Protective Potential of Lycopene Enriched Tomato Extract against Dexamethasone Induced Hepatic and Renal Damage in Mice

A. Koul[1*], J. Kaur[1] and N. A. Chugh[1]

[1]*Department of Biophysics, Punjab University, Chandigarh-160014, India.*

Authors' contributions

This work was carried out in collaboration among all authors. The project was conceived and designed by author AK. Author JK was the project fellow who along with author NAC performed the experimental work including animal handling. Finally, all the three authors evaluated the data and shaped it into a manuscript. All authors read and approved the final manuscript

Article Information

Original Research Article	

ABSTRACT

Aim: The present study was carried out to determine the protective potential of Lycopene enriched tomato extract (LycT) against hepatic and renal damage caused in mice by dexamethasone administration.
Study Design: Male LACA mice were randomly divided into four treatment groups (n=6-7 animals per group) depending upon the treatment they received. Group I (control) animals served as control and were orally administered with olive oil (vehicle) thrice a week for five weeks. Group II (DEX) animals were intraperitoneallly (i.p.) administered with dexamethasone at a dose of 5 mg/kg b.w. on alternate days for three weeks. Group III (LycT) animals were orally (p.o.) administered with LycT at a dose of 5 mg/kg b.w. on alternate days for five weeks. Group IV (LycT+DEX) animals were co-administered with LycT (p.o.) and dexamethasone (i.p.) according to the above mentioned dose regimen
Results: Dexamethasone caused hepatic and renal damage as evident from disturbed histoarchitecture, deranged levels of organ function markers (alkaline phosphatase, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, total and direct

___

Corresponding author: E-mail: drashwanikoul@yahoo.co.in;

bilirubin, urea, creatinine) and enhanced level of cell damage (lactate dehydrogenase) and oxidative stress (lipid peroxidation) markers. Increased blood glucose level, decreased hepatic glycogen level along with inhibited activities of enzymes involved in glycolysis (hexokinase, phosphoglucoisomerase) indicated altered glucose metabolism in DEX group. The mitigation in histoarchitectural alterations, cell damage and oxidative stress markers, improved levels of organ function markers, blood glucose level along with ramped up antioxidant defense system indicated the protective potential of LycT against dexamethasone induced ill effects.

Conclusions: These results point towards beneficial effects of LycT against dexamethasone induced damage to hepatic and renal tissues in mice.

1. INTRODUCTION

The use of corticosteroids is associated with many short- and long-term side effects [1]. The range and severity of adverse effects experienced with use of corticosteroids is dependent upon dosage and duration of treatment [2]. Many of the side effects are reversible after the termination of corticosteroid treatment while other side effects may be permanent. When steroids are used for long periods of time, or taken on multiple occasions, more serious side effects may occur. Dexamethasone is a synthetic corticosteroid that has appreciable anti-inflammatory and immunosuppressive actions. It is considered as one of the most important drugs required in a basic health system and World Health Organization (WHO) has included it in its list of essential medicines [3]. For several decades, dexamethasone is being used in various ailments including management of inflammatory conditions. Unfortunately, the use of dexamethasone has been linked to adverse effects including hypertension, hyperglycemia, muscle breakdown, hepatic steatosis, electrolyte imbalance, delayed healing, increased susceptibility to infections and even psychiatric disturbances [2,4]. It has been reported that long term use of glucocorticoids causes damage to liver and kidneys [2]. Studies have demonstrated that prenatal and neonatal dexamethasone exposure in rodents may lead to renal damage and potential renal failure in later life [5-7]. Dexamethasone leads to impaired glucose and lipid metabolism, increased hepatic gluconeogenesis, adiposity (central and visceral), fat accumulation in liver which can contribute to insulin resistance resulting in hyperglycemia induced disorders [8]. High levels of circulating glucocorticoids including dexamethasone accelerate metabolic rates, increase free radicals and decrease antioxidant defense capacity which consequentially leads to oxidative stress [9,10,11]. Studies have demonstrated that dexamethasone administration enhanced the levels of oxidative stress indicators in liver and kidneys of rodents [12-16]. Considering the clinical usefulness of dexamethasone, it becomes essential to minimize its associated adverse effects. This has instigated the quest for agents that could lessen the accompanying undesired effects and warrant its safe use.

Natural products or dietary components are appreciated for their suitability in counteracting drug induced organ toxicities and chronic diseases owing to their easy availability and acceptability [17,18,19]. Worldwide, tomatoes are commonly used for their flavor and nutritive values. Carotenoids are powerful antioxidants that confer yellow, orange and red color to fruits and vegetables. Among the carotenoids, lycopene has the highest antioxidant activity which has been attributed to the presence of maximum number of double bonds in its structure. Lycopene is the most abundant carotenoid found in tomatoes (*Lycopersicon esculentum L.*). It is also present in rosehips, watermelon, papaya, pink grapefruit, guava, apricot etc [20]. The strong radical scavenging and antioxidant ability of lycopene is responsible for its several health benefiting effects. The cancer chemopreventive properties of carotenoids has been very widely reviewed and several epidemiological and experimental studies demonstrate the same [21]. Studies conducted in our laboratory have shown considerable cancer chemopreventive action of lycopene enriched tomato extract (LycT) against hepatic and skin cancer in mice [14,22-25]. The beneficial effects of lycopene against xenobiotic induced toxicities has also been reported [26-28]. We have previously reported the protective effects of LycT against doxorubicin nephrotoxicity in mice [29]. This was evident from improved urea and creatinine levels,

reduced histopathological damage and lipid peroxidation level, and upregulation of antioxidant defense system.

The diversified biological effects of tomato and tomato-based products and the recognized health potential of carotenoids makes it worthwhile to examine their potential protective effects. Therefore, this study was designed to explore the effects of LycT (carotenoid enriched extract) against dexamethasone induced hepatic and renal damage in mice.

2. MATERIALS AND METHODS

2.1 Chemicals

Dexamethasone was obtained from Sigma Chemical Co. (St Louis, MO, USA). Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), ethylenediaminetetraacetic acid (EDTA), nicotinamide adenine dinucleotide (NADH), oxidized glutathione (GSSG), reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), sodium acetate, thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA) etc were obtained from local reputed firms (Sisco Research Laboratory, Central Drug House, HiMedia). Other chemicals used for reagent preparation were obtained from the above mentioned local firms and were of highest purity/analytical grade. Kits for estimation of kidney and liver function markers were obtained from Reckon Diagnostics Private Limited (Gujarat, India). Fresh tomatoes for the preparation of LycT were obtained from the local market.

2.2 Preparation of Lycopene Enriched Tomato Extract (LycT)

LycT was prepared in our laboratory from red tomatoes by following the method described previously [22,23]. Tomato paste was prepared from fresh and unpeeled tomatoes. This paste was transferred to a 1000 ml amber coloured flask and wrapped with aluminium foil so as to avoid exposure to light. This was followed by heating the tomato paste at 80ºC for 1 hour and then cooling to 4ºC. It was then treated with a solvent mixture of hexane/acetone/ethanol (2:1:1) containing 2.5% BHT. This mixture was then continuously stirred for 1 hr on a magnetic

stirrer at 4ºC. After adding distilled water, the mixture was again stirred for another 30 minutes. After this, separation of polar and non-polar layers was done by leaving the flask undisturbed. LycT appeared in the top organic layer and was transferred to a large glass plate in order to enable fast vaporisation of hexane leaving behind red-orange droplets of LycT. All the steps of extract preparation including its storage were done in dark to avoid light exposure. LycT was maintained at 4ºC until its further use. Tomato extracts including the one prepared for the present study have been previously characterised using spectroscopic techniques [22,30]. NMR and FT-IR spectroscopy demonstrated the presence of groups characteristic of lycopene (=CH, =CHCH$_2$, =CCH$_3$, =CCH$_2$, trans C=C, carbon-carbon double bond stretching, –CH$_2$ bending, CH$_3$/CH$_2$ stretch, –CH$_3$ group etc). Ultraviolet-Visible (UV-Vis) spectroscopy revealed that this extract exhibited absorbance maxima at 444, 470 and 503 nm. In order to avoid interference from other carotenoids the content of lycopene in the extract was evaluated at 503 nm using 1.72×10^5 L mol^{-1} cm^{-1} as the extinction coefficient [22-26]. As determined using UV-Vis spectroscopy it was observed that this extract contained an average lycopene content of 12-14 mg/kg of tomato. It has been reported that use of dietary fats enhances lycopene absorption into the intestinal mucosa [31]. Considering this, olive oil was used to reconstitute LycT before its oral administration to animals. Pilot studies carried out in our laboratory have indicated no consequential alterations between control (untreated) and olive oil administered animals (data not included).

2.3 Animal Model and Experimental Conditions

Random bred male LACA mice (25–30 g each) procured from Central Animal House, Panjab University, Chandigarh (India) were housed in polypropylene cages. The cages were bedded with clean rice husk and the animals were provided *ad libitum* drinking water and standard animal pellet diet (Ashirwad Industries Ltd., Tirpari, Distt. Ropar, Punjab, India). The animal room was maintained at a temperature of 21±1ºC and humidity of 50-60%. All the experimental protocols were initially approved by the Institutional Ethics Committee of Panjab University, Chandigarh (India) and conducted according to the Indian National Science Academy guidelines for the use and care of

experimental animals. The animals were acclimatized for one week before the commencement of the treatment regimen.

After acclimatization, the animals were randomly divided into four treatment groups (n=6-7 animals per group) depending upon the treatment they received Fig. 1. Group I (control) animals served as control and were administered with olive oil (0.1ml; vehicle) orally on alternate days for five weeks. Group II (DEX) animals were intraperitoneally i.p. administered with dexamethasone at a dose of 5 mg/kg b.w. on alternate days for three weeks. Group III (LycT) animals were orally p.o. administered with LycT at a dose of 5 mg/kg b.w. on alternate days for five weeks. Group IV (LycT+DEX) animals were co-administered with LycT (p.o.) and dexamethasone (i.p.) according to the above mentioned dose regimen. The dose for dexamethasone administration was selected from literature reports [32]. The dose for LycT administration was standardized in our laboratory and selected based on our previous reports [22-26].

