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BIOCHEMICAL AND HISTOLOGICAL ALTERATIONS IN LIVER AND KIDNEY OF GROWING RABBITS FED ON OVERABUNDANT DRIED CITRUS LIMON

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ABSTRACT: Fruit and vegetables are under continuous analysis worldwide to figure out or detect new treatments to animal and human diseases. This study was intended to examine effects of dried C. limon (DCL) on New Zealand White rabbits liver and kidney functions of 10 and 20g DCL/kg diet. The rabbits were randomly allocated into three treatments groups of control and two dried Citrus limon groups (10 and 20 g DCL/kg diet). Feed and water were served ad-libitum throughout the 8 weeks of experimental period. At the end of the administration, there were a significantly increment of GOT, GGT and ALP activities. Contrary, total bilirubin was significant ($p<0.01$) decreased with feeding growing rabbit on DCL levels. While, there were no significant differences ($p>0.05$) among all dietary treatments on GPT, LDH, BA and CB values; substantial intension in KC and P levels were noticed as related to DCL supplementation. Furthermore, the values of creatinine, urea, uric acid, Ca, K, Na and Cl had no differences ($p>0.05$) among all groups. Also, serum total protein, albumin and globulin levels had the same trend. Likewise, the histological results of liver and kidneys revealed no cellular abnormality in the entire treatment groups as compared to control group. Generally, it is shown that DCLcan be included up to 20 g/kg in rabbit diets without any adverse or deleterious effect on the histomorphology of the liver and kidney of rabbits, as it stimulates the cells architecture thereby preserving and increasing the cellular profile.

In conclusion, these results suggest that the addition of DCL to the growing rabbit's diet could significantly enhance liver and kidney function.

Key words: Citrus limon – Rabbit – Histology – Biochemical.

INTRODUCTION

The effect of fruit and vegetables on performance, physiological response and antioxidant status of animals is currently a topic of great scientific and practical importance, due to the trend in reducing the use of antibiotics in feed as well as in therapy (Yang et al., 2009). Studies on the plant products efficacy have given conflicting results (Huyghebaert et al., 2011), and this is a stimulus to implement research activities in this direction. Lemon trees are fruit trees that produce small, oval, yellow citrus fruits. *Citrus limonis* is the scientific name of it. The lemons are a type of berry called a hesperidium. Lemons can be used in various ways in our life. They can be used as preventative and domestic medicine because they are very rich in vitamin C, bioflavonoids, acids and volatile oils that help the body fight infections. Since they are very acidic, they cause an alkalizing effect upon the body. The sweetened juice relieves gingivitis, stomatitis, and inflammation of the tongue. Flavonoids are widely dispersed group of polyphenol compounds with health-related characteristics that are based on their antioxidant activity, e.g. inhibition of platelet aggregation, anticancer, anti-inflammatory and antiviral activities. The flavonoids present in Citrus are Flavanones, flavones, and flavonols, although flavones and flavonols are found in low concentrations as compared to flavanones, but are potent antioxidants and free radical scavengers (Armando et al., 1998). It has been exhibited that Citrus fruit have highest antioxidant activity (Chun et al., 2008), due to the presence of abundant flavonoids, vitamin C and carotenoids (Xu et al., 2008). Common species of the genus Citrus are *Citrus indica*, *Citrus aurantifolia*, and *Citrus limon*, in which *Citrus limonis* available in Egypt and commonly known as lemon. It has significant economic value for its essential oil and is reported to be the source of magnesium, potassium, vitamin C, folic acid, limonoids and flavonoids (Deyhim et al., 2008). Citrus limonhas shown usefulness as antidote against certain venom, due to its platelet inhibitory effect (Arias and Ramon, 2005), however it needs further confirmation. More attention has also been paid on antioxidant capacity of Citrus limon (Berhow and Smolensky, 1995; Xu et al., 2008), since increase dietary antioxidant constituents could help to prevent diseases (Hernandez et al., 2009; Gonzalez et al., 2010). Citrus limon benefits are due to its wide range of bioflavonoids, including rutin, hesperidin, quercitrin, eriocitrin, narirutin, didymin and naringin (Nijveldt et al., 2001; Tripoli et al., 2007). Two more isomers of hesperidin, neohesperidin and homoeriodicty olrutinoside have also been identified in Citrus limon (Gonzalez et al., 2010). Blood biochemical and histological changes are reflections of the effects of dietary treatments on animals in terms of the type and amount of feed ingested and available for the animal to meet its physiological, metabolically necessities (Ewuola et al., 2004). Thus, dietary components have measurable effects on blood components; hence blood constituents are widely used in nutritional evaluation, and survey of animals (Olorode et al., 1995). Farm animals are being fed by human with forages, Citrus limon which may contain certain elements and metals that can have adverse effect on the wellbeing of the animal and in turn influence the digestive/metabolic life of the animals negatively. Glutamic Oxaloacetic Transaminase (GOT) is found in appreciable quantities in the heart, pancreas, muscle, and liver. It is the serum enzyme that reflects the functionality of heart and liver. Glutamic Pyruvic Transaminase (GPT) is found principally in the liver and in many tissues,

