Histological and Histochemical Features of the Adrenal Gland, Kidney and Liver of the Many Laboratory Animals, Comparative Study

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Abstract

Objective: *The aims of study to demonstrate and comparison the histological structures, histochemical and biochemical characteristics of adrenal glands, kidneys, and liver between the many laboratory animals and rodents (*rabbit*, cape hare, hamster, mice and guinea pig) directly involves living environment, metabolic and nutritional needs.* ***Materials and methods:*** *Seven animals from each species.* ***Results:*** *Histological results showed that the adrenal glands were encircled by capsule, three zones of cortex; glomerulosa, fasciculate, and reticularis, the medulla was made up of epinephrine and nor-epinephrine secreting cells. The kidney has been covered by a tiny amount of collagen and reticular fibers in all study animals, except in cape hare; the capsule was formed up of thick connective tissues. Renal corpuscles can be classified into; cortical, mid-cortical, and juxtamedullary. Proximal convoluted tubules were the longest, lined by cuboidal epithelial tissue had brush border. Henle loop comprises of a thin and thick limbs, were longer in mice and guinea pig compared to in other animals, and distal convoluted tubules were short and not brush border. Collecting tubules continues from the terminal part of distal tubule. All the corpuscle and nephron's lumen had a bigger diameter in cape hare compared with other animals. Bowman's capsules contained a more collagen fibers, as well as a thin space around the glomeruli in cape hare, but other animals' have large renal gaps and capsules contained fewer collagen fibers. The liver was enveloped by a thin capsule containing simple squamous epithelium with fibroconnective tissues, the parenchyma consisted of hepatocytes, as well as the central vein around these cells, divided by hepatic sinusoids and Kupffer cells protruded alongside the endothelial cells that lined the sinusoids. Histochemical results; brush border in kidneys and liver cells were take positive reaction to PAS. Biochemical tests appeared level of each the enzymes, and uric acid were significant differences between the animals. Because the kidney has little ability to filter out urea, an increase in urine volume is necessary as the urea load grows. The metabolic rate differs greatly amongst species.* ***Conclusion:*** *The findings demonstrated the hare's ability to adapt to its terrestrial habitat, as these qualities reduce the quantity of glomerular filtration, and differ in rate of metabolism caused by environmental factors or the animal's nutrition.*

Key words: *Kidneys, Liver, Histochemical, Kupffer cells, Nephron, Laboratory animals*

INTRODUCTION

Laboratory animals are commercially valuable animals employed in scientific experiments and medical testing (1,2). Rabbits and some rodents, such as guinea pigs, were classed as fermenters of nutrients that are digested in the hindgut, depending on the microorganisms present (3,4). Continuity and excretion, the primary roles of the urinary system, depends strictly on the structural makeup of the pelvis, notwithstanding the fact that different anatomical features can be equivalent, but a great concern for humans must be assumed when compared to animals. In kidneys, vital chemicals like glucose and sodium chloride are preferentially reabsorbed and preserved (5,6). Proteins are nitrogenous complexes containing organic substances found in all animals and plants, and functional proteins include enzymes and hormones that govern chemical reactions. The adrenal glands are the endocrine glands generate adrenaline and steroids hormones; regulate the body's Na+ and K+ ions and effects on the metabolic activity of carbohydrates, proteins, and fats, and the kidney helps to maintain homeostasis through a complex mechanism that includes filtration, absorption, and secretion. It also regulates the body's fluid and electrolyte balance and is the site of production of renin, a substance that regulates blood pressure, and erythropoietin, which stimulates the production of erythrocytes (7-11). The nephrons and collecting tube comprise nearly all of the kidney's functional components (12).

The liver has two types of secretions: external secretion (bile) and internal secretion (protein and amino acid secretion). It regulates metabolic activity and is responsible for the elimination of hazardous and damaging chemicals from the body (13,14), the liver contain some enzymes that help in chemical reactions; Aminotranminase, Asparate liver enzymes and Alanine Aminotransminase (15,16). Several researches have looked into the links between certain organic compound levels and age, gender, and animal species (17-29).

There has been very little research published on the features and biochemical tests of the kidneys and liver in different animals. The goal of this study was to provide light on kidneys and liver structure by histological and histochemical methods and the functions of the kidneys and liver. Various mammals are employed to research the structure and function of kidneys; often, the conclusions are critical and immediately applied to humans, and the value of the kidney and liver tissue of laboratory animals to be used in experimental researches was significant, and this knowledge was essential to provides data to the investigative researches.

MATERIALS AND METHODS

Ethical approval

All procedures were performed in compliance with animal care, College of Veterinary Medicine, Al-Muthanna University, Iraq; during February 2024.

Study animals: This investigation was performed utilizing Ten animals of each of the healthy adult rabbit *Oryctolagus cuniculus* which were nonruminant herbivore, tends for select the low fiber portion of the plant materials; Cape hare *Lepus capensis* was a nocturnal herbivore; and rodents the hamster *Mesocricetus urtus*; mice *Mus musculus* and guinea pig *Cavia aperea* which a descendant of wild cavy. The animals were obtained alive from marks in Al-Muthanna city. Rodents are classified as one of the most essential mammals used in laboratories, conducting tests, and scientific research.

Samples collection: Blood samples (5 ml each animal) were obtained directly before slaughter from the heart, ear, or jugular vein in sterile tubes (anticoagulant-free test tubes) to assess differences in the levels of several biochemical tests and physiological factors. The samples were left to coagulate before being separated by a centrifuge at 5000 cycles per minute for five minutes. The serum was then stored in clean tubes devoid of any contaminant at a temperature of 20 degrees Celsius. The serum samples were examined using spectrophotometers. The blood sample was centrifuged, the serum and urine, as well as urea, uric acid and ammonia, blood urea nitrogen test measures urea nitrogen levels in the bloodstream were measured in tissue samples and body fluids within two days of the collection using a miniaturized modification of (16). Potassium, sodium, magnesium dioxide Mg^{2+} were measured using flame photometry on urine and serum that had been suitably diluted with ion-free water, as well as on tissue samples that had been dried and extracted with 0-1 N nitric acid. The spectrophotometer was used to determine uric acid and creatinine concentrations in order to identify kidney activity and enzymes (Aminotransminase (AST) and Alanine Aminotransminase (ALT). Animals were subsequently anesthetized with a ketamine and xylazine overdose before being slaughtered for histochemical analysis (30).