2.4 Sample Preparation

The animals were kept on overnight fasting before obtaining blood for serum preparation and excising out liver and kidney tissues for biochemical and histoarchitectural studies. 0.5% proparacaine hydrochloride ophthalmic solution was used to anaesthetize the mouse eye and whole blood was obtained from the retro-orbital plexus using a glass capillary. Blood was collected in a plain (without anti-coagulant) microcentrifuge tube and left undisturbed (allowing clot formation) for 4 hours at 37ºC. After this, the tubes were kept at 4ºC for 30 minutes and then centrifuged at 3000 x g for 15 minutes. The supernatant (serum) thus obtained was stored at -20°C until use. Tissue perfusion was done with cold 0.9% NaCl. The tissues were then blot dried, weighed and processed. The tissues were homogenized in 50 mM Tris buffer (pH 7.4) to obtain 10% homogenate (w/v). Aliquots of 10% homogenate were kept at 4°C for analysis of reduced glutathione and the remaining homogenate was subjected to cold centrifuge at 10,000 x g for 30 minutes. The pellet was discarded and the supernatant [post mitochondrial fraction (PMF)] was used for the biochemical analysis of antioxidant defense system, lipid peroxidation and carbohydrate metabolizing enzymes. A part of liver tissue

was processed for estimation of glycogen content.

2.5 Glucose Metabolism

2.5.1 Glucose

Serum glucose level was analyzed using a commercially available assay kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit is based on the procedure described by Trinder [33]. Glucose oxidase oxidizes β-D-glucose to gluconic acid and hydrogen peroxide. The hydrogen peroxide thus produced is acted upon by peroxidase and oxygen is liberated. The liberated oxygen is transferred to chromogen system consisting of 4-aminoantipyrene and phenolic compound to produce a red colored quinoneimine dye whose absorbance is read at 505 nm. The glucose concentration is proportional to the absorbance of the colored product and is expressed as mg/dL.

2.5.2 Glycogen

The glycogen content in the hepatic tissue was estimated using the protocol described by Seifter et al. [34]. This method involved a series of steps including alkali digestion of tissue, ethanolic precipitation of glycogen, hydrolysis of the precipitate and determination of glucose using anthrone. A part of liver tissue was immersed in 30% KOH solution for alkali digestion of tissue. This was followed by precipitation using ice cold ethanol (95%) and centrifugation at 3000 x g for 20 minutes. The pellet obtained was dissolved in water and vortexed. The precipitate was then hydrolysed by adding sulphuric acid. 0.2% anthrone solution was then added and the absorbance of the reaction mixture was read at 640 nm. A standard of glucose was plotted to determine the glucose content in the precipitate. A conversion factor of 1.11 (Morris factor) was used to convert glucose values into glycogen content [35]. The glycogen content is expressed as mg glycogen/100 mg liver.

2.5.3 Glucose metabolizing enzymes

2.5.3.1 Hexokinase

The hexokinase activity in hepatic and kidney tissues was estimated according to the method described by Crane and Sols [36] and Brandstrup et al. [37]. Hexokinase catalyzes the ATP dependent reaction of glucose into

glucose-6-phosphate. The inorganic phosphorous (Pi) is reacted with ammonium molybdate to form phosphomolybdate which upon reduction with 1-amino-2-napthol-4-sulphonic acid (ANSA) forms a blue colored product whose absorbance is measured at 660 nm. The reaction mixture that was used to incubate hexokinase containing PMF with substrate included 0.1 M histidine-tris EDTA buffer, 25 mM ATP, 0.4 mM $MgCl_2$, 20% sodium sulfide and distilled water. This was then divided into two parts-blank and test. The reaction of blank was terminated at zero seconds by adding 10% TCA, while the test was allowed to incubate at room temperature for 30 minutes, followed by termination of reaction by adding TCA. The tubes were then centrifuged at 3000 x g for 5 minutes and the supernatant obtained was reacted with 2.5% ammonium molybdate solution and ANSA. The increase in the absorbance is directly proportional to the hexokinase activity. A standard curve using Pi was plotted and the amount of Pi in the test samples was calculated from the standard curve. The enzyme activity is expressed as nano moles of Pi liberated/min/mg protein.

2.5.3.2 Phosphoglucoisomerase

The phosphoglucoisomerase activity in hepatic and kidney tissues was estimated according to the procedure described by Horrocks et al. [38]. This enzyme catalyzes the isomerization conversion of glucose-6-phosphate to fructose-6-phosphate. To the test and blank tubes buffered substrate containing glucose-6-phosphate and borate buffer (10 M, pH 7.8) was added. This was followed by addition of PMF to the tubes. Then to the blank, coloring reagent containing HCl and resorcinol-thiourea was added. The tube containing test sample was allowed to incubate for 30 minutes at room temperature, followed by the addition of the coloring reagent. The tubes were then incubated for 15 minutes at 75ºC in a water bath. The absorbance of the colored product formed was then taken. Fructose-6-phosphate in the presence of concentrated HCl gets dehydrated and converts to hydroxyl methyl furfural. This upon further condensation with resorcinol forms a cherry colored complex whose absorbance is measured at 470 nm. The increase in absorbance is proportional to phosphorglucoisomerase activity and is expressed as nano moles of fructose formed/min/mg protein.

2.6 Liver Function Markers

2.6.1 Serum glutamate oxaloacetate transaminase (SGOT)

SGOT activity was analyzed using a commercially available enzyme assay kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit is designed according to the procedure described by Karmen et al. [39]. L-aspartate and α-ketoglutarate react in the presence of SGOT to yield oxaloacetate and L-glutamate. Oxaloacetate is reduced by malate dehydrogenase to yield L-malate accompanied by the oxidation of NADH to NAD. The decrease in the absorbance of NADH at 340 nm is proportional to SGOT activity and is expressed as International Units/L (IU/L).
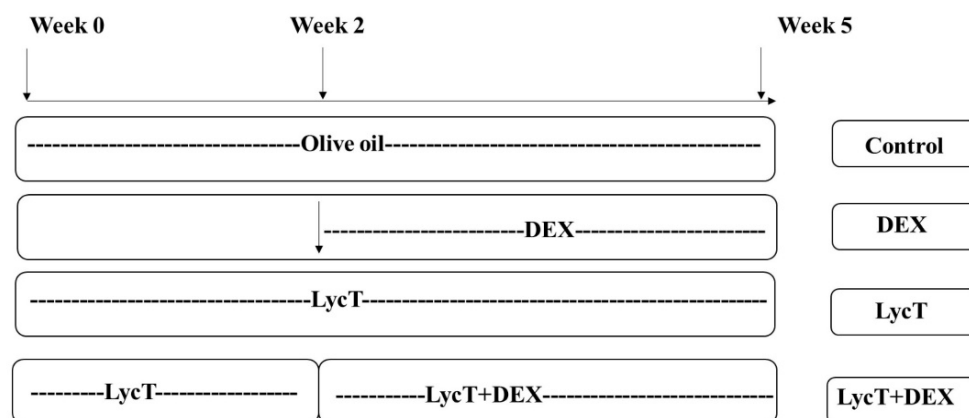

Fig. 1. Treatment regimen of mice

2.6.2 Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT activity was analyzed using a commercially available enzyme assay kit (Reckon Diagnostics Private Limited (Gujarat, India). The kit is designed according to the procedure described by Henry et al. [40]. L-alanine and α-ketoglutarate react in the presence of SGPT to yield pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate accompanied by the oxidation of NADH to NAD+. The decrease in the absorbance of NADH at 340 nm was proportional to SGPT activity and is expressed as IU/L

2.6.3 Alkaline Phosphatase (ALP)

ALP activity in serum was analyzed using a commercially available enzyme assay kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit was designed according to the procedure recommended by German Society for Clinical Chemistry [41]. ALP in the presence of magnesium ions hydrolyses p-nitrophenyl phosphate into p-nitrophenol and phosphate. The increase in absorbance at 405 nm due to generation of p-nitrophenol is proportional to ALP activity and is expressed as IU/L.

2.6.4 Bilirubin

Total and direct bilirubin in serum were analyzed using a commercially available kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit is based on the procedure described by Jendrassik and Grof [42]. Bilirubin reacts with diazotized sulphanilic acid to form a colored azocompound whose absorbance is measured at 546 nm and reflects the concentration of bilirubin. Bilirubin concentration is expressed as mg/dL.

2.7 Cell Damage Marker-Lactate Dehydrogenase (LDH)

LDH activity in serum was estimated according to the procedure described by Bergmeyer [43]. Pyruvate is reduced by LDH to yield lactate accompanied by the oxidation of NADH to NAD+. The reaction mixture contained potassium phosphate buffer (50 mM, pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH and an appropriate amount of sample. A blank was also run parallel without sample in it. The rate of decrease in absorbance at 340 nm due to the formation of NAD+ is indicative of LDH activity and is expressed as nanomoles of NADH oxidized/min/mg protein.

2.8 Kidney Function Markers

2.8.1 Urea

Serum urea level was analyzed using a commercially available kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit is based on the modified Berthelot method as described by Chaney and Marbach [44]. Urease breaks down urea into ammonia and carbon dioxide. In the presence of sodium nitroprusside, ammonia reacts with hypochlorite and salicylate to form dicarboxyindophenol, a colored compound whose absorbance is read at 578 nm which is proportional to the urea content. The concentration of urea in the serum is expressed as mg/dL.

2.8.2 Creatinine

Serum creatinine level was analyzed using a commercially available kit [Reckon Diagnostics Private Limited (Gujarat, India)]. The kit is based on the method described by Folin [45]. In alkaline medium, creatinine reacts with picric acid leading to the formation of a red creatinine picrate complex (Jaffe's Reaction) whose absorbance is read at 510 nm. The intensity of the color developed is proportional to the creatinine content. The concentration of creatinine in the serum is expressed as mg/dL.

2.9 Oxidative Stress Marker and Antioxidant Defense System

2.9.1 Lipid peroxidation (LPO)

LPO level in hepatic and renal tissues was estimated according to the method described by Trush et al. [46]. Tris-Cl buffer (0.1 M, pH 7.4) containing NADPH (0.3 μM) was added to PMF and this was then was subjected to incubation at 37°C for 1 hour. This was followed by ice cold precipitation by addition of cold TCA-HCl mixture and centrifugation at 1500 x g for 15 minutes. The supernatant was then reacted with 0.67% Thiobarbituric Acid (TBA) and the reaction mixture was boiled for 30 minutes. The oxidative deterioration of lipids leads to the formation of cycloperoxides which on cleavage form malondialdehyde (MDA). MDA reacts with (TBA) to generate pink colored MDA-TBA chromophore whose absorbance is read at 532

nm. The concentration of MDA-TBA chromophore is indicative of LPO and is expressed as nanomoles MDA-TBA chromophore formed/mg protein using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

2.9.2 Reduced glutathione (GSH)

GSH level in hepatic and renal tissues was estimated as the total non-protein sulphydryl groups according to the method described by Moron et al. [47]. The tissue homogenates were precipitated using 25% trichloroacetic acid and centrifugation at 1500 x g for 10 minutes. The free-SH groups were assayed in the supernatant by adding 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer. Reduction of 5, 5´-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) by the –SH groups of GSH forms a yellow-colored chromophore, 5-thionitrobenzoic acid whose absorbance is read at 412 nm. A standard curve using GSH was run to determine the levels in the test samples and expressed as nanomoles of GSH/mg protein.