thus it is of limited use for rabbit disease (Jekins, 2000). When there is liver cell damage, the serum or plasma level of both GOT and GPT rises tremendously. Thus, GPT is more specific for detecting liver inflammation, liver cell damage, and necrosis such as that caused by parasites and hepatic lipidosis (Jekins, 2000). Alkaline phosphatase (ALP) is also found in appreciable amounts especially in the liver, small intestines, kidney, placental tissues, and osteoblasts; the organs usually release the enzyme into the blood. Some ALPs are normally excreted in the bile. However, according to Ravel (1995), ALP is found in many tissues in the body, with the highest concentrations found in the liver, biliary tract, epithelium, bone, and intestinal mucosa. GOT is found in several organs and tissues (for example, liver, skeletal muscles, heart, and red blood cells). ALT is predominantly found in the liver, with a moderate-sized component in the kidney, and small quantities in the heart and skeletal muscles. When rabbits become sick with suspected liver disease(s), chemistry panel (part of the blood work) is performed to determine liver disease that caused elevated levels of GOT, GPT and ALP (Meredith and Rayment, 2000). Blood biochemistry could be used to monitor the progress of disease before final evaluation by pathology of arteries and organs (Aguilera et al., 2002; Marinou et al., 2010; Tsantila et al., 2010). Albumin is a protein specifically made by the liver and is easily and cheaply analyzed in the laboratory. It is mainly constituted of 60 % total protein. Decreased levels of albumin are found in chronic liver disease (cirrhosis) and also nephritic syndrome where it is lost in urine. Low albumin results to oedema since the intravascular oncotic pressure is higher than the pressure in extravascular space. Albumin diffuses through damaged membrane and is filtered by the kidney because of its molecular size of 65000 kD (Monica, 2009). Total bilirubin refers to both unconjugated and conjugated bilirubin. Conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several diseases, in which the bilirubin conjugation is impaired, cause similar elevations of circulating unconjugated bilirubin. Bile tract obstruction or damage to hepatocellular structure also causes increases in levels of both direct and indirect bilirubin in the circulation (Balisteri and Shaw, 1987). In the liver, bilirubin is conjugated with glucoronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. An increase in conjugated bilirubin is highly specific for disease of the liver or bile ducts. Hepatocellular injury or cholestasis is suspected when more than 50% of total bilirubin is conjugated bilirubin (Fody, 2005). Rabbits are one of the most important farm animals, which produces meat at 10-15 times or more its own weight in a year through progenies. They produce high class protein characterized as lean meat and excellent fur. The primary function of the kidney is the formation of urine. The kidney performs several functions which aid maintains physiological integrity of the extracellular fluid volume. These processes are: conservation of water, fixed cations, glucose, and amino acids; conservation being used in the broad sense to imply the return to the body fluids of the amount of the substance required by the body (Kluwe, 2001). Elevations in blood urea nitrogen (BUN) concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephlosclerosis, tubular necrosis, glomerular-nephritis (renal causes) and urinary tract obstruction (postrenal causes). Urea is used to detect any abnormality of the kidneys. Urea nitrogen in

plasma and serum levels is shown to be higher in men than women (Kamath et al., 2001). Thus, the applications of laboratory tests can be used to evaluate the functional status of several organs notably the liver and kidneys. This study was aimed to examine the suitability of using dried Citrus limon in diets of growing rabbits to ascertain its suitability as an additive, and its effects on the liver and kidney function.

MATERIALS AND METHODS

Experimental animals

The experimental work was carried out at the farm of Animal and Poultry Production Department, Faculty of Agriculture, Minia University in a semi closed housed on galvanized wire cages (40 × 50 × 35 cm) provided with feeders and automatic drinking watery system, and were kept under the same managerial, hygienic and environmental conditions. A period of 14-16 hours of day light was provided. Feed and water were available all time ad libitum during the experimental period (8 weeks).

Preparation of dry lemon

Lemonwas provided from a private commercial market at El-Minya Governorate Egypt. The lemon was dried at 40ºC until constant weight. The dry lemon was finally milled, sieved (1 mm mesh) and stored in a well tight polyethylene bags at room temperature 25°C. Composite sample of lemon powder was taken in sample plastic bag for nutritional analysis.

Rabbitsdiet and lemon Supplementation

Three batchesofrabbitsdiet each of 500 kg were formulated to contain; 44% ground yellow corn, 40.5% wheat bran, 13.5% soybean meal (44% crude protein), 0.5% lime stone, 1% sodium chloride and 0.5% vitamin & mineral premix. Citrus limon powder was added and thoroughly hand mixed with other feed ingredients of each batch at 0, 10 and 20g DCL/kg diet. Experimental diets were packed in polyethylene bags until feeding.

Animals and treatments

Forty eight unsexed growing New Zealand White (NZW) rabbits aged eight weeks weighed in average 1543.33 ± 25g were randomly blocked by weight into three groups (16 animals each), where the 1st group fed a basal ration free of dried Citrus limon (control), while the 2nd and 3rd groups were fed on the same basal ration supplemented with 10g and 20g DCL/ kg diet respectively. The experiment lasted to 16 weeks of age.

Blood sampling

At the end of experimental period (8 to 16 weeks of age) 5ml of blood samples were collected in non-heparinized tubes from one animal each replicate, then allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20º Cuntil analysis of liver and kidney functions.