Histological study: Samples were obtained from various parts of the adrenal glands, cortex and medulla of the kidney and lobes of liver, dehydrated by successive spirals of ethyl alcohol, cleared by xylen, infiltration and embedding with paraffin wax, and sectioned by rotary microtome (31).

Tissue staining: The stains that used; Masson's trichrome; demonstrate the collagen and smooth muscle, verhofes stain for the elastic tissues (31).

Histochemical staining:

Periodic acid schiff (PAS) was used to treat carbohydrate, glycoprotein, mucopolysaccharides, and basement membranes. Sections were treated with an aqueous solution (1%) of periodic acid for 30 minutes. The sections are then rinsed to remove any acid residue, then Schiff's reagent was applied for 30 minutes, followed by potassium metabisulfite 0.55% for 1 minute (31).

Alcian blue pH 2.5: The procedure for acidifying mucopolysaccharides with alcian blue pH 2.5 is as follows: Deparaffinize with xylene, then rehydrate in graded ethanol, alcian blue for thirty minutes, tap water for five minutes, then nuclear fast red for ten minutes. Water for one minute. Dehydrate using a gradient of ethanol (31).

The neutral and acidic mucopolysaccharides were compared using the accompanied alcian blue plus periodic acid schiff (AB-PAS) technique, Deparaffinize and rehydrate using distilled water. Alcian blue, pH2.5, microwave. Allow forty-five seconds of intense power and 2 to 5 minutes of stand time. Wash approximately 5 minutes under running tap water, and rinse with distilled. Periodic acid in 0.5% for five minutes. Wash with distilled water. Schiff's Reagent and Microwave Allow to rest for 2-5 minutes following 45 seconds of intense exercise. Wash with running tap water for 5 minutes, and rinse using distilled water (31).

Tissue preparation for scanning electron microscopy (SEM)

The specimens of liver were preserved in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2%) solutions for 48 hours. Following that, the sample was rinsed twice with 0.1 M phosphate buffer solution. The tissues were maintained in a 1% osmium tetroxide buffer for 1 hour before being treated with acetone series and dehydrated using critical point drying. The specimens were plated with gold palladium and examined under a scanning electron microscope (SEM 3400) during various magnifications (32).

STATISTICAL ANALYSIS:

This study was analyzed with using the two-ways analysis of variance (ANOVA) test at a 1% level of significance (compare between several animals). Data are processed and analyzed by using statistical program social science (33).

RESULTS AND DISCUSSION

Adrenal glands were encircled by a big, well developed capsule and an adrenal cortex. This cortex has three zones; zona glomerulosa, zona fasciculate, and zona reticularis. The zona glomerulosa was the layer immediately beneath the capsule. The cells in this zone were massive and conspicuous, with large nuclei, and were columnar cords surrounded by many capillaries. The zona fasciculate was the biggest zone of the adrenal cortex, consists of long straight cords of massive polyhydral secretory cells. The zona reticularis was the deepest layer, consists of a short anastomosing network of secretory cells cords divided by sinusoids. These cells were frequently more strongly stained than other zones. The medulla was made up of large pale staining it polygonal cells arranged in tiny anastomosing cords backed by reticular fibers; these cells are separated by sinusoids capillaries that intervene between adjacent cords; and parasympathetic ganglionic cells, which are large cells that can be found alone or in groups (Fig. 1-4). In the adrenal medulla, epinephrine-secreting cells appeared as big spherical cells with light acidic cytoplasm and ovoid huge conspicuous dark nuclei with many nucleoli. The smaller cells with darker nuclei represent nor-epinephrine cells, which were mixed in with the earlier cells, and sinusoidal capillaries (Fig. 5,6), and no found differ in histological structure between the study animals, this agree with (34-36). The adrenal glands are the endocrine glands that generate several hormones, including adrenaline and steroids; aldosterone (regulate the body's Na+ and K+ ions (water and electrolyte balance) and cortisol (effects on the metabolic activity of carbohydrates, proteins, and fats) (37).

The kidney of cape hare was encased with thick collagenous fibrous tissue shell, but in other animals by thin collagenous fibrous tissue shell (Table 1, histogram 1) (Fig.7), A considerable volume of blood flows through the kidney, which is responsible for circulatory blood filtration. The notifications were similar (28) in guinea pigs. The present research identified a connective tissue capsule because of its remarkable elasticity and toughness in controlling the form and size of the kidney across different animals. Hare's capsule was thicker than that of other animals, and there was a substantial volume of blood flow in the kidney, which is responsible for circulatory blood filtration (Fig. 7). This is consistent with the findings of (29) in dog. This wide range of thicknesses may have an impact on renal function.

The renal corpuscle was made up of the glomerulus, a tuft of capillaries, and a double-layered Bowman's capsule. Three types of renal corpuscles have been recognized based on their position in renal cortex; Cortical, midcortical, and juxtamedullary renal (Fig.8-12) This discovery is consistent with the findings of (5, 29, 30, 38) on many mammalian species. The size variation leads that glomerular filteration surface would be much larger in large juxtamedullary population of the glomeruli, which could lead to preferential filtration in these nephrons, and because they have long loop, results indicated maximal concentrate capacity, is consistent with (39) said the rate of filtration of juxtamedullary nephron is (8) times that of nephron in cortex.

The kidney is made up of functioning filtering units called nephrons that divided into cortical labyrinth, which included renal corpuscles with vascular bole and aurinary pole, complicated

tubules of the nephrons, medullary rays had been parallel arrays of tubules entered the cortex and formed the core of the lobule, had straight tubules and collecting ducts, and had more mid-cortical instead of juxtamedullary nephrons alongside the Henle's loops (Fig.9-12). The afferent arteriole enters the renal corpuscle and forms the glomerulus, that is surrounded by Bowman's capsule. In mice, hamster and guinea pig no space between layers of capsule (Fig.6-10). Unlike the capsule in rabbit and hare consisted of two layers (inner and outer) of the capsule of Bowman with renal space (Fig.8).