2.9.3 Glutathione peroxidase (GPx)

GPx activity in hepatic and renal tissues was estimated according to the method described by Lawrence and Burk [48]. The reaction mixture contained 50 mM potassium phosphate buffer, 0.15 M GSH, 0.125 M sodium azide, 0.132 M NADPH, 2 mM H_2O_2, glutathione reductase (10 units/ml) and an appropriate amount of PMF. GPx catalyses the conversion of hydrogen peroxide to water in the presence of reduced GSH to form GSSG and accompanied by the oxidation of NADPH to NADP+. The oxidation of NADPH which reflects the activity of GPx was measured by the decrease in absorbance at 340 nm. GPx enzyme activity is expressed as nano moles of NADPH consumed/min/mg protein using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.9.4 Glutathione reductase (GR)

GR activity in the hepatic and renal tissues was measured by the method of Williams and Arscott [49]. GR catalyzes the NADPH dependent reduction of GSSG to GSH. The assay reaction mixture was composed of 100 mM phosphate buffer (pH 7.6), 60 mM EDTA, 2 mM NADPH, 60 mM GSSG, 20 mg/ml BSA, distilled water and appropriate amount of PMF. The enzyme activity was determined by following the decrease in absorbance at 340 nm due to NADPH oxidation and expressed as nanomoles of NADPH consumed/min/mg of protein using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.9.5 Catalase (CAT)

Catalase activity in the hepatic and renal tissues was determined by the method described by Luck [50]. An appropriate amount of PMF was added to 50 mM phosphate buffer containing hydrogen peroxide. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. The activity of catalase is reflected as the decomposition of hydrogen peroxide which is measured at 240 nm. The enzyme activity was expressed as IU/mg protein using an extinction coefficient of 0.0394 $mM^{-1}cm^{-1}$.

2.9.6 Superoxide dismutase (SOD)

SOD activity in hepatic and renal tissues was determined according to the method described by Kono [51]. This enzyme activity estimation is based on the inhibitory action of SOD on the reduction of nitroblue tetrazolium (90 mM) by the superoxide anions which are produced by the photo-oxidation of hydroxylamine hydrochloride (20 mM, pH 6.0) forming a blue color complex whose absorbance is read at 560 nm. The enzyme activity is expressed as IU/mg protein.

2.10 Protein Estimation

The protein content in the samples was analyzed according to the method described by Lowry et al., [52]. Proteins in the test sample (homogenate and PMF) were reacted with copper ions in an alkaline medium (Lowry's Reagent) and incubated at 37ºC for 10 minutes. After this Folin's reagent was added and the reaction mixture was incubated at 37ºC for 30 minutes. The aromatic amino acids reduce phosphomolybdate-phosphotungsticacid present in Folin's reagent to produce a blue colored complex whose absorbance is noted at 620 nm. A standard curve of bovine serum albumin (BSA) was prepared to determine the protein concentration in the samples.

2.11 Histopathological Studies

After excising out the hepatic and renal tissues, they were immediately immersed in buffered formalin for fixation. Post fixation, the tissues were dehydrated using ascending series of alcohol concentration, followed by clearing using benzene. Embedding of the tissues was done
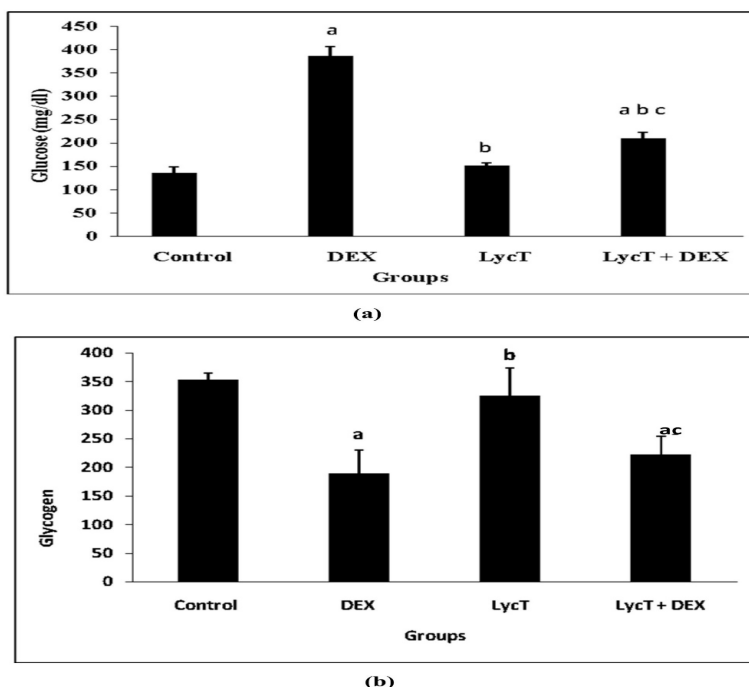
Fig. 2. Modulatory effect of LycT and/or DEX on (a) blood glucose level and (b) hepatic glycogen level

Data is represent as mean ± SD (n=5). Data is analysed by one way ANOVA followed by post hoc test. $^a p≤0.05$ significant with respect to control group ; $^b p≤0.05$ significant with respect to DEX group; $^c p≤0.05$ significant with respect to LycT group

with paraffin wax. Using a hand driven microtome, 5 μm thick paraffin sections were obtained and transferred to glass slides. These slides were then deparaffinized in xylene and stained using hematoxylin and eosin according to the procedure described by Humanson [53].

2.12 Statistical Analysis

Data is expressed as Mean ± S.D. SPSS software was used for the statistical evaluation. One-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test was used for statistical analysis. p≤0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Assessment of Glucose Metabolism

3.1.1 Blood glucose level

A significant increase in blood glucose levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in glucose levels when compared to the animals in

DEX group and increased when compared to control and LycT group. No changes were observed in the glucose levels of LycT group when compared to control group Fig. 2a.

3.1.2 Hepatic glycogen level

A significant decrease in hepatic glycogen levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group did not exhibit any change in glycogen levels when compared to DEX group and decreased when compared to control and LycT groups. No changes were observed in glycogen levels of LycT group when compared to control group Fig. 2b.

3.1.3 Glucose metabolizing enzymes

3.1.3.1 Hexokinase

A significant decrease in hepatic and renal hexokinase levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in hepatic hexokinase levels when compared to DEX group and decreased

when compared to control and LycT groups. Renal hexokinase levels remained unaltered in LycT+DEX group when compared to DEX group. No changes were observed in the hepatic and renal hexokinase levels of LycT group when compared to control group Figs. 3a; 3c.

3.1.3.2 Phosphoglucoisomerase

A significant decrease in hepatic and renal PGI levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in PGI levels when compared to DEX group and remained unaltered when compared to control and LycT groups Figs. 3b; 3d.

3.2 Assessment of Liver Function Markers

3.2.1 SGOT and SGPT

DEX administration caused a significant increase in serum levels of SGOT and SGPT when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in these levels when compared to the animals in DEX group and increased when compared to control and LycT group. No changes were observed in the SGOT and SGPT levels of LycT group when compared to control group Figs. 4a-b.

3.2.2 Alkaline phosphatase

A significant increase in serum ALP levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited no change in serum ALP levels when compared to the animals in DEX group and increased when compared to control and LycT group. No changes were observed in these levels in LycT group when compared to control group Fig. 4c.

3.2.3 Bilirubin

A significant increase in total and direct bilirubin levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in total and direct bilirubin levels when compared to the animals in DEX group. Direct bilirubin levels increased in LycT+DEX group when compared to control and LycT groups while the total bilirubin levels remained altered between these groups. No significant difference was observed in the bilirubin levels in

LycT group when compared to control group Fig. 4e-f.

3.3 Cell Damage Marker

A significant increase in serum LDH levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in serum LDH levels when compared to the animals in DEX group and increased when compared to control and LycT group. No changes were observed in these levels in LycT group when compared to control group Fig. 4d.

3.4 Assessment of Renal Function Markers

A significant increase in serum urea and creatinine levels was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in urea and creatinine levels when compared to the animals in DEX group. Urea levels remained unchanged in LycT+DEX group when compared to control and LycT groups. Creatinine levels increased in LycT+DEX group when compared to control and LycT group No change in these levels was observed between control and LycT groups Fig. 6a-b.

3.5 Histopathology

Liver from control and LycT groups exhibited normal histoarchitecture. Clear cut hexagonal hepatic lobules, separated by interlobular septa and traversed by portal veins were observed. Polyhedral hepatocytes, kupffer cells and sinusoids were normal in appearance Portal triad comprised of portal vein, hepatic artery and bile duct. Liver from DEX group revealed areas with kupffer cell infiltration, abnormal lipid accumulation. The hexagonal arrangement of hepatocytes appeared disturbed. Liver from LycT+DEX group did not exhibit any significant departure from the normal histoarchitecture Fig. 5.

The kidneys from control and LycT groups exhibited normal histoarchitecture. Regions of outer cortex and deeper medulla were clearly visible with no signs of damage. Cortical labyrinth revealed proximal and distal convoluted tubules (PCT and DCT) along with renal corpuscle. The renal corpuscle consisted of bowman's capsule and tuft of capillaries

known as glomerulus. The medullary region forming the renal pyramids consisted of loops of Henle and collecting ducts. Disorganization and atrophy of renal histoarchitecture was observed in DEX group. Renal lesions including marked glomerular damage and shrinkage, increased capsular space, hyaline casts, hemorrhage, tubular atrophy were observed in DEX group. In LycT+DEX group some lesions such infiltration, empty spaces (damaged histoarchitecture) were observed but the damage was less in comparison to DEX group Figs. 7 and 8.

3.6 Oxidative Stress Marker

A significant increase in hepatic lipid peroxidation was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in lipid peroxidation levels when compared to the animals in DEX group and remained unaltered when compared to control and LycT groups. No changes were observed in LycT group when compared to control group Table 1.