Liver Function Tests (LFTs)

Estimation of liver functions by measuring, the activities of liver enzymes such as GOT, GPT, ALP, LDH, GGT, BA, TB and DB using diagnostic kits (Vitro, Germany). Also, total protein, albumin, globulin, albumin globulin ratio were determined using commercial kits (Bio-Med, Egypt), where, alanine and aspartate aminotransferases were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957), Gamma glutamyltransferase activity was determined by the method of Rosalki et al. (1970) using gamma -glutamyl-p-nitroanilide as substrate, alkaline phosphatase by the phenolphthalein monophosphate method (Babson, 1965). The activity of LDH was assayed by the method of Horecker and Kornberg (1948), total protein was determined by the Biuret method (Peters, 1968), albumin by the bromocresol green

method (Doumas et al., 1971), total bilirubin and direct bilirubin concentrations (Fischbach et al., 1996). All analytical testes were done using T80 UV Spectrophotometer UK, at Animal and Poultry Production Department, Faculty of Agriculture, Minia University.

Kidney Function Tests (KFTs)

The serum was analyzed for levels of urea, uric acid, creatinine, creatinine kinase, calcium, phosphate, potassium, sodium and chlorine and results were expressed as mg/dL, U/L and mmol/L respectively, using commercially Bio-Med reagent kits Egypt according to determined according to (Tietz, 1986, 1990). While, blood electrolytes were determined using Vet scan VS2 Blood chemistry and electrolyte analyzer (ABAXIS-Germany) at Animal and Poultry Production Department, Faculty of Agriculture, Minia University.

Histopathological Examinations

For the histopathological analysis, the tissue samples from liver and kidney were collected from the slaughtered rabbits (8 rabbits per treatment) at the end of experimental period (8 to 16 weeks of age) and fixed in 10% BPS formalin saline buffer. Tissues were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene, and embedded in paraffin. The casting of blocks was carried out in stainless block models. The rotary type fully automated microtome (model FAM 47, Acculab, USA) was used for cutting the paraffin sections. Then blocks were trimmed, and sections of 3 mm thickness. Six to seven inches continuous long ribbons of the material were cut and laid on constant temperature water bath (around 50°C). The sections were separated with a heated scalpel after they spread completely. The cut sections were mounted on the clean glass slides using Mayer's egg albumin as the section adhesive. The mounted slides were dried in paraffin oven at 58-60°C for 1 h. The tissue sections were stained by the Harris hematoxylin and eosin staining method using fully automated staining machine (model LST 94, Hestion, Australia). The paraffin sections were deparaffinized with the xylene before hydration through graded alcohol to distilled water. This was followed by the dehydration in ascending grades of alcohol. The clearing was performed in the xylene, and a drop of distrene plasticizer xylene mountant was placed on a coverslip and the section on the slide pressed on it. The slide was inverted, and the cover slip was pressed with a rod to remove the air bubbles if any trapped. Sections were examined at a magnification of ×40 under a LED Fluorescence microscope fitted with the stage micrometer. Microscope; photomicrographs of the samples were recorded using an Optika Research LED Fluorescence Microscope (model B-500TIFL) (Diab et al., 2012).

Statistical Analysis

The study was conducted based on a completely randomized design (CRD) with three treatments and 16 replicates per treatment 1 animal each. Data were analyzed by Statistical Analysis System software (SAS, Version 9.1.3, 2003) using the generalized linear model (GLM) procedure. However, significant differences among treatment means for each trait in experiment was detected using Duncan's multiple rang test (1955). The statistical model was as follow:

$X_{ij} = \mu + T_i + E_{ij}$

Where; X_{ij} = value observed in each experimental unit, μ = mean population, T_i = the effect of each treatment, and E_{ij} =the effect of experimental errors.

RESULTS AND DISCUSSION

Liver function

The obtained data revealed that, no significant difference ($p>0.05$) between dietary treatments was observed in the values of GPT, LDH, BA, DB, TP, albumin, globulin and

AGR (Table 1). The non-significant difference may be due to the biological activities of phenolic compounds present in the additive which may have contribution to the enhancement of the rabbit health status. Even though, as shown in Table 1, there are significant differences ($p<0.05$) between dietary treatments were observed in the mean values of GOT, ALP, GGT and TB. Adding DCL supplementation to growing rabbits depicted increased enzyme levels with increasing DCL level. The significant differences ($p<0.05$) observed in the GOT, ALP, GGT and TB still remains within the normal values as obtained by Ewuola and Egbunike (2008), and Ezenwanne and Ucheya (2012), in clinically healthy rabbits. The value of the serum enzymes was in line with earlier report of Ewuola and Egbunike (2008); and Ezenwanne and Ucheya (2012). The results of the histological examination of the liver (Fig. 2) did not show any visible alteration in the liver cells among all treated groups. These results may give approve the critical role of flavonoids present in Citrus which that are based on their antioxidant activity, e.g. inhibition of platelet aggregation, anticancer, anti-inflammatory and antiviral activities. The flavonoids are Flavanones, flavones, and flavonols, although flavones and flavonols are found in low concentrations as compared to flavanones, but are potent antioxidants and free radical scavengers (Armando et al., 1998). In addition, it has been exhibited that citrus fruit have highest antioxidant activity (Chun et al., 2008), due to the presence of abundant flavonoids, vitamin C and carotenoids (Xu et al., 2008).