Proximal convoluted tubules are the first longest categorize lined by tall cuboidal epithelial tissue and show up to be occupied by brush border (Fig. 9,10). This provides a greater surface area for accommodating the membrane channels whose function is responsible for absorbent into the cell small molecules that come from filtrate in the tubular lumen, indicating such a difference was likely attributable to a higher cell density and an impressive evolution of apical brush border membrane (35,40), these may lead The henle loop is made up of two types of limps: thin and thick, with various lengths (Fig. 11). This variance is related to the aquatic environment of these animals, and the short Henle loop in rabbits, mice, and guinea pigs did not require considerable water re-absorption as compared to cape hare, which discharge concentrated urine. In mammalian, the protein intake increase the rate of glomerular filtration, that has been linked to the advancement of the renal illness. The study demonstrate which postprandial increased the glomerular filtration rate disappears and mild a vegetable protein compare to animal products with significant differences ($P < 0.05$) (Table 1, histogram 1). This suggests that this kidney was holding water and requires more reabsorption. These kidneys played a significant role in the retention of biological function during the creation of concentrated urine.

The medulla comprises of the renal pyramids. The region between pyramids inhabited by renal columns composed of collecting ducts, straight tubules, and capillaries perpendicular to the collecting tubule, Collecting tubule from the distal tubule end and has been divided into two parts: cortical and medullary ducts (Fig. 8,9). Similar observations have been made by (41-46), the features stated demonstrated that the hare kidney has a significant reabsorption competence, resulting in the production of extremely concentrated urine, allowing it to adapt to the arid environment by conserving more water, and increasing the degree of infiltration to reintroduce a large amount of fluid into the bloodstream to resist dehydration. This indicates that the kidney is retaining water and requires further reabsorption. Hare's pee was highly concentrated, and they were able to withstand several days of water restriction while adapting their water intake to ambient temperature. This allows them to generate less urine but at a higher concentration. Water loss is further decreased by reducing overall evaporative water loss. Fluid selections enter the renal space via a capillary wall and visceral layer. The mesangial cells play phagocytic roles, and as vasoconstrictors, the macula dense cells are highly sensitive to the concentration of ions and the amount of fluid in the tube, sending chemical messages causing the enzyme rennin to be released into the circulation (47). The distal tubules undergo ion exchange, which absorbs sodium and releases potassium ions. This system influences the organism's overall salt and water content, and secretes hydrogen and ammonium ions into tubular urine (48, 49).

The liver was enveloped by a thin capsule containing simple squamous epithelium with thin connective tissue has fibroconnective tissues, with parenchyma consisted of hepatocytes, as well as the central vein around these cells, divided by hepatic sinusoids and reticular fibers. Liver cells

were radially elevated surrounding the central vein, and Kupffer cells protruded alongside the endothelial cells that lined the hepatic sinusoids (Fig. 13-18). Table (1) shows significant differences in capsule thickness and diameters of the central vein, hepatic artery, and hepatic duct across study animals. Differences amongst animals may be attributed to evolutionary variances. Kupffer cells' physiological purpose is protective function, expressed with eating foreign body and harmful compounds which arrive from portal artery. The liver is divided into lobules with few or no connective tissue septa. Central veins are randomly distributed within the lobules. Liver cells were polyhedral and have rounded nucleus organized as irregular cords. The portal triads, located in the angles of the hepatic lobules; include branch of the portal vein, hepatic artery, and bile duct (Fig. 14-18). The cytoplasm of the hepatocyte is clearly vacuolized, whereas cells next to bile ducts possess a homogeneous cytoplasm and nucleus that are possibly light euchromatic or dark heterochromatic. The wider size of the portal triad in hare's liver than in other animals' livers, because of the smaller diameter of the portal venule and hepatic arteriole, allowing for more connective tissues (Table 1), which is consistent with findings of (17). This could be increased metabolic activity as well as roles of the hepatocyte, which correlate to differences in size and surface area among the animals that help to maintained the body homeostasis.

Histochemical examination of the liver demonstrated less each of carbohydrates, and glycogen contents, and activity of the alkaline phosphatase within the cytoplasm of rabbit liver showed as a strong reaction for PAS, compared to a weak positive responses for PAS in a cytoplasm of the mice liver (Fig. 13-18), which agrees with findings of the previous investigators (18) who stated that greater metabolic activity and a greater surface area lead to rapid glycogen depletion. This means that the discrepancy could be caused by environmental factors or the animal's nutrition. Because of its role in metabolizing digestive tract contents, the liver is regarded as an excellent indicator of nutritional disease.

The biochemical analysis of serum uric acid, urea, Sodium and potassium and creatinine for all study animals showed that the level of serum uric acid significantly increased ($P<0.05$) compare between the animals (Table 2, histogram 2). Nutrition did not affect the levels of serum protein quantity, polarization of albumin, and suggests that the high percentages may be suggestive of the particular adapting of the desert environment. The content of urea concentrations in the blood varies according to living things, that the quantity of urea in the blood of cape hare is much higher than the level in the blood of other kinds of animals, as they indicated (5, 20) to the presence of significant differences in the concentration of urea in the bloodstream, as well as the large variation in the nature of nitrogenous values created in the body as a consequence of different metabolic processes, that is directly related to the internal equilibrium of the animal and how much its adaptation to its environment External, whether it resides in an aquatic or terrestrial habitat, necessitates a difference in concentrations of standards.

Hare's serum creatinine levels significantly increased ($P<0.05$) compared to other animals. Other investigations have found that accumulating urea in the blood increases uric acid and creatinine levels in diverse environments. The concentration of PAST, ALT in the blood of mice was found to be significantly lower at a probability level of $0.05>p$ than in other animals (Table 2, histogram 2). Blood proteins have a crucial role in the synthesis of enzymes, hormones, and antibodies, as well as maintaining osmotic pressure. Blood urea is reabsorbed and returned to the bloodstream in the capsular fluid, which has a similar composition to plasma fluid, while certain

chemicals are released. From the cells of tubules and from secretory chemicals delivered by the blood; urea secreted in the Loop of heel, as the cells of this loop are active to absorb water, as well as the secretion of urea and its concentration. The amount of nitrogen ingested affects the percentage of urea in the blood, and the difference in urea clearance is related to the concentration of urea in plasma and the percentage of renal filtration, as well as the amount of nitrogen ingested. It was also discovered that the urea concentration in blood increased when the starved animals. Ammonia is produced in all tissues as a byproduct of protein catabolism, a poisonous chemical that is removed. The body's conversion to urea. Urea is found in the majority of bodily fluids and tissues due to the way it spreads osmotically.