A significant increase in renal lipid peroxidation was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant decrease in lipid peroxidation levels when compared to the animals in DEX group and remained unaltered when compared to control and LycT groups. No changes were observed in LycT group when compared to control group Table 2.

3.7 Non-enzymatic and Enzymatic Antioxidant Defense System

3.7.1 Reduced glutathione

A significant decrease in hepatic reduced glutathione level was observed in DEX group when compared to control group. Animals of LycT+ DEX group exhibited a significant increase in reduced glutathione levels when compared to the animals in DEX and LycT group and remained unaltered when compared to control group. No changes were observed in LycT group when compared to control group Table 1.

A significant decrease in renal glutathione level was observed in DEX group when compared to control group. Animals of LycT+ DEX group exhibited a significant increase in reduced glutathione level when compared to the animals in DEX and LycT group and remained unaltered

when compared to control group. No changes were observed in LycT group when compared to control group Table 2.

3.7.2 Catalase

A significant decrease in hepatic catalase activity was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in catalase activity when compared to the animals in DEX group and remained unaltered when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 1.

A significant decrease in renal catalase activity was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in catalase activity when compared to the animals in DEX group and decreased when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 2.

3.7.3 Superoxide dismutase

A significant decrease in hepatic superoxide dismutase activity was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in SOD activity when compared to the animals in DEX group and remained unaltered when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 1.

A significant decrease in renal superoxide dismutase activity was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in superoxide dismutase activity when compared to the animals in DEX group, remained unaltered when compared to LycT group and decreased when compared to control group. No changes were observed in LycT group when compared to control group Table 2.

3.7.4 Glutathione peroxidase

No change in hepatic glutathione peroxidase activity was observed in any of the treatment groups Table 1.
A significant decrease in renal glutathione peroxidase activity was observed in DEX group

when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in GPx activity when compared to the animals in DEX group and decreased when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 2.

3.7.5 Glutathione reductase

A significant decrease in hepatic glutathione reductase activity was observed in DEX group when compared to control and LycT groups. Animals of LycT+ DEX group exhibited a significant increase in glutathione reductase activity when compared to the animals in DEX

group and remained unaltered when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 1.

A significant decrease in renal glutathione reductase activity was observed in DEX group when compared to control group and remained unaltered in comparison to LycT group. Animals of LycT+ DEX group exhibited significant increase in glutathione reductase activity when compared to the animals in DEX group and remained unaltered when compared to control group and LycT groups. No changes were observed in LycT group when compared to control group Table 2.
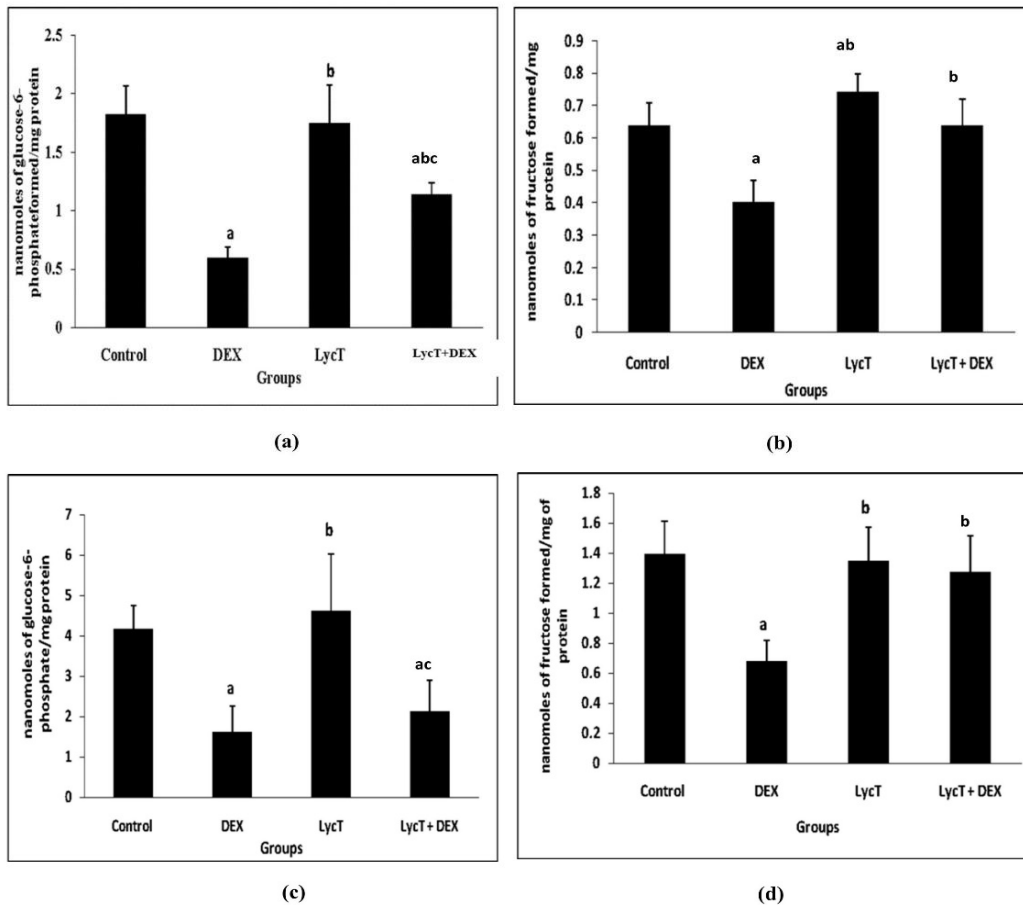
(a)

(b)

(c)

(d)

Fig. 3. Modulatory effect of LycT and/or DEX on hexokinase and phosphoglucoisomerase in (a,b) liver (c,d) kidney

Data is represented as Mean±SD (n=5). Data is analysed by one way ANOVA followed by post hoc test. [a]p ≤ 0.05 significant with respect to control group; [b]p≤0.05 significant with respect to DEX group; [c]P≤0.05 significant with respect to LycT group
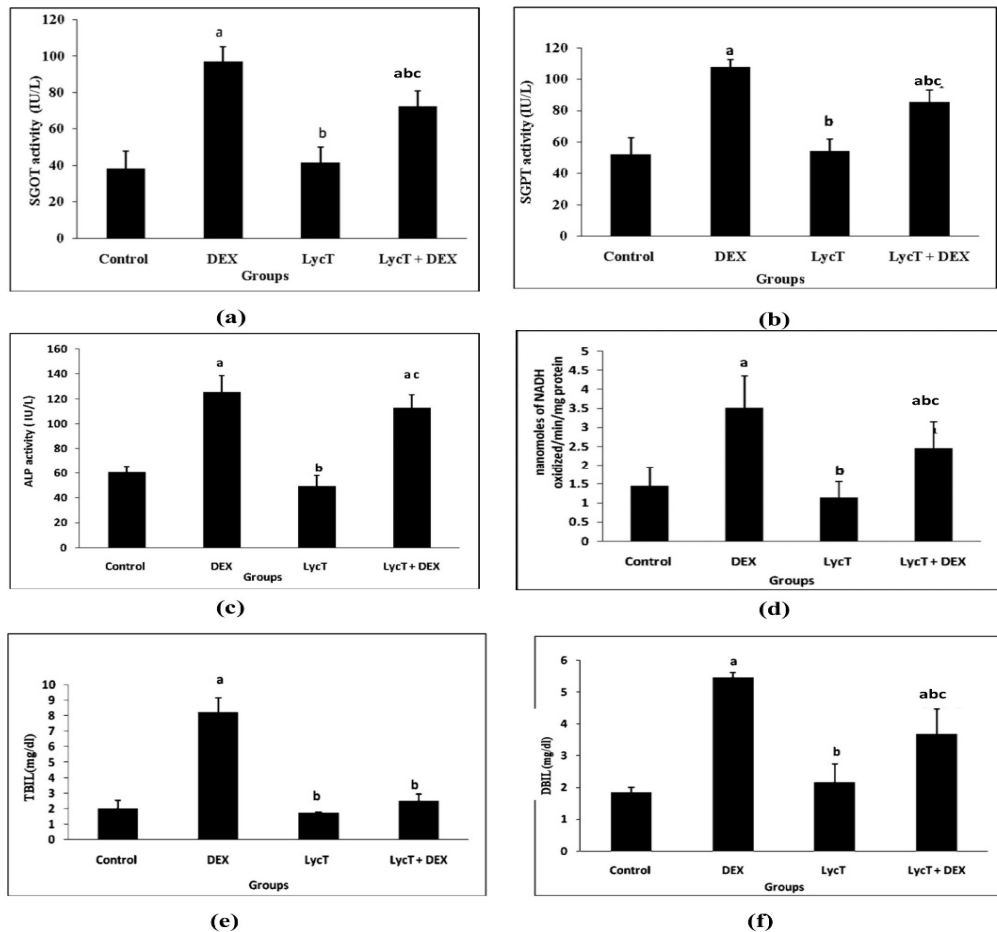
Fig. 4. Modulatory effect of LycT and/or DEX on (a) SGOT (b) SGPT (c) ALP (d) LDH (e) total bilirubin (e) direct bilirubin
Data is represent as Mean±SD (n=5). Data is analysed by One way ANOVA followed by post hoc test. [a]p≤0.05 significant with respect to control group; [b]p≤0.05 significant with respect to DEX group; [c]p≤0.05 significant with respect to LycT grourp

4. DISCUSSION

Despite its immense clinical benefits, dexamethasone is used with extreme caution because of the accompanying adverse effects. The need to use dexamethasone with mitigated or no side effects has provoked the search for agents that could enable its safe and effective use. The data presented here indicates the potential of LycT (carotenoid rich extract) in ameliorating dexamethasone induced hepatic and renal damage in mice.

In the present study, enhanced blood glucose and reduced hepatic glycogen levels in DEX group indicated altered glucose metabolism by liver in response to dexamethasone administration. Consumption of dexamethasone and other glucocorticoids leads to increased glucose production and reduced glucose uptake by peripheral tissues. Glucocorticoids induce peripheral insulin resistance by inducing post-receptor defects in insulin actions, inhibition of insulin secretion by pancreatic β-cells, GLUT4 translocation, impaired insulin signaling etc [54-57]. Steroids also regulate the synthesis and release of hormones associated with the development of hyperglycemic disorders [58]. Insulin resistance that causes decreased supply of glucose to liver in response to dexamethasone may be considered responsible for the decreased glycogen levels observed in the present study [59]. Also, reports suggest that glucose metabolizing enzymes are severely

impaired during glucocorticoid consumption and this was evident in the present study as well. It has been previously demonstrated that administration of glucocorticoids to rats and rabbits provoked inhibition of glucose utilizing enzymes such as hexokinase and phosphoglucoisomerase [60,61]. The inhibited enzyme activities results in impaired glucose oxidation leading to hyperglycemia [62-65].