Kidney function

The effect of dietary dry citrus limon on kidney function of growing rabbits such as creatinine kinase, creatinine, urea, uric acid, Ca, P, K, Na and Cl are shown in Table 2; the data revealed that adding DCL to growing rabbit diets at all levels recorded a significant improvement ($p<0.05$) in the values of C K and P. However, no significant ($p\geq0.05$) alleviation was detected in the values of Creatinine, Urea, Uric Acid, K, Na, and Cl as a resulted of the previous addition. The result from this study demonstrates that C. limon treatment does not induce electrolyte imbalance in treated rabbits. Sodium is the most abundant extracellular ion, and it plays an important role in muscle contraction. Similarly, potassium, an abundant intracellular ion, plays a vital role in muscle contraction. The results of electrolyte levels were in a normal range of rabbits resulting from the serum level of sodium seen in this study thus provides evidence that the use of citrus limon dose not present risk for renal function in the experimental groups that the sodium and potassium levels were not significant when compared to the control groups. Creatinine and urea concentration were extremely insignificant in all the treated groups as compared to the control group at ($p<0.01$) as represented in Table 2. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function (Henry, 2001; and Wallech, 2009). Moreover, the result reviled that administration of C. limon to rabbits had a positive effect on the kidney in accordance with Solomon et al. (2015) who concluded that, administration of C. limon juice to rabbits has a positive effect on the kidney and added that, it could be suggested that the breeder should supplement animal feed with this beneficial fruit as it will help improve animal health and prevent any incidence of kidney malfunctioning. Also, Manners (2007) reported that citric acid present in C. limon helps in dissolving kidney stones and Benavente-Garcia et al. (2007) reported that ascorbic acid present in C. limon is a natural antioxidant. It also showed that lemon juice has an effect on the level of uric

acid in the kidney. Kang et al. (2007) reported that when lemon has been fully metabolized in the body, the pH of the body is raised. This implies that reduced level of uric acid improves kidney function. The microscopic appearance of the kidneys has shown that supplementation with 10 and 20g DCL/kg diet revealed normal cellular profile, no abnormality seen as control group and this confirm the results of kidney function tests, there were no adverse effects of excises dry citrus limon up to 20g/kg diet.

Histological alterations

The basic structure of liver sections after 8 weeks of dietary treatment, showed numerous hepatic lobules (Figure 2). The central vein is located in the middle of the lobule. The hepatocytes are polygonal in shape with granulated, eosinophilic cytoplasm and centrally located nuclei with one or two nucleoli and delicate strands of chromatin. Also, Kupffer cells appeared between the hepatocytes as spindle-shaped cells Fig. 2 (A, B and C). These cells act as an effective particulate filtration system in the liver tissue to prevent bacteria and other foreign materials to penetrate from the central vein to the systemic circulation .This is due mainly to the phagocytic activity of Kupffer cells, which might explain the better immunity of treated compared to control rabbits in terms of higher plasma globulin level. Moreover, the kidney consists of an outer cortex and an inner medulla. The outer cortex of control kidneys contains the renal corpuscles which appear as large spherical structure and renal tubules (proximal and distal convoluted tubules). Each renal corpuscle is surrounded by the Bowman's capsule composed of simple squamous epithelial cells. It encloses the urinary space and the capillary tuft of the glomerulus which consists of blood capillaries Fig. 2 (D, E and F). The previous finding may insure that the citrus fruit have highest antioxidant activity (Chun et al., 2008), due to the presence of abundant flavonoids, vitamin C and carotenoids (Xu et al., 2008).

CONCLUSION

The results obtained from this study show that dried citrus limon levels do not disrupt the activities of the liver and kidneys. This indicates its safe usage as growth additive in growing rabbits.

Table (1): Liver function alteration of New Zealand white rabbits fed on 0, 10 and 20g/kg diet of dry Citrus limon

Treatments	GPT (U/L)	GOT (U/L)	ALP (U/L)	LDH (U/L)	GGT (U/L)	BA (µmol/L)	TB (mg/dl)	DB (mg/dl)
Control	7.266	11.093^{b}	10.100^{b}	140.256	23.000^{b}	3.560	0.736^{a}	0.233
DCL 10g/kg diet	7.333	11.533^{a}	17.150^{a}	139.428	26.000^{a}	3.553	0.626^{b}	0.223
DCL 20g/kg diet	7.466	11.933^{a}	26.933^{a}	138.153	25.333^{a}	3.583	0.596^{b}	0.220
±SEM	0.074	0.093	3.504	17.360	0.693	0.055	0.014	0.011
P value	0.234^{NS}	0.001^{**}	0.036^{*}	0.116^{NS}	0.049^{*}	0.922^{NS}	0.001^{**}	0.704^{NS}

a, b and c values within a column with different superscripts differ significantly at $p<0.05$, SEM: Standard error of the mean. P= P; DCL= Dried Citrus limon; GPT= alanine aminotransferase; GOT= aspartate aminotransferase; ALP= alkaline phosphatase, LDH= lactate dehydrogenase; GGT= γ-glutamyltransferase; BA=bill acid; TB=total bilirubin and DB= direct bilirubin.