Table (2) demonstrated the biochemical and physiological results of blood samples in various species as they frequently correlate with the efficiency and adaptation of the animal, the level of Aminotransminase (AST) and Alanine Aminotransminase (ALT) enzymes in the blood of cape hare has significantly increased compared to other animals, nevertheless, it noticed a non-significant increase in the levels of these enzymes in a blood serum, which may reflect the state of effort displayed, which is natural. The condition is a significant effort on the kidneys, and then the liver and skeletal muscles, since the measurements of these enzymes are considered an indicator to evaluate the impact of tissues in internal organs. These findings were consistent with (21), which demonstrated that calculating enzyme levels assesses the state of degradation in interior organ tissues. Alternatively, this increase could be attributed to enzymatic processes, which aid in the removal of harmful substances produced by the digestion of feed containing chemicals. Increased urea levels in blood serum. This is due to the animal's adaptability to living in harsh environmental conditions and the stress it is exposed to, and it may have a part in the body's water balance through its ability to retain water (22-26).

The animal wagets subjected to various environmental changes as it moves through desert areas. The results indicate that the levels of urea content. The high urea level in blood is due to the fact that blood returns a portion of the urea created by the liver to the rumen, where the nitrogen returned to the stomach is used to produce microbial protein. Protein affects blood urea content due to big renal glomeruli that comparing the existence of an association between the influence of urea, the concentration of urine, and the growth of the renal pelvis. The kidneys are responsible for maintaining magnesium dioxide equilibrium. Throughout proximal tubule and thick ascending limb, magnesium dioxide is reabsorbed paracellularly, $Mg2+$ returns transcellularly via transient binding potential melastatin in distal tubule. Furthermore, these tubules were fraction $Mg2+$ reabsorption is higher in hare, allowing it to fine-tune $Mg2+$ excretion more effectively, with significant differences between animals, this agree (27).

SEM shows the liver as a parenchymatous organ with the liver cells were scattered among portal space and central vein, monolayered walls of biliary ducts were made up of epithelial biliary cells. Endothelial cells, with their fenestrated cytoplasmic were contribute to the structural formation of the sinusoids; Kupffer cells, within the sinusoids; and Per sinusoidal cell in the Disse space. The hepatic lobule's matrix was made up of the laminae and vascular lacunae that connect portal space to the centrolobular vein, while an interlobular bile ducts and central vein serve as its axes. The intimate spatial connections between hepatic laminae as well as vascular lacunae provide the hepatic architecture a degree of plasticity and flexibility. Putting physiological and pathological factors aside, the lobule of liver can take on various forms (classic, portal, or acinus) depend on

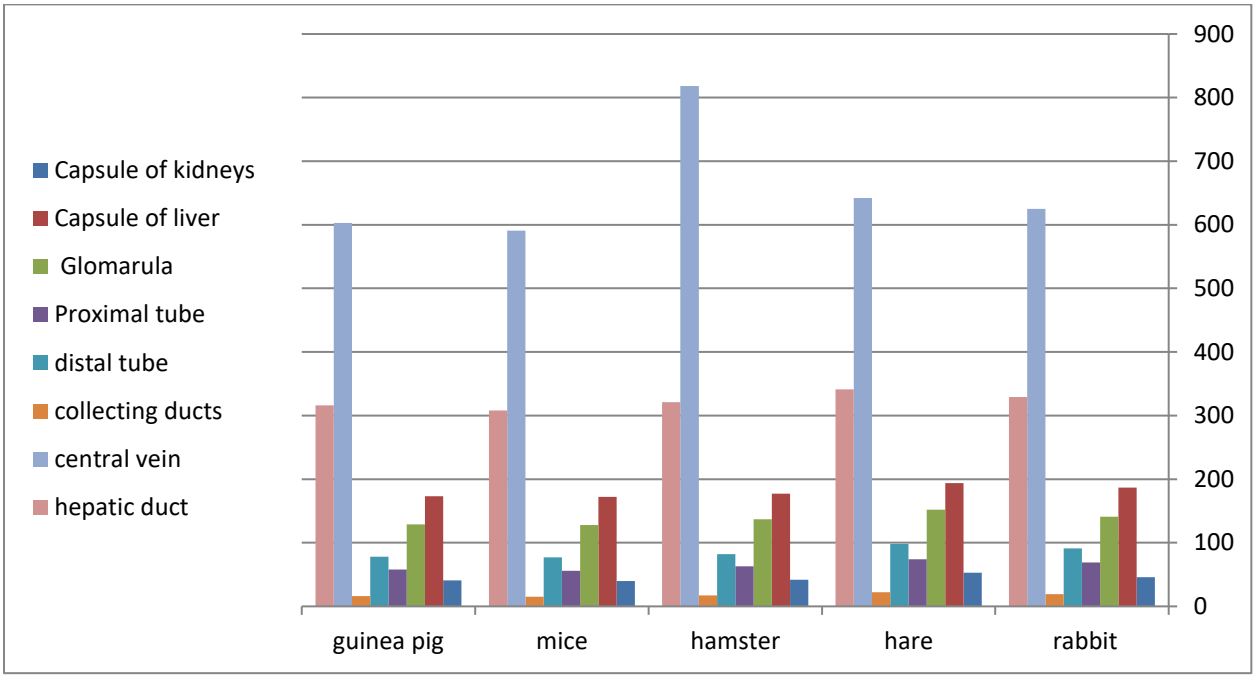
the changes in blood pressure variations throughout the ramification of hepatic vein and resulting variations in blood flow through sinusoids (Fig.19-22), Similar observations have been made by (32).