Cortisol treatment inhibited the phosphorylation of glucose and fructose-6-phosphate possibly due to reduced enzyme activities of hexokinase and 6-phosphofructokinase [62,64]. The decreased activities of hexokinase and phosphoglucoisomerase enzymes in DEX group may be attributed to the insulin insensitivity/resistance caused by dexamethasone [66].
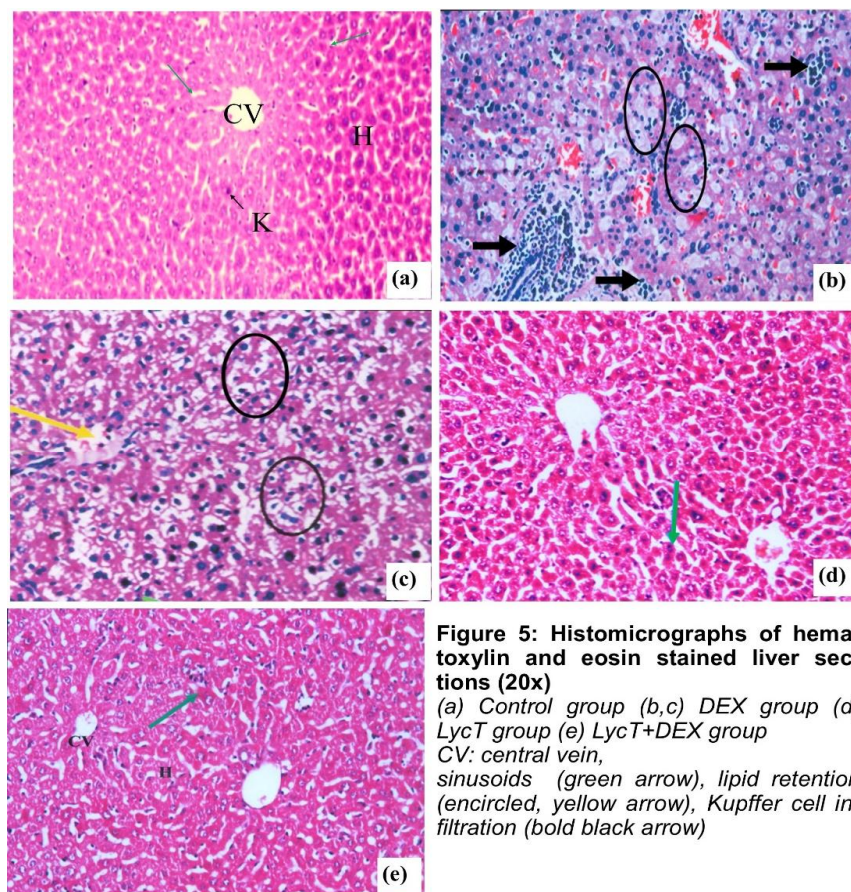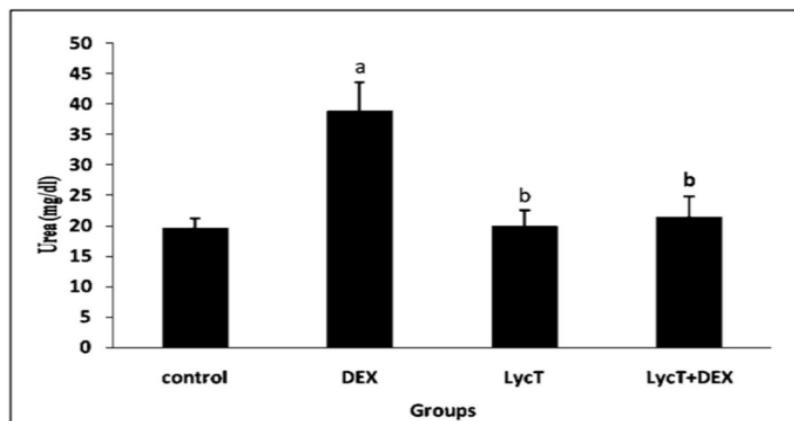

Figure 5: Histomicrographs of hema-toxylin and eosin stained liver sec-tions (20x)
(a) Control group (b,c) DEX group (d) LycT group (e) LycT+DEX group
CV: central vein,
sinusoids (green arrow), lipid retention (encircled, yellow arrow), Kupffer cell in-filtration (bold black arrow)
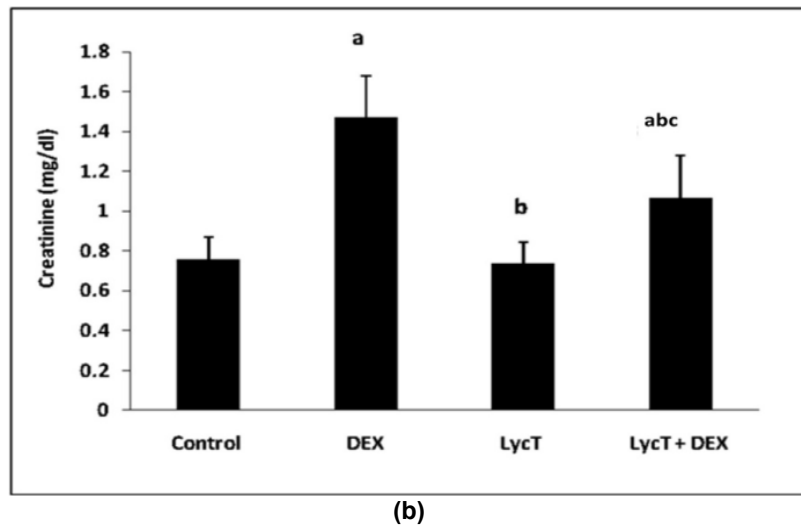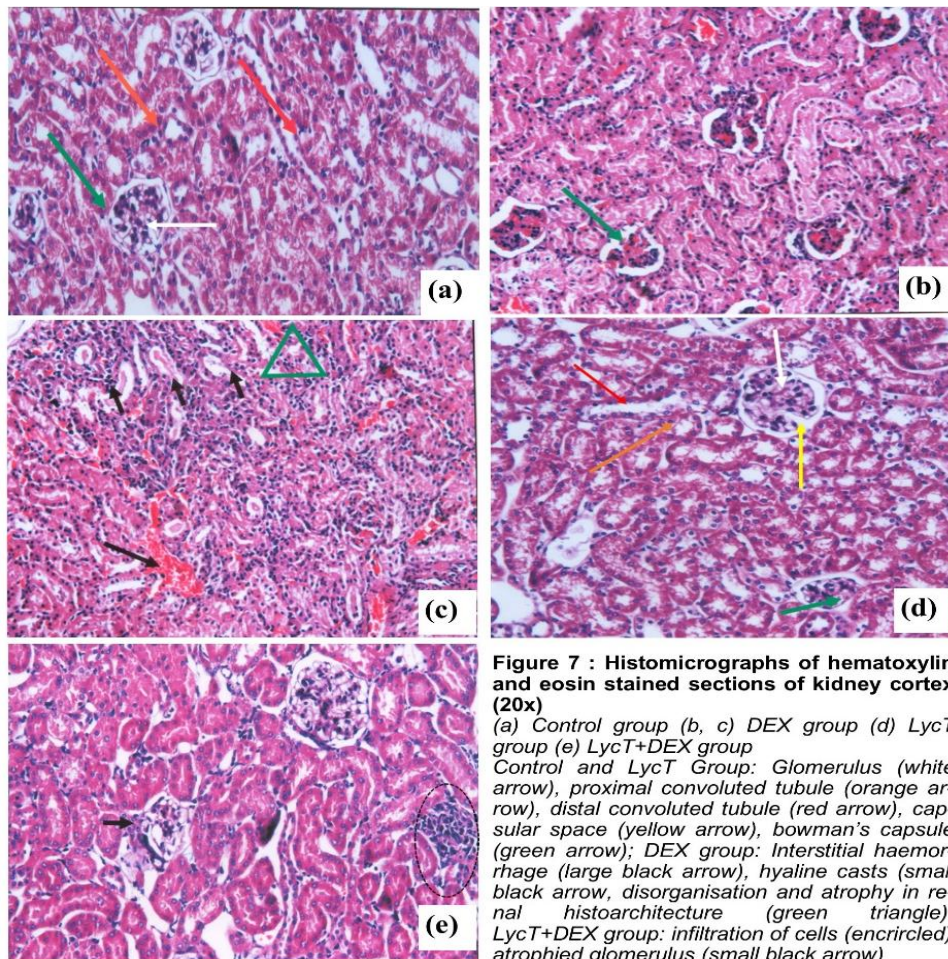

(a)

(b)

Fig. 6. Modulatory effect of LycT and/ or DEX on (a) urea and (b) creatinine levels in serum
Data is represent as Mean±SD (n=5). Data is analysed by One way ANOVA followed by post hoc test. [a]p≤0.05 significant with respect to control group; [b]p≤0.05 significant with respect to DEX group; [c]p≤0.05 significant with respect to LycT grourp