Table (2): Kidney function alteration of New Zealand white rabbits fed on 0, 10 and 20g/kg diet of dry Citrus limon

Treatments	CK (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	UA (mg/dl)	Ca (mmol/L)	P (mmol/L)	K (mmol/L)	Na (mmol/L)	Cl (mmol/L)
Control	158.20^{c}	2.01	51.23	3.39	3.63	1.20^{b}	4.53	144.00	103.00
DCL10g/kgdiet	175.73^{b}	2.252	52.82	3.36	3.53	1.50^{a}	4.46	144.66	101.00
DCL20g/kgdiet	198.33^{a}	2.23	53.84	3.25	3.83	1.60^{a}	4.30	145.33	103.66
±SEM	6.88	0.28	2.43	0.08	0.13	0.10	0.10	1.81	0.69
P Value	0.01^{*}	0.53^{NS}	0.75^{NS}	0.52^{NS}	0.35^{NS}	0.02^{*}	0.31^{NS}	0.87^{NS}	0.07^{NS}

a, b and c Values within a column with different superscripts differ significantly at $p<0.05$, SEM: Standard error of the mean. P= P value; DCL= Dried Citrus limon; CK= Creatinine Kinase; Ca= calcium; P= phosphorus, K= potassium; Na= sodium; Cl=chloride and UA=Uric Acid.

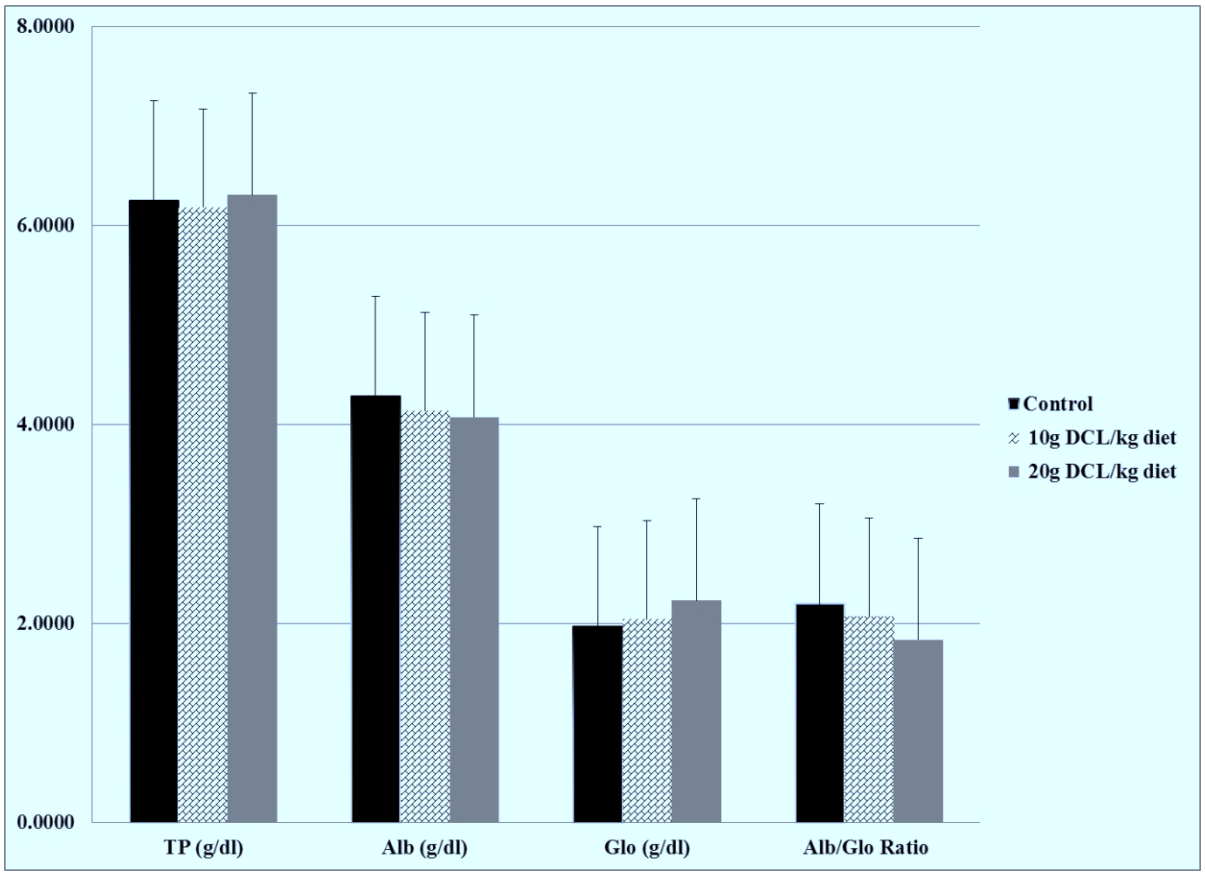

Figure (1): Protein profile of New Zealand white rabbit's serum as affected by dietary 0, 10 and 20g/ kg diet of dry *Citrus limon.*