The portal triad may quickly distinguish the portal space, interlobular biliary duct, that connects to the bile canalicular network via the bile ductules, is made up of cuboidal epithelial cells. The many microvilli covering the cell surface toward the canalicular lumen demonstrate the cells' absorptive/secretory characteristics. Connective tissue fibers are scattered throughout the portal space's vascular and biliary constituents. Their quantity varies by species and race, with mice having fewer levels. These connective fibers surround all of these components and also separate between them vast mall areas, may exert an effect in collecting of the interstitial fluid.

Table 1: Measurement of thickness of capsule of the kidneys and liver between the rabbit, cape hare, hamster, mice and guinea pig

Parts / Species	Capsule of kidney	Capsule of liver	Glomarula	Proximal tube	Distal tube	Collecting duct	Central vein	Hepatic duct
Rabbit	46.2±0.1 A	187.2 ± 1.5 B	141.4±1.5 A	69.1±0.2 C	91.4±2.3 A	19.2±0.3D	625.7 ± 0.9 E	329.6 ± 0.5 A
Cape Hare	53.1±0.2 A	194.2 ± 0.2B	152.2±0.1 A	74.5±0.1 C	98.2±1.1 A	22.4±0.1D	642.3 ± 1.1 E	341.3 ± 1.1 A
Hamster	42.2±0.5 A	177.2 ± 1.4B	137.3±1.4 A	63.4±0.3 C	82.2±0.3 A	17.2±0.4d	618.4 ± 0.2 e	321.4 ± 0.3 A
Mice	40.3±0.1 A	172.2 ± 0.2b	128.5±1.3 A	56.1±0.4 C	77.3±2.4 A	15.3±0.2d	591.2 ± 0.3 e	308.3 ± 1.4 A
Guinea Pig	40.8±0.4 A	173.2 ± 1.3b	129.6±1.2 A	58.3±0.1 C	78.1±0.1 A	16.1±0.1d	603.1 ± 1.6 e	316.2± 0.7 A

The values with capital letters in the same column denote to the significant difference ($P>0.05$) whereas values with small letters denote to the nonsignificant differences ($p<0.05$).

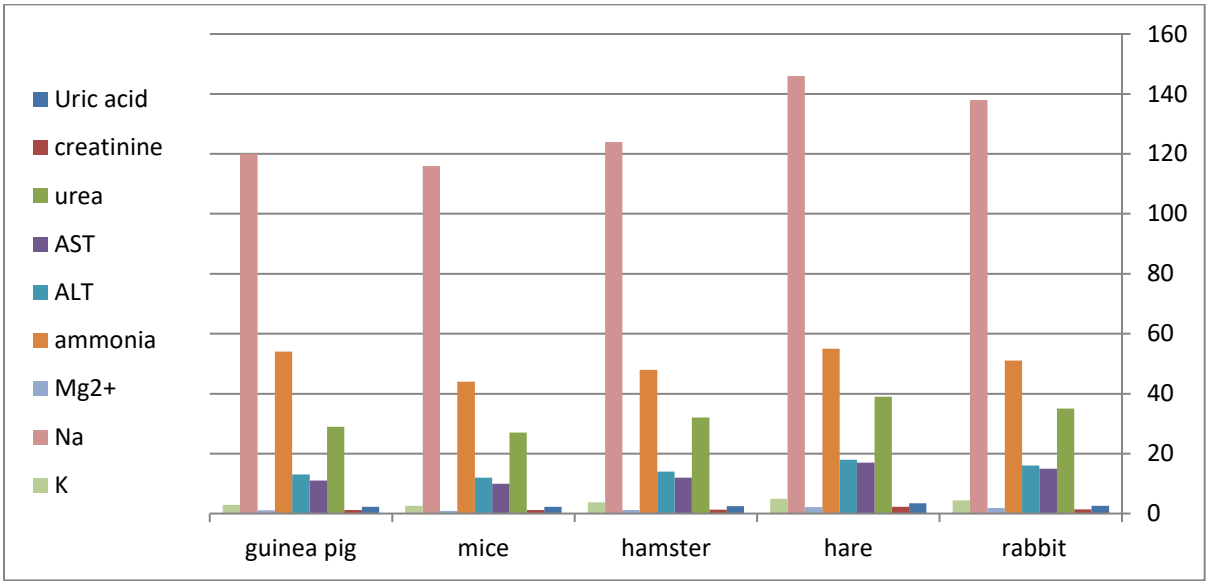


Histogram 1: Measurement of thickness of capsule of the kidneys and liver between the rabbit, cape hare, hamster, mice and guinea pig

Table (2): The biochemistry parameters in kidney and liver of rabbit, cape hare, hamster, mice and guinea pig Mg-dl (X^{-} ± S.E)

Measure / species	Uric acid	Creatinin	Urea	AST	ALT	ammonia	Mg^{2+}	Na	K
in rabbit	2.68±0.1A	1.4±0.3 A	35.1±0.2 B	15.4±0.3A	16.4±0.3 A	51.4±0.3 A	1.8±0.01 A	138.2±1.2 B	4.4±0.01 A
Hare	3.42±0.3A	2.3±0.1 A	39.5±0.4 B	17.2±0.5A	18.7±0.1 A	55.6±0.6 A	2.2±0.02 A	146.1±0.1 B	4.9±0.01 A
Hamster	2.55±0.4 A	1.3±0.4 A	32.2±0.1 B	12.3±0.1A	14.6±0.5 A	48.3±0.7 A	1.2±0.05 A	124.3±4.1 b	3.8±0.02 a
Mice	2.21±0.1 A	1.2±0.1 A	27.1±0.3 B	10.6±0.4A	12.2±0.6 A	44.4±0.9 A	0.9±0.01a	116.4±2.2 b	2.6±0.05 a
Guinea pig	2.23±0.1 A	1.2±0.6 A	29.3±0.6 B	11.8±0.2A	13.1±0.4 A	45.2±0.1 A	1.1±0.06a	120.5±3.2b	2.9±0.03 a

The values with capital letters in the same column denote to the significant difference (P>0.05) whereas values with small letters denote to the nonsignificant differences (p<0.05).