Figure 7 : Histomicrographs of hematoxylin and eosin stained sections of kidney cortex (20x)
(a) Control group (b, c) DEX group (d) LycT group (e) LycT+DEX group
Control and LycT Group: Glomerulus (white arrow), proximal convoluted tubule (orange arrow), distal convoluted tubule (red arrow), capsular space (yellow arrow), bowman's capsule (green arrow); DEX group: Interstitial haemorrhage (large black arrow), hyaline casts (small black arrow, disorganisation and atrophy in renal histoarchitecture (green triangle); LycT+DEX group: infiltration of cells (encrircled) atrophied glomerulus (small black arrow)
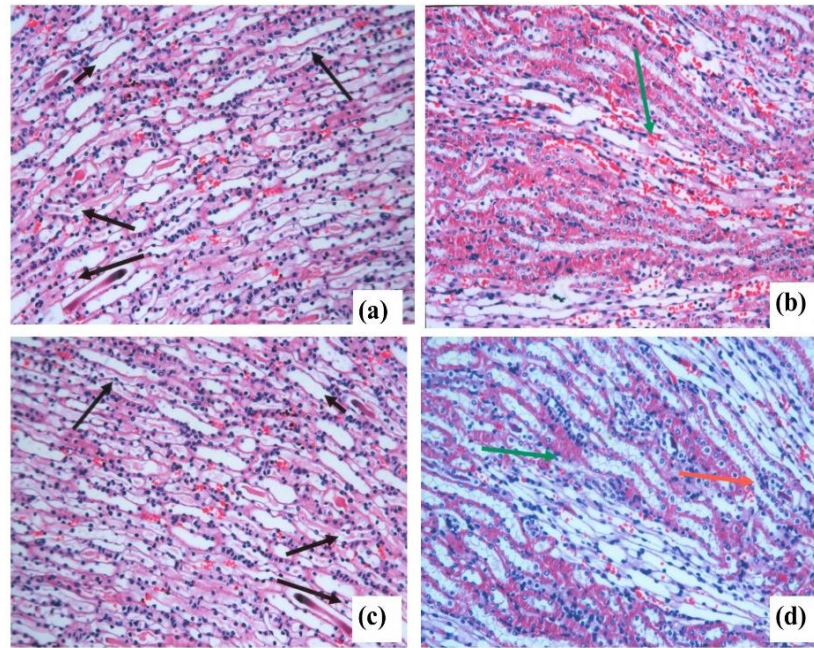
Fig. 8. Histomicrographs of hematoxylin and eosin stained sections of kidney medulla (10x)
(a) Control group (b) DEX group (c) LycT group (d) LycT + DEX Group
Control and LycT Group : collecting dust (small black arrow), loop of henle (large black arrow); DEX group;haemorhagic stroma (green arrow); tubular atrophy observed; LycT + DEX group; tubular atrophy (green arrow); stroma appears without haemorrhage (orange arrow)

Table 1. Effect of LycT and/or DEX on lipid peroxidation and antioxidant defense system in hepatic tissue

Groups	Control	DEX	LycT	LycT + DEX
Lipid Peroxidation (nanomoles of MDA-TBA chromophore formed/mg protein)	0.650 ± 0.090	1.34 ± 0.166[a]	0.70 ± 0.05[b]	0.560 ± 0.043[b]
Reduced glutathione (nanomoles of GSH/mg protein)	4.84 ± 0.446	3.91 ± 0.235[a]	4.44 ± 0.647	5.08 ± 0.648[bc]
Catalase (IU/mg protein)	41.6 ± 4.42	31.4 ± 5.60 [a]	43.9 ± 6.23[b]	39.8 ± 6.39[b]
Superoxide Dismutase (IU/mg protein)	11.0 ± 1.40	8.58 ± 1.58[a]	13.2 ± 0.399[b]	11.7 ± 1.61[b]
Glutathione Peroxidase (nanomoles of NADPH consumed/min/mg protein)	23.6 ± 0.35	20.3 ± 2.26	22.6 ± 1.55	22.3 ± 5.15
Glutathione Reductase (nanomoles of NADPH consumed/min/mg protein)	31.4 ± 3.75	21.7 ± 4.90[a]	30.1 ± 4.54[b]	30.0 ± 5.30[b]

Data is represented as Mean±SD (n=5). Data is analysed by One-Way ANOVA followed by post hoc test. [a]$p \leq 0.05$ significant with respect to control group; [b]$p \leq 0.05$ significant with respect to DEX group; [c]$p \leq 0.05$ significant with respect to LycT group

Table 2. Effect of LycT and/or DEX on lipid peroxidation and antioxidant defense system in renal tissue

Groups	Control	DEX	LycT	LycT + DEX
Lipid Peroxidation (nanomoles of MDA-TBA chromophore formed/mg protein)	0.525 ± 0.112	0.88 ± 0.078 [a]	0.617 ± 0.078 [b]	0.620 ± 0.14 [b]
Reduced glutathione (nanomoles of GSH/mg protein)	4.88 ± 1.160	3.40 ± 0.668 [a]	4.23 ± 0.746	5.24 ± 0.977 [bc]
Catalase (IU/mg protein)	40.6 ± 3.68	22.7 ± 3.92 [a]	37.2 ± 2.92 [ab]	28.6 ± 4.01 [abc]
Superoxide Dismutase (IU/mg protein)	8.42 ± 0.929	3.48 ± 0.300 [a]	7.48 ± 1.04 [b]	6.81 ± 0.545 [ab]
Glutathione Peroxidase (nanomoles of NADPH consumed/min/mg protein)	20.8 ± 3.99	5.23 ± 1.64 [a]	24.5 ± 4.62 [b]	8.13 ± 1.25 [ac]
Glutathione Reductase (nanomoles of NADPH consumed/min/mg protein)	29.5 ± 1.67	21.2 ± 1.43 [a]	25.3 ± 2.74	31.4 ± 7.87 [b]

Data is represented as Mean±SD (n=5). Data is analysed by One-Way ANOVA followed by post hoc test. [a]p ≤0.05 significant with respect to control group; [b]p≤0.05 significant with respect to DEX group; [c]p ≤0.05 significant with respect to LycT group

Decreased blood glucose levels and enhanced activities of glucose metabolizing enzymes in LycT+DEX group suggested that LycT was able to mitigate hyperglycemic conditions, possibly improving glucose metabolism that was dysregulated because of dexamethasone administration. The beneficial effects of lycopene/lycopene rich products as observed in the present study are in corroboration with previous reports. Muhsin and Sefa [67] have reported normalization of blood glucose levels in diabetic rats by lycopene treatment. Rats administered with lycopene rich tomato homogenate exhibited improved glucose tolerance [68]. Zeng et al. [69] have demonstrated that lycopene improved insulin sensitivity in mice that were fed a high fat diet. Lycopene is known to raise insulin levels and concomitantly decrease body glucose levels [70,71]. Tomatoes have been considered beneficial in hyperglycemic/diabetic conditions because they mitigate diabetes induced tissue damage, atherosclerosis, inflammation, oxidative stress etc. The various constituents present in tomatoes such as ascorbic acid, lycopene, β-carotene, flavonoids, several small bioactive molecules, minerals (such as magnesium and potassium) etc are favorable in diabetic conditions [72].

Enhanced levels of liver function markers were observed in serum of animals belonging to DEX group. Abnormally enhanced levels have been observed during hepatic toxicity and could be a result of damaged and leaky hepatic cell membrane [73-75]. Hepatic damage as revealed by altered histoarchitecture and raised SGOT and SGPT levels has been reported after dexamethasone administration to rats [76]. Dexamethasone associated hepatic necrosis in rats has been associated with increased serum levels of SGOT and SGPT [77]. Hasona and Morsi [16] and Hasona et al. [14] have reported elevations in liver function markers in dexamethasone administered rats. Abou-Seif [78] have also demonstrated that dexamethasone induced liver injury was reflected by raised SGOT, SGPT and LDH levels along with disturbed histoarchitecture. LycT supplementation to dexamethasone treated mice decreased the enhanced levels of these markers which is suggestive of mitigation of hepatic damage and preserved membrane integrity. We have previously demonstrated the

effect of LycT in protecting membrane integrity and decreasing the levels of liver function markers in serum during NDEA induced hepatocellular carcinoma in mice [79]. Lycopene supplementation was effective in improving hepatic function which was impaired during hepatitis caused by lipopolysaccharide [80]. The derangement in hepatic function markers in NDEA and phenobarbital treated rats was improved upon lycopene supplementation [81]. A previous report from our laboratory demonstrated that lycopene was effective in mitigating DMBA induced hepatotoxicity [82]. Elevated levels of creatinine and urea in serum serve as indictors of renal malfunctioning [83]. The abnormally raised serum levels of renal function markers suggesting renal damage in DEX group is in agreement with previous reports [14]. Improvement in the levels of renal function markers in LycT+DEX group demonstrated the protective potential of LycT against DEX induced renal damage. These results are in harmony with those previously reported. As observed from improved renal function markers and improved histoarchitecture it was apparent that lycopene was effective in mitigating several drug induced nephrotoxicity in animals [27,84-87]. We have also previously reported that LycT conferred protection against doxorubicin induced renal toxicity in mice [29]. Light microscopy studies of hepatic and renal tissues indicated marked damage in these tissues in DEX group. Such deviations in histoarchitecture signifying damage have been reported previously [29,88-90]. The tissue sections from LycT+DEX group revealed less damage as compared to the DEX group. Considering the improved levels of organ function markers and mitigated damage in histoarchitecture in LycT+DEX group, it may be regarded that LycT conferred protection against dexamethasone induced deleterious effects.

In the present study, increased MDA levels in hepatic and renal tissues of DEX group indicated enhanced lipid peroxidation which is suggestive of oxidative stress in the tissues. This was accompanied by inhibition of antioxidant enzymes and reduced glutathione. The declined activities/levels of antioxidant defense system components partially explain the deleterious effects caused by dexamethasone. Recent reports have demonstrated that dexamethasone administration led to elevated MDA levels and suppression of antioxidant defense enzymes in rats [14,16]. Dexamethasone induced apoptosis

in thymocytes was observed to be associated with mitochondrial dysfunction, decreased antioxidant enzyme activities, increased ROS production, and lipid peroxidation [10]. NADPH oxidases are involved in dexamethasone mediated increase in ROS and apoptosis in various tissues [91,92]. JNK-P38 MAPK signaling pathway has been implicated in dexamethasone induced oxidative damage in mice [93]. Free radical scavenging enzymes are the first line of cellular defense against oxidative injury and inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage [94]. SOD is involved in catalyzing the dismuation of $O^{2.-}$ to H_2O_2. CAT and GPx enzymes catalyze the decomposition of H_2O_2 to water and oxygen and thus protect the cell from oxidative damage. GR is the enzyme directly involved in reduction of GSSG to GSH. Low activity of GPx is responsible for disturbance of the prooxidant/antioxidant balance [95]. GSH is a ubiquitous thiol containing tri-peptide that serves as an essential role in maintaining cell integrity because of its reducing properties. It is an important antioxidant molecule involved in the protection against LPO reactions. Hepatic and renal tissues of mice belonging to DEX group exhibited decrease in GSH and various antioxidant enzymes. These observations are in concordance with other reported studies [9,11,13,15,9].

