as protective and curative agents and treated with dexamethasone.

Conclusion

The results of the present study reveal that tomato contained potential antioxidant bioactive compounds particularly lycopene, which if properly utilized could provide source of biologically active nutraceutical ingredient/medicine application. It also shows its titanic importance as therapeutic agent in preventing or curing the diseases caused due to oxidative stress as induced due to side effect of some medicinal drugs like dexamethasone Also, suggests use anthocyanin which extracted from pomegranate peel as protective and curative agent against oxidative stress of long term of using dexamethasone. Also, results showing that sometimes anthocyanins reveal more potent than lycopene and vice versa lycopene in other parameters was more potent than anthocyanin. These current results showed that the potential of these substances should be used as medicine against the diseases caused by free radicals.

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تأثير اللايكوبين والأنثوسيانين على وظائف الكبد والكلى في ذكور وإناث الجرذان البيضاء المعالجة بالديكساميثازون

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تمت دراسة تأثير اللايكوبين والأنثوسيانين كعوامل وقائية وعلاجية على ذكور وإناث الجرذان البيضاء المعاملة بالديكساميثازون لمدة سبعة أسابيع كمعاملة وقائية ، بينما تمت المعاملة العلاجية لمدة ثلاث أسابيع ثم المعاملة باللايكوبين والأنثوسيانين لمدة سبعة أسابيع على وظائف الكبد إنزيمات نقل مجموعة الأمين (AST وALT) ووظائف الكلى (الكرياتينين) ، وأوزان الجسم أظهرت النتائج أن المعاملة بالديكساميثازون أدت إلى نقص أوزان الجسم ، بالإضافة إلى ذلك لوحظ ارتفاع مستويات AST وALT والكرياتنين في السيرم. وقد أدى استخدام اللايكوبين والأنثوسيانين كمضادات أكسدة طبيعية مستخلصة من مخلفات قشور الطماطم والرمان إلى تعديل التأثير للمستويات المرتفعة من AST، ALT، والكرياتينين مقارنة بمجموعة الكنترول المعاملة بالديكساميثازون ، والكنترول غير المعاملة. وفي ذات الوقت ، فإنه رغم النقص الذي حدث في أوزان جسم الفئران نتيجة المعاملة بالديكساميثازون فقد صاحبه تحسناً طفيفا في وزن الفئران نتيجة المعاملة باللايكوبين والأنثوسيانين مقارنة بمجموعة الديكساميثازون. وقد أظهرت النتائج أنه يمكن استخدام كل من اللايكوبين والأنثوسيانين كمواد وقائية أو علاجية رخيصة الثمن ومتاحة وذلك لمواجهة الآثار الجانبية غير المرغوب فيها للإجهاد التأكسدي الناجم عن المعاملة بالديكساميثازون.