Histogram (2): The biochemistry and physiology parameters in kidney of rabbit, cape hare, hamster, mice and guinea pig Mg-dl

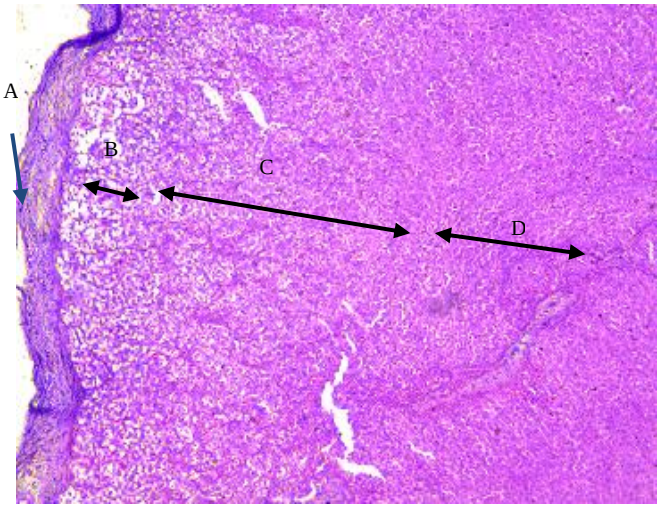


Fig.(1): Cross section of adrenal gland of rabbit; capsule (A), glomerulosa (B), fasciculate (C), reticularis (D), H@E 100X.

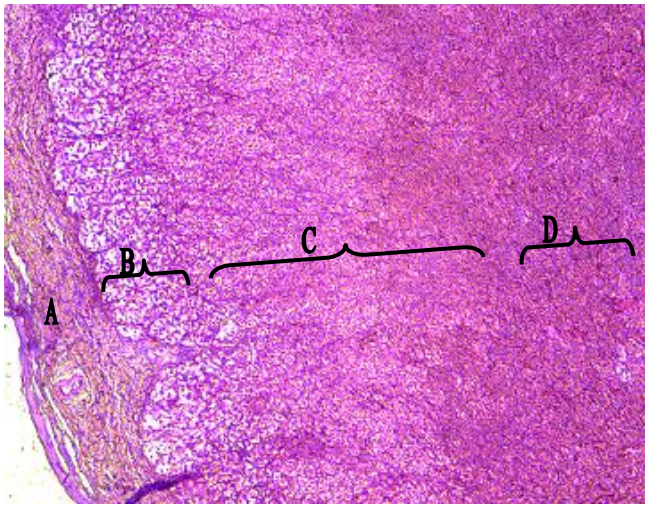


Fig.(2): Cross section of adrenal gland of hare; capsule (A), glomerulosa (B), fasciculate and reticularis (C), medulla (D), Masson 100X.

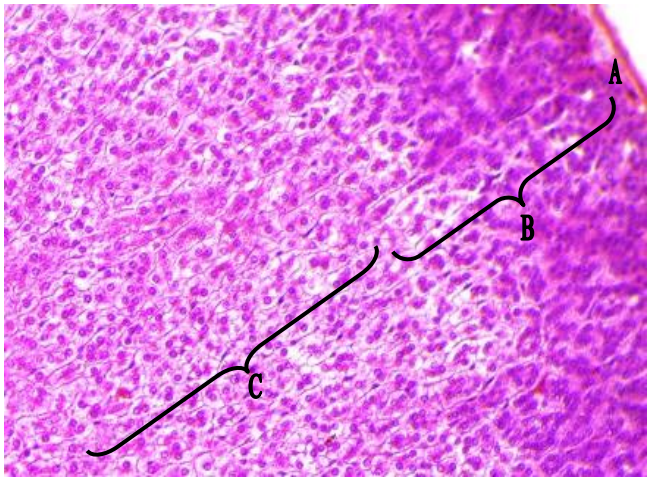


Fig.(3): Cross section of adrenal gland of guinea pig; capsule (A), cortex (B), medulla (C), H@E 100X.

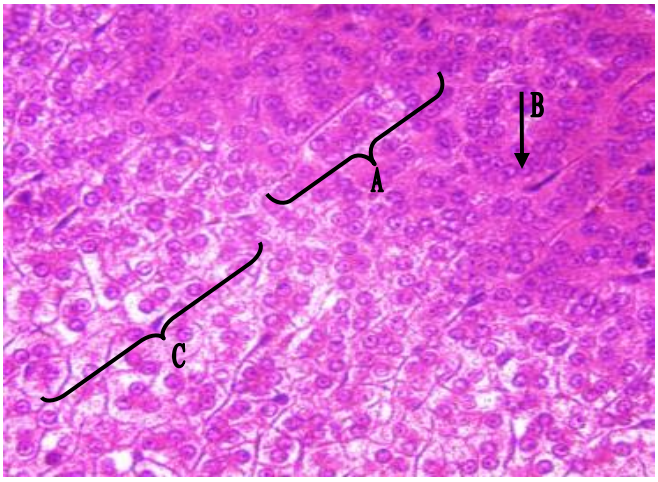


Fig.(4): section of zona glomerulosa in adrenal cortex of mice (A), myloid cells (B), medulla (C), H@E, 200X.

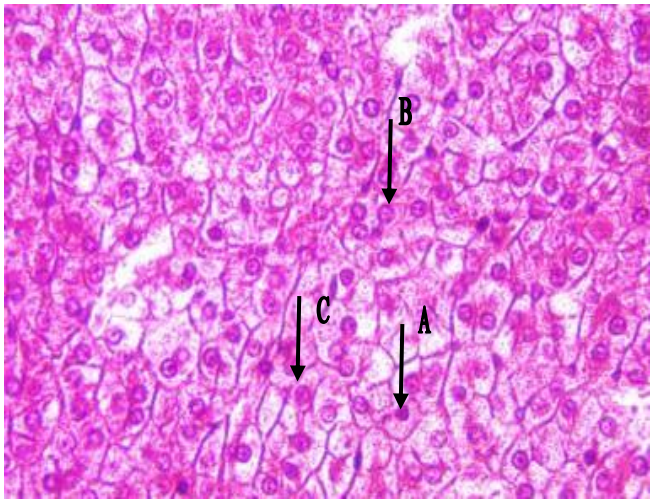


Fig.(5): Cross section of adrenal medulla; nor-epinephrine cell (A), epinephrine cell (B), sinusoid (C), H@E,400X.