The fall in MDA levels in LycT+DEX group in comparison to DEX group can be correlated to the upregulation of enzymatic and non-enzymatic antioxidant defense system. We have previously reported that LycT was effective in mitigating doxorubicin induced nephrotoxicity in mice by boosting the enzymatic and non-enzymatic antioxidant defense system [29]. Reducing xenobiotic induced increased LPO by intervention of lycopene has been reported previously by us [82]. Lycopene efficiently scavenges peroxyl radicals and thus, contributes to the defense against LPO induced by dexamethasone [96]. Kujawska et al. [97] have reported antioxidative effects of lycopene enriched tomato paste against nitrosamine induced oxidative stress in rats. Owing to its extended conjugated polyene chain lycopene is highly reactive towards oxygen and free radicals [98]. It also has an exceptionally high singlet oxygen quenching ability (Stahl and Sies, 2003). The other carotenoids present in tomatoes and tomato-based products also exhibit antioxidant activities [99,100]. As observed from the present

study and other reports available in literature it is likely that the strong antioxidant potential of lycopene and other carotenoids present in tomatoes may play an important role in counteracting oxidative stress induced deleterious effects.

5. CONCLUSION

The mitigation in damage to histoarchitecture, along with improvement in specific organ function markers and glucose metabolism suggest the protective potential of LycT against dexamethasone induced deleterious effects. The strengthening of antioxidant defense response as observed from decreased lipid peroxidation levels and upregulation of enzymatic and non-enzymatic defense system suggest a possible mechanism for its protective effects. Although these observations indicate alleviation of dexamethasone induced adverse effects by LycT, however further studies are warranted.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All the experimental protocols were initially approved [(IAEC/411); 11/9/13] by the Institutional Ethics Committee of Panjab University, Chandigarh (India) and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rice JB, White AG, Scarpati LM, Wan G, Nelson WW. Long-term systemic corticosteroid exposure: A systematic literature review. Clin Ther. 2017;39(11): 2216-2229.
2. Yasir M, Goyal A, Bansal P, Sonthalia S. Corticosteroid adverse effects. In: Stat pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2020.
3. World Health Organization model List of essential medicines, 21st List, 2019. Geneva: World Health Organization; 2019.
    Licence: CC BY-NC-SA 3.0 IGO.
4. Drozdowicz LB, Bostwick JM. Psychiatric adverse effects of pediatric corticosteroid use. Mayo Clin Proc. 2014;89(6):817-834.
5. Kamphuis PJ, De Vries WB, Bakker JM, Kavelaars A, van Dijk JE, Schipper Me, et al. Reduced life expectancy in rats after neonatal dexamethasone treatment. Pediatr Res. 2007;61:72–76.
6. Liu Y van, Goor H, Havinga R, Baller JFW, Bloks VW, van der Leij FR, et al. Neonatal dexamethasone administration causes progressive renal damage due to induction of an early inflammatory response. Am J Physiol Renal Physiol. 2008;294(4):F768-76.
7. De Vries WB, Van den Borne P, Goldschmeding R, De Weger RA, Bal MP, van Bel F, et al. Neonatal dexamethasone treatment in the rat leads to kidney damage in adulthood. Pediatr Res. 2010;67(1):72-76.
8. Ferris HA, Kahn CR. New mechanisms of glucocorticoid induced insulin resistance: Make no bones about it. J Clin invest. 2012;122(11):3854-3857.
9. Eid Y, Ebeid T, Younis H. Vitamin E supplementation reduces dexamethasone-induced oxidative stress in chicken semen. British Poultry Science. 2006; 47(3): 350-356.
10. Yi J, Zhu R, Wu J, Wu J, Xia W, Zhu L, et al. In vivo protective effect of betulinic acid on dexamethasone induced thymocyte apoptosis by reducing oxidative stress. Pharmacol Rep. 2016;68(1):95-100.
11. Beytut E, Yilmaz S, Aksakal M, Polat S. The possible protective effects of vitamin E and selenium administration in oxidative stress caused by high doses of glucocorticoid administration in the brain of rats. J Trace Elem Med Biol. 2018;45:131-135.
12. Kakali De, Roy K, Saha A, Sengupta C. Exploring effects of different antioxidants on dexamethasone-induced lipid

peroxidation using common laboratory markers. Acta Pol Pharm. 2004;61(1):77-86.

13. Tayade PM, Jagtap SA, Borde S, Chandrasekar N, Joshi A. Effect of psoralea corylifolia on dexamethasone-induced insulin resistance in mice. J King Saud Univ Sci. 2012;24:251–255.

14. Hasona NA, Alrashidi AA, Aldugieman TZ, Alshdokhi AM, Ahmed MQ. Vitis vinifera extract ameliorate hepatic and renal dysfunction Induced by dexamethasone in albino RATS. Toxics. 2017;5:11

15. Safaeian L, Zolfaghari B, Karimi S, Talebi A, Ghazvini MA. The effects of hydroalcoholic extract of *Allium elburzense* wendelbo bulb on dexamethasone-induced dyslipidemia, hyperglycemia, and oxidative stress in rats. Res Pharm Sci. 2018;13(1):22–29.

16. Hasona N, Morsi A. Grape seed extract alleviates dexamethasone-induced hyperlipidemia, lipid peroxidation, and hematological alteration in rats. Ind J Clin Biochem. 2019;34(2):213–218.

17. Arora N, Koul A. A 'complex solution' to a 'complex problem': Tackling the complexity of cancer with botanicals. Eur J Cancer Prev. 2014;23(6):568-578.

18. Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya- Martínez MT, Gutiérrez-Salinas J, Bautista M,et al. Review of natural products with hepatoprotective effects. World J Gastroenterol. 2014;20(40):14787–14804.

19. Simon JP, Prince SE. Natural remedies for non-steroidal anti-inflammatory drug-induced toxicity. J Appl Toxicol. 2017;37(1):71-83.

20. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. Journal of the American Dietetic Association. 1993; 93(3):284-296.

21. Tanaka T, Shnimizu M, Moriwaki H. Cancer chemoprevention by carotenoids. Molecules. 2012;17:3202-3242.

22. Gupta P, Bansal MP, Koul A. Spectroscopic characterization of lycopene extract from *Lycopersicum esculentum* (Tomato) and its evaluation as a chemopreventive agent against experimental hepatocarcinogenesis in mice. Phytother Res. 2013a;27:448–456.

23. Gupta P, Bansal MP, Koul A. Evaluating the effect of lycopene from *Lycopersicum esculentum* on apoptosis during NDEA induced hepatocarcinogenesis. Biochem Biophys Res Commun. 2013b;434:479–485.

24. Bhatia N, Gupta P, Singh B, Koul A. Lycopene enriched tomato extract inhibits hypoxia, angiogenesis, and metastatic markers in early stage N-nitrosodiethylamine induced hepatocellular carcinoma. Nutr Cancer. 2015;67:1270–1277.

25. Gupta P, Bhatia N, Bansal MP, Koul A. Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma. World J Hepatol. 2016;8:12–22.

26. Koul A, Bansal MP, Aniqa Chaudhary H, Chugh NA. Lycopene enriched extract suppresses chemically induced skin tumorigenesis in mice. International Journal of Vitamin Research. 2020;90(5-6):493-513.

27. Moawad KM. Possible prophylactic effects of vitamin E or lycopene treatment on renal toxicity induced by CCl4 administration in albino rats. World J Zool. 2007;2

28. Jamshidzadeh A, Baghban M, Azarpira N, Bardbori AM, Niknahad H. Effect of tomato extract on oxidative stress induced toxicity in different organs of rats. Food Chem Toxicol 2008;46:3612-3615

29. Koul A, Shubhrant Gupta P. Phytomodulatory potential of lycopene from *Lycopersicum esculentum* against doxorubicin induced nephrotoxicity. Indian J Exp Biol. 2013;51:635-645.

30. FAO/WHO food category system. General standards for food additives codex stan. Food and Agriculture Organization of the United Nations (FAO), Rome, and the World Health Organization (WHO), Geneva. 2009;192:9-48.

31. Shi J, Le Maguer M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. Crit Rev Biotechnol. 2000;20: 293-334.

32. Poggioli R, Ueta CB, Drigo RA, Castillo M, Fonseca TL, Bianco AC. Dexamethasone reduces energy expenditure and increases susceptibility to diet-induced obesity in mice. Obesity (Silver Spring). 2013;21(9):E415–E420.

33. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annual Clinical Biochem. 1969;6:24-25.

34. Seifter S, Dayton BS, Novic B, Muntwyler E. The estimation of glycogen with the anthrone reagent. Archs Biochem.1949;25:191-200.

35. Morris DL. Quantitative determination of carbohydrates with dreywood's anthrone reagent. Science.1948;107:254-255.

36. Crane RK, Sols A. Animal tissue hexokinases. In: Colowick SP and kaplan ND methods in enzymology. Academic Press. 1955;1:277-286.

37. Brandstrup N, Kirk JE, Bruni C. The hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individuals of various ages. J Gerontol. 1957;12:166-171.

38. Horrocks JE, Ward J, King J. A routine method for the determination of phosphoglucose isomerase activity in body fluid. J Clin Pathol. 1963;16:248-251.

39. Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. J Clin Invest. 1955;34:126-133.

40. Henry RJ, Chiamori M, Golub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamic oxaloacetic transaminase, glutamic pyruvate transaminase and lactic acid dehydrogenase. Am J Clin Pathol. 1960;34:381-398.

41. Recommendations of the German society for clinical chemistry: Standardization of methods for the estimation of enzyme activity in biological fluids. J. Clinical Chemistry, Clinical Biochemistry. 1972;8:182-192.

42. Jendrassik L, Grof P. Biochem Z. 1938; 297:81-89.

43. Bergmeyer HU. Methods of enzymatic analysis Ed. 2 vevlag chemie, weinheim: Academic press, LONDON; 1965.

44. Chaney AL, Marbach CP. Modified reagents for determination of urea and ammonia. Clinical Chemistry. 1962;8:130-132.

45. Folin O, Beitrag zur Chemie, Des Kreatinins, Kreatins Im Harne. Hoppe-seyler's zeitschrift fur physiologische chemie. 1904;41:223-242.

46. Trush MA, Mimnaugh EG, Ginsburg E, Gram TE. In vitro stimulation by paraquat of reactive oxygen-mediated lipid peroxidation in rat lung microsomes. Toxicol Appl Pharmacol. 1981;60:279-286.

47. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979;582:67-78.

48. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium deficient rat liver. Biochem Biophys Res Commun. 1976;71:952-958.

49. Williams CM Jr, Arscott ID. Glutathione reductase. Methods in enzymology. XVIIB. 1971;503-509.

50. Luck H. In: Methods of enzymatic analysis, Ed: HU Bergmeyer academic press, New York. 1971;885-893.

51. Kono Y. Generation of superoxide radicals during auto oxidation of hydroxylamine and an assay of super oxide dismutase. Arch Biochem Biophys. 1978;186:189-195.