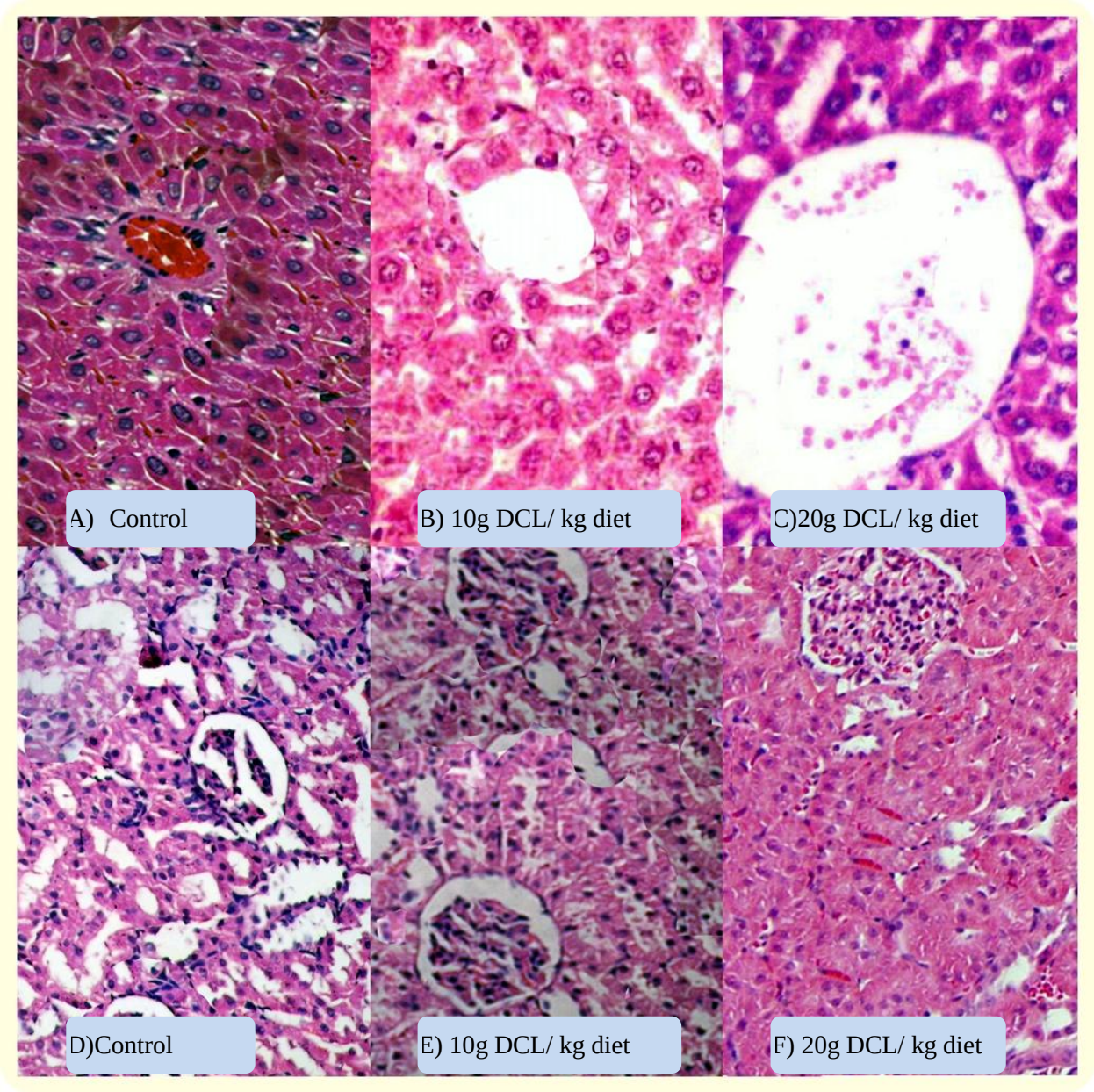

Figure (2): Photomicrograph of liver (A, B and C) and kidney (D, E and F) sections showing normal histological structure stained with general stain (H&E)

REFERENCES

Aguilera, C. M.; Ramirez-Tortosa, M. C.; Mesa, M. D.; Ramirez-Tortosa, C. L; and Gil, A., (2002). Sunflower, virgin-olive and fish oils differentially affect the progression of aortic lesions in rabbits with experimental atherosclerosis. Atherosclerosis, 162: 335-344.

Arias, B.A.; and Ramon, L. L., (2005). Pharmacological properties of citrus and their ancient and their medical use in the Mediterranean region. J. Ethnopharmacol., 97: 89-95.

Armando, C.; Maythe, S.; and Beatriz, N. P., (1998). Antioxidant Activity of Grapefruit Seed Extract on Vegetable Oils. J. Sci. Food Agri., 77: 463-467. http://dx.doi.org/10.1002/(SICI)1097-0010(199808)77:4<463::AID-JSFA62>3.0.CO;2-1.

Babson, L. A., (1965). Phenolphalein monophosphate methods for the determination of alkaline phosphatase. Clin. Chem., 11: 789- 796.

Balisteri, W. F.; and Shaw, L. M., (1987). Liver function. In Tietz, N.W. (ed.) Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company, pp: 729-761.

Benavente-Garcia, O. A.; Castillo, J. V.; Alcaraz, M. E.; Vincente, V. O.; Del Rio, J. A.; and Ortuno, A. S., (2007). Beneficial action of citrus flavonoids on multiple cancer-related biological Pathways. Current cancer Drug Targets, 7(8): 795- 809.