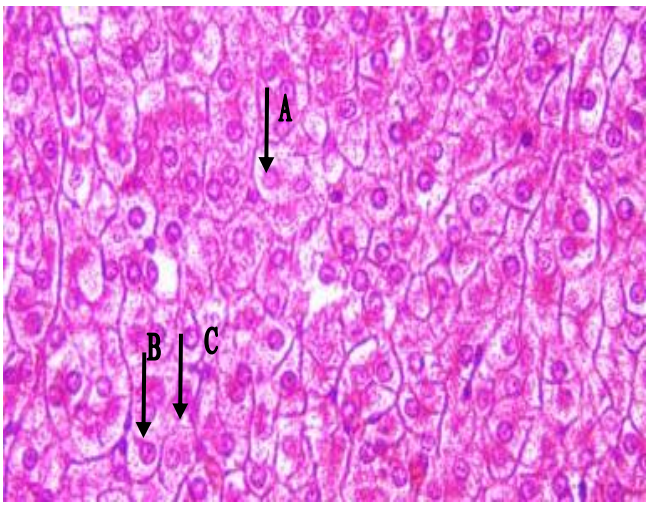


Fig.(6): Cross section of adrenal medulla; nor-epinephrine cell (A), epinephrine cell (B), sinusoid (C), H@E,400X.

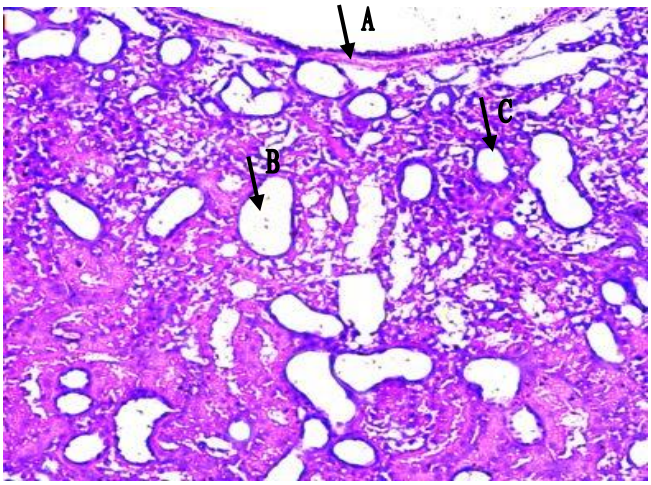


Fig.(7): Cross section of hare kidney; thick capsule of connective tissue (A), distal tube (D), proximal tube (E), Masson 100X.

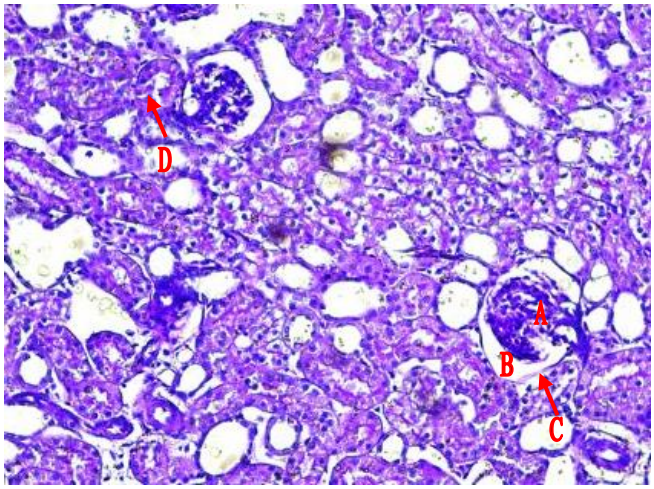


Fig.(8): section of rabbit kidney; glomerular part (A), Renal space (B), Bowman's capsule (C), Juxtaglomerular cells (D), PAS,100X.

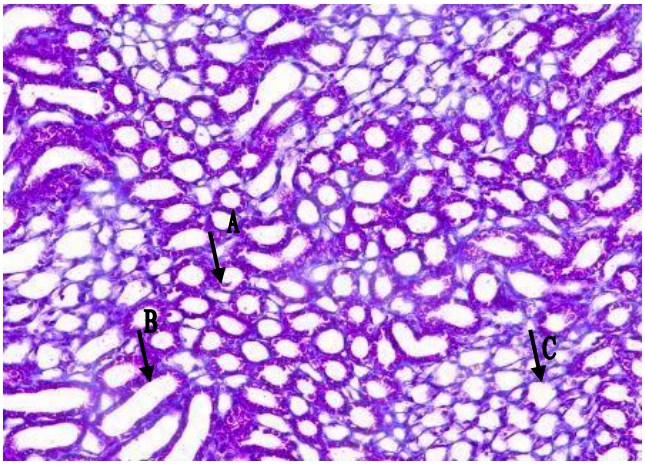


Fig.(9): section of hamster kidney; proximal tube (A), Distal tube (B), fiber (C), AB,100X.

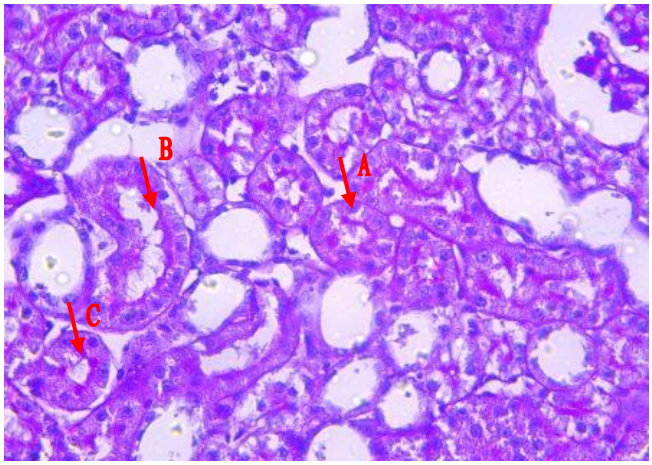


Fig.(10): section of mice kidney; collecting duct (A), Loop Henle (B), Juxtaglomerular cells (C), PAS-AB.200X.