52. Lowry OH, Rosebrough NJ, Farr AN, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem. 1951;193:265–275.

53. Humanson, G.L. (1962) Animal tissue techniques. Animal Tissue Techniques. 21–34.

54. Rafacho A, Roma LP, Taboga SR, Boschero AC, Bosqueiro JR. Dexamethasone-induced insulin resistance is associated with increased connexins 36 mRNA and protein expression in pancreatic rat islets. Can J Physiol Pharmacol. 2007;85:536-545.

55. Ghaisas MM, Ahire YS, Dandawate PR, Gandhi SP, Mule M. Effects of combination of thiazolidinediones with melatonin in dexamethasone-induced insulin resistance in mice. Indian J Pharm Sci. 2011;73(6):601-607.

56. Gosmanova EO, Tangpricha V, Gosmanov AR. Endocrine-metabolic pathophysiologic conditions and treatment approaches after kidney transplantation. Endocr Pract. 2012;18(4):579-590.

57. Yates CJ, Fourlanos S, Hjelmesæth J, Colman PG, Cohney SJ. New-onset diabetes after kidney transplantation—changes and challenges 2012;12(4):820-828.

58. Kuo T, Mc Queen A, Chen TC, Wang JC. Regulation of glucose homeostasis by

glucocorticoids. Adv Exp Med Biol. 2015;872:99-126.

59. Yi CX, Foppen E, Abplanalp W, Gao Y, Alkemade A, la Fleur SE, et al. Glucocorticoid signaling in the arcuate nucleus modulates hepatic insulin sensitivity. Diabetes. 2012;61(2):339-345.

60. Ilyin VS. Hormonal regulation of liver hexokinase activity. Adv Enzyme Regul. 1964;2:151-175.

61. Salleh M, Ardawi M, Majzoub MF, Newsholme EA. Effect of glucocorticoid treatment on glucose and glutamine metabolism by the small intestine of the rat. Clin Sci (Lond). 1988;75(1):93-100.

62. Kipnis D, Ann NY. Acad. Sci. 1959;82;354–365.

63. Morgan H, Regen D, Henderson M, Sawyer T, Park CJ. Regulation of glucose uptake in muscle. Biol. Chem. 1961;236(8):2162–2168.

64. Randle P, Morgan H. Regulation of glucose uptake by muscle. Vitamins and Hormones. 1962;20:199–249

65. van Raalte DH, Brands M, van der Zijl NJ, Muskiet MH, Pouwels PJW, Ackermans MT, et al. Low-dose glucocorticoid treatment affects multiple aspects of intermediary metabolism in healthy humans: A randomised controlled trial. Diabetologia. 2011;54(8):2103-2112.

66. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue; Diabetes Res Clin Pract. 2011;93(Suppl 1):S52-59.

67. Muhsin A, Sefa C. Effects of lycopene on plasma glucose, insulin levels, oxidative stress and body weights of streptozotocin-induced diabetic rats. 2012;42(2):1406-1413.

68. Hashimoto N, Tominaga N, Wakagi M, Ishikawa‑Takano Y. Consumption of lycopene‑rich tomatoes improved glucose homeostasis in rats via an increase in leptin levels. Journal of Natural Medicines. 2020; 74:252–256.

69. Zeng Z, He W, Jia Z, Hao S. Lycopene improves insulin sensitivity through inhibition of stat3/srebp-1c-mediated lipid accumulation and inflammation in mice fed a high-fat diet. Experimental and Clinical Endocrinology and Diabetes. 2017;125(9):610-617.

70. Cuevas-Ramos D, Almeda-Valdes P, Chavez-Manzanera E, Meza-Arana CE, Brito-Cordova G, Mehta R, et al. Effect of tomato consumption on high-density lipoprotein cholesterol level: A randomized, single-blinded, controlled clinical trial. Diabetes Metab Syndr Obes. 2013;6:263-273.

71. Martin-Pozuelo G, Gonzalez-Barrio R, Barbera GG, Albalat A, Garcia-Alonso J, Mullen W, et al. Tomato juice consumption modifies the urinary peptide profile in sprague-dawley rats with induced hepatic steatosis. Int J Mol Sci. 2016;17:(1789).

72. Banihani SA. Tomato (Solanumlycopersicum L.) and type 2 diabetes. International Journal of Food Properties. 2018;21(1):99-105.

73. Hamza RZ, Al-Harbi MS. Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian Pacific Journal of Tropical Biomedicine. 2015;5(7):521-531

74. Wahid A, Hamed AN, Eltahir HM, Abouzied MM. Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl$_4$-induced chronic hepatotoxicity in rats. BMC Complement Altern Med. 2016;16(263).

75. Uchida NS, Silva-Filho SE, Cardia GFE, Cremer E, De Souza Silva-Comar FM, Silva EL, et al. Hepatoprotective effect of citral on acetaminophen-induced liver toxicity in mice. Evid Based Complement Alternat Med. 2017;2017:1796209.

76. Al-Fartosi KG, Al-Muswie RT, Al-rekabi EA. Muslim mohsen; Khalid et al., Effect of dexamethasone and contraceptive pills in female rats (*Rattus norvegicus*). World J Pharm Sci. 2017;5(3):171-175.

77. Jackson ER, Kilroy C, Joslin DL, Schomaker SJ, Pruimboom-Brees I, Amacher DE. The early effects of short-term dexamethasone administration on hepatic and serum alanine aminotransferase in the rat. Drug and Chemical Toxicology. 2008;31(4):427-445.

78. Abou-Seif HS, Hozayen WG, Hashem KS. Thymus vulgaris extract modulates dexamethasone induced liver injury and restores the hepatic antioxidant redox system. Journal of Basic and Applied Sciences. 2019;8:21

79. Gupta P, Bansal MP, Koul A. Lycopene modulates initiation of N-

nitrosodiethylamine induced hepatocarcinogenesis: Studies on chromosomal abnormalities, membrane fluidity and antioxidant defense system. Chemico Biol Interact. 2013c;206(2):364-374.

80. Sheriff SA, Devaki T. Lycopene stabilizes liver function during D-galactosamine / lipopolysaccharide induced hepatitis in rats. JTUSCI. 2013;7:8–16.

81. Das BK, Koti BC, Gadad PC. Role of lycoperscion esculentum in diethylnitrosamine induced and phenobarbital promoted hepatocellular carcinoma. Indian Journal of Health Sciences. 2016;9:147-152.

82. Koul A, Tanwar L, Arora N. Lycopene mediated modulation of 7, 12 dimethylbenz(a)anthracene induced hepatic clastogenicity in male Balb/c mice. Nutr Hosp. 2010;25(2):304-310.

83. Salazar JH. Overview of urea and creatinine. Laboratory medicine. 2014; 45(1):e19-e20.

84. Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin induced cardiotoxicity and nephrotoxicity. Toxicology. 2006;218(2-3):164-171.

85. Karahan I, Atessahin A, Yilmaz S, Ceribasi AO, Sakin F. Protective effect of lycopene on gentamycin induced oxidative stress and nephrotoxicity. Toxicology. 2005;215(3): 198-204.

86. Gerbed MSA. Protective effect of lycopene on deltamethrin induced histological and ultrastructural changes in kidney tissue of rats. Toxicol Ind Health. 2012;160-173.

87. Dai C, Tang S, Deng S, Zhang S, Zhou Y, Velkov T, et al. Lycopene attenuates colistin-induced nephrotoxicity in mice via activation of the Nrf2/HO-1 pathway. Antimicrob agents chemother. 2015;59: 579–585.

88. Welta K, Weissa J, Martinb R, Hermsdorfc T, Drewsa S, Fitzl G. Ginkgo biloba extract protects rat kidney from diabetic and hypoxic damage. Phytomedicine. 2007;14:196–203.

89. Bala S, Chugh NA, Bansal SC, Garg ML, Koul A. Radiomodulatory effects of Aloe vera on hepatic and renal tissues of X-ray irradiated mice. Mutation Research. 2018;811:1-15.

90. El-Kordy EA. Effect of suramin on renal proximal tubular cells damage induced by cisplatin in rats (Histological and Immunohistochemical Study). J Microsc Ultrastruct. 2019;7(4):153–164.

91. Bai SC, Xu Q, Li H, Qin YF, Song LC, Wang CG, et al. NADPH oxidase isoforms are involved in glucocorticoid-induced preosteoblast apoptosis. Oxidative Medicine and Cellular Longevity; 2019.
Article ID 9192413

92. Macedo FN, De Souza DS, Araújo JEDS, Dantas CO, Santos RMD, Silva-Filha E, et al. Mesquita TRR NOX-dependent reactive oxygen species production underlies arrhythmias susceptibility in dexamethasone-treated rats. Free Radic Biol Med. 2020;152:1-7.

93. Zhu L, Yi X, Zhao J, Yuan Z, Wen L, Pozniak B, et al. Betulinic acid attenuates dexamethasone-induced oxidative damage through the JNK-P38 MAPK signaling pathway in mice. Biomed Pharmacother. 2018;103:499-508.

94. Rodrigo R, Libuy M, Feliú F, Hasson D. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage disease markers. 2013;35(6):773-790.

95. Benabdeslam H, Abidi H, Garcia I, Bellon G, Gilly R, Revol A. Lipid peroxidation and antioxidant defenses in cystic fibrosis patients. Clinical Chemistry and Laboratory Medicine. 1999;37(5):511–516.

96. Bose KSC, BK Agrawal. Effect of long term supplementation of tomatoes (cooked) on levels of antioxidant enzymes, lipid peroxidation rate, lipid profile and glycated hemoglobin in type 2 diabetes mellitus. West Indian Med J. 2006;55(4):274-278.

97. Kujawska M, Ewertowska M, Adamska T, Sadowski C, Ignatowicz E, Jodynis-Liebert J. Antioxidant effect of lycopene-enriched tomato paste on N-nitrosodiethylamine-induced oxidative stress in rats. J Physiol Biochem. 2014;70:981–990.

98. Peters U, Leitzmann MF, Chatterjee N, Wang Y, Albanes D, Gelmann EP, et al. Serum lycopene, other carotenoids, and prostate cancer risk: A nested case-control study in the prostate, lung, colorectal, and ovarian cancer screening

trial. Cancer Epidemiol Biomarkers Prev. 2007;16(5):962-968.

99. George B, Kaur C, Khurdiya DS, Kapoor HC. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chemistry. 2004;84:45-51.

100. Gama JT, Tadiotti AC, Sylos CM. Comparison of carotenoid content in tomato, tomato pulp and ketchup by liquid chromatography. Alim. Nutr. Araraquara. 2006;17(4):353-358.