Berhow, M. A.; and Smolensky, D., (1995). Development and substrate specificity of hesperetin-7-O-glucosyltransferaseactivity in Citrus limon tissues using high performance liquid chromatographic analysis. Plant Sci., 112: 139-147.

Chun, O. K. Y.; Chung, S.; Claycombe, J.; KJ and Song, W. O., (2008). Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. Adults. Nutrition, 138: 753-760.

Deyhim F, Mandadi K, Faraji B, Patil BS (2008). Grapefruit juice modulates bone quality in rats. J. Med. Food 11: 99-104.

Diab KAE, Elmakawy AI, Abd-Elmoneim OM, Sharaf HA (2012). Assessment of Genotoxicity and Histopathological Changes Induced by Polyethylene Glycol (PEG6000) in Male Mice. J CytolHistol 3:153. doi:10.4172/2157-7099.1000153

Doumas, BT, Watson, WA and Biggs HC, (1971). Albumin standards and Management of serum albumin with bromocresol green. Chin. Chim. Acta. 31:87-96.

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics.11, 1- 42.

Ewuola, EO, and Egbunike GN (2008). Haematological and serum biochemical response of growing rabbit bucks fed dietary fumonisin B1. African Journal of Biotechnology, 7: 4304-4309.

Ewuola, EO, Folayan OA, Gbore FA, Adebunmi AI, Akanji RA, Ogunlade JT, and Adeneye JA (2004). Physiological response of growing West African goats fed groundnut shell-based diets as the concentrate supplements. BOWEN Journal of Agriculture, 1: 61-69.

Ezenwanne, EB, and Ucheya RE, (2012). A study of the serum concentrations of some hepatic enzymes in does of aqueous leaf extract of Vernoniaamygdalina in rabbits. International Journal of Veterinary Advances, 4: 80-83.

Fischbach, F., (1996). A manual of laboratory and diagnostic tests. 5th edition. Philadelphia Lippincott:382-384.

Fody, E.P., (2005). Liver function. In Bishop, M.L., Fody, E.P. and Schoeff, L. (eds) Clinical Chemistry: Principles,

Procedures and Correlations. 5th ed. Philadelphia: Lippincott Company, pp 334-336.

Gonzalez ME, Domínguez PR, Moreno DA and García VC (2010). Natural bioactive compounds of Citrus limon for food and health. J. of Pharm. and Biomed. Anal., 51: 327-345.

Henry, JB, (2001). 20th ed. Philadelphia, PA: W. B. Saunders Company. Clinical diagnosis and management by laboratory methods.

Hernandez, MV, Montes MMA and Elizalde GMP (2009). Study of the thermal degradation ofcitrus seeds. Biomass. Bioenergy., 33: 1295-1299.

Horecker, B. L. and A. Kornberg (1948). The extinction coefficient of the reduced band of pyridine nucleotides. Journal of Biological Chemistry,vol.175, pp.385–390.

Huyghebaert, G., Ducatelle, R., and Van Immerseel, F., (2011). An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187:182-188.

Jekins, J, (2000). Rabbit and Ferret liver and gastrointestinal testing. In: A Fudge, Edn., Laboratory Medicine: Avian and Exotic Pets. Philadelphia. WB Saunders; pp 291-304.

Kamath, P, Wiesner, R., Malinchoc, M., Kremers, W., Therneau, T., and Kosberg, C., (2001). A model to predict survival in patients with end stage liver disease. Hapatology 33:464-470

Kang, DE, Sur RL, Haleblian GE, Fitzsimons NJ, Borawski KM, and Preminger GM (2007). Long-term Lemonade hypocitraturic nephrolithiasis. J. Urol., 177 (4): 1358-1360.

Kluwe, WO, (2001). Renal Function tests as indictors of Kidney injury in sub-acute toxicity. Toxicol. Appl. Pharmacol., 57:414-424.

Manners, GD, (2007). Citrus limonoids: analysis bioactivity and biomedical prospects. J Agric. Food Chem., 55(21): 8285-8294.

Marinou, KA, Georgopoulou K, Agrogiannis G, Karatzas T, Iliopoulos D, Papalois A, Chatziioannou A, Magiatis P, Halabalaki M, Tsantila N, Skaltsounis LA, Patsouris E, and Dontas IA, (2010). Differential effect of Pistaciavera extracts on experimental atherosclerosis in the rabbit animal model: an experimental study. Lipids in Health and Disease, 9: 73. doi: 10.1186/1476-511X-9-73

Meredith, A, and Rayment L, (2000). Liver disease in rabbits. Seminars in Avian and Exotic Pet Medicine, 9: 146-152.

Monica,C. (2009). District Laboratory Practice in Tropical Countries, pp: 356-358.

Nijveldt, R.N.V., D.E.C.V. Hoorn, P.G. Boelens, K.V. Norren, and P.A.V. Leeuwen, (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr., 74: 418-25.

Olorode, BR, Ajagbonna OP, and Babatunde GM, (1995). Comparism of air-dried poultry droppings in broiler rations: Effect on performance, organ weight and hematological parameters. International Journal of Animal Science, 10: 289-293.

Peters, T., (1968). Determination of total protein in serum. Clinical Chemistry, 14:1147.

Ravel, R, (1995). Clinical Laboratory Medicine: Clinical application of laboratory data. 6thEdn., St Louis. Mosby Year Book Inc; pp 309-330.

Reitman S, Frankel S (1957). A colorimetric method for determination of serum oxaloacetic and glutamic pyruvic

transaminases. Am J Clin Pathology 28:56–63

Rosalki, S.B., D. Rav, D. Lehman, and M. Prentice, (1970). Determination of serum gamma-glutamyl trans peptidase activity and its clinical applications, Ann. Clin. Biochem. 7 143–147.

SAS (2003). Institute. Inc. SAS/STAT user's guide: Statistics (versin6.4thed.) Vol.2. SAS Insitute. Inc. Cary. NC. 2003.

Solomon, I.P., Oyebadejo S.A., Ukpo E.M. and Joseph O.S. (2015). Changes in serum electrolyte, creatinine and urea of fresh Citrus limonjuice administered to growing rabbits (Oryctolaguscuniculus). International Journal of Agricultural Science Research Vol. 4(8), pp. 180-183.

Tietz, N. W., (1986). textbook of clinical chemistry. W. B. Saunders, Philadelphia, p 1271- 1281.

Tietz, N. W., and Saunders, W. B., (1990). clinical guide to laboratory tests: 2nd ed. Philadelphia: 26-29.

Tripoli, E, Guardia ML, Giammanco S, Di MD and Giammanco M (2007). Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry, 104: 466-479.

Tsantila N, Karantonis HC, Perrea DN, Theocharis SE, Illopoulos DG, Latrou C, Antonopoulou S, and Demopoulos CA, (2010). Atherosclerosis regression study in rabbit upon olive pomace polar lipid extract administration. Nutrition Metabolism and Cardiovascular Diseases, 20: 740-747.

Wallech, J, (2009). 7th ed. Philadelphia: Lippincott Williams and Wilkins; Interpretation of diagnostic tests.

Xu, G, Liu D, Chen J, Ye X, Ma Y and Shi J (2008). Juice component and antioxidant capacity of citrus varieties cultivated in China. Food chemistry, 106: 545-441.

Yang, Y., Iji, P.A., and Choct, M., (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World. Poultry Sci. J. 65:97-114.

الملخص العربى

التغيرات الهستولوجية والبيوكيميائية لكبد وكلي الأرانب النامية المغذاه على تركيزات مرتفعة من الليمون المجفف

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أجريت هذة التجربة لدراسة بعض التغيرات الهستولوجية و البيوكيميائية الناتجة عن تغذية الأرانب النامية على تركيزات مرتفعة من الليمون المجفف وكانت مده التجربة 8 أسابيع. حيث تم إستخدام عدد 48 أرنب (نيوزيلاندى أبيض عمر 56 يوم) حيث تم تقسيمهم عشوائياً إلى ثلاث مجموعات كل مجموعة 16 أرنب وكان تسكين الأرانب فى بطاريات من السلك المجلفن (35 × 50 × 40سم) في عنبرمغلق وكان توزيع المجموعات كالتالى:-

1. المجموعة الأولى كنترول.
2. المجموعة الثانية تم تغذية الأرانب على العليقة الكنترول +10 جم ليمون مجفف / كجم عليقة.
3. المجموعة الثالثة تم تغذية الأرانب على العليقة الكنترول +20 جم ليمون مجفف / كجم عليقة.

تم جمع عينات الدم فى نهاية التجربة من صوان الأذن لقياس بعض وظائف الكبد و الكلى ممثله فى نشاط بعض الإنزيمات (الإنزيمات الناقلة لمجموعة الأمين و الفوسفاتايز القاعدى والجاما جلوتامات و اللكتات ديهيدروجيناز و الكيرياتينين كيناز) (GOT, GPT, ALP, GGT, LDH and CK) و كل من بروتين السيرم الكلى و الألبومين و الجلوبيولين و اليوريا و حمض اليوريك و الكرياتينين والكالسيوم و الفوسفات والصوديوم و البوتاسيوم وكذا الكلور.
وكذالك تم إجراء تجربة ذبح لعدد 24 أرنب (8 / مجموعة) لفحص التغيرات الظاهرية و النسيجية للكبد و الكلى.
و خلصت نتائج التجربة إلى أن إستخدام الليمون المجفف بتركيزات 10 و 20 جم ليمون مجفف / كجم عليقة لم تؤثر ($p>0.05$) معنوياً على معظم وظائف الكبد و الكلى، حيث زاد نشاط كلاً من الـ GOT و ALP و CK كذالك ارتفع تركيز الفوسفات فى حين إنخفض تركيز الصفراء الكلية إلا أن هذه التغيرات كانت فى الحدود الطبيعية للأرانب النامية.
أما عن التغيرات الهستولوجية فلم تكن هناك أى تغيرات ملحوظة لأنسجة وخلايا الكبد و الكلى.
ونستنتج من هذة الدراسة أنه يمكن إستخدام الليمون المجفف حتى 20 جم/ كجم عليقة فى تغذية الأرانب النامية حيث أنها حسنت من وظائف الكبد و الكلى دون أى آثار ضاره عليهما.