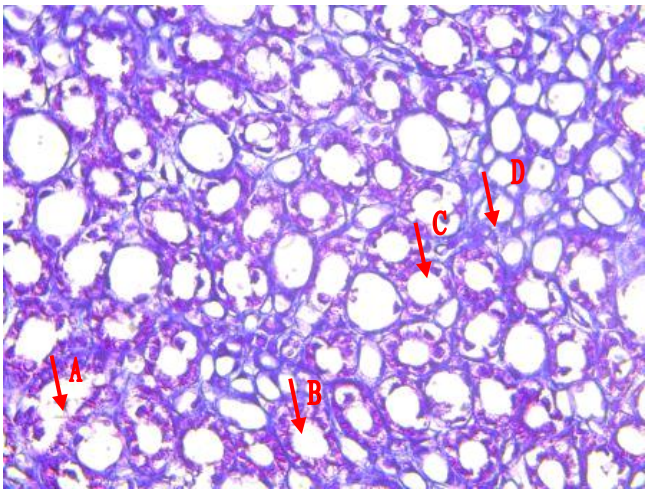


Fig.(11): section of hare kidney; distal tube(A), proximal tube (B), collecting duct (C), fiber (D), Masson, 200X.

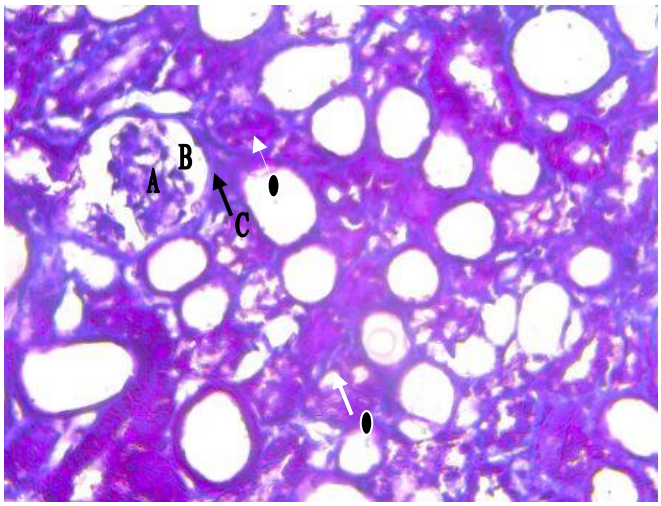


Fig.(12): section of hare kidney; glomerulus (A), renal space (B), Bowman capsule (C), Masson, 400X.

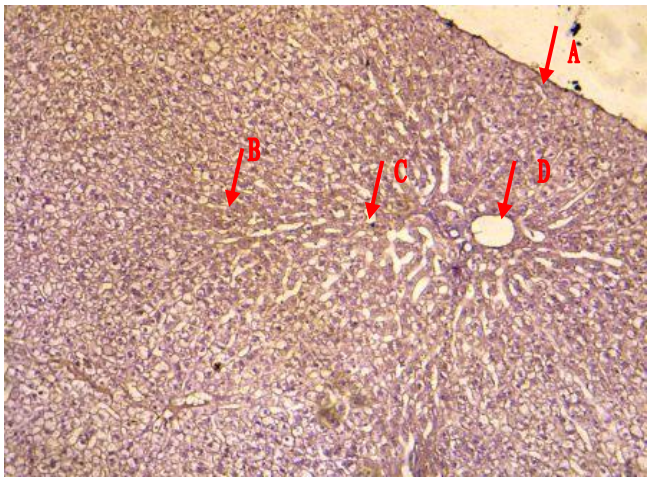


Fig.(13): Cross section of rabbit liver; capsule (A), hepatocytes (B), sinsoiods (C), central vein (D), PAS, 200X.

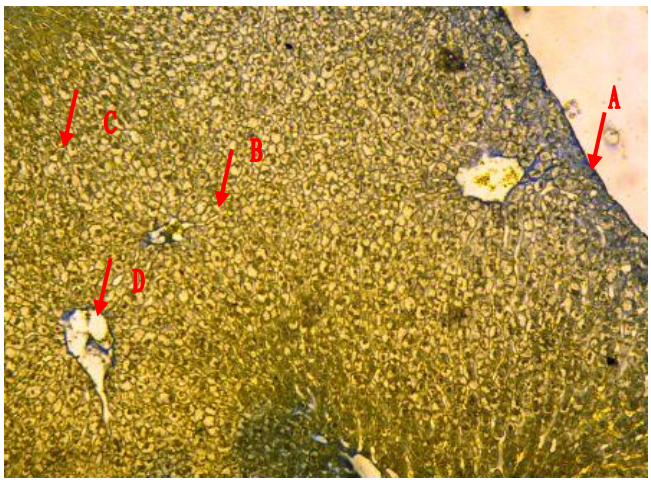


Fig.(14): Cross section of hare liver; capsule (A), hepatocytes (B), sinsoiods (C), central vein (D), AB, 200X.

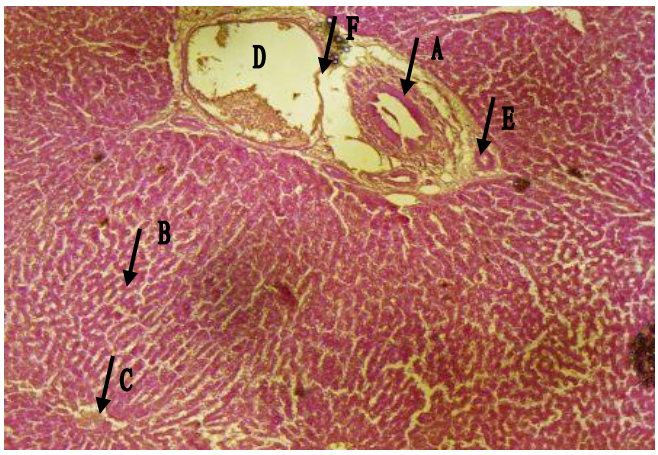


Fig.(15): section of hamster liver; duct (A), hepatocytes (B), sinsoiods (C), central vein (D), artery (E), endothelium (F), PAS-AB, 200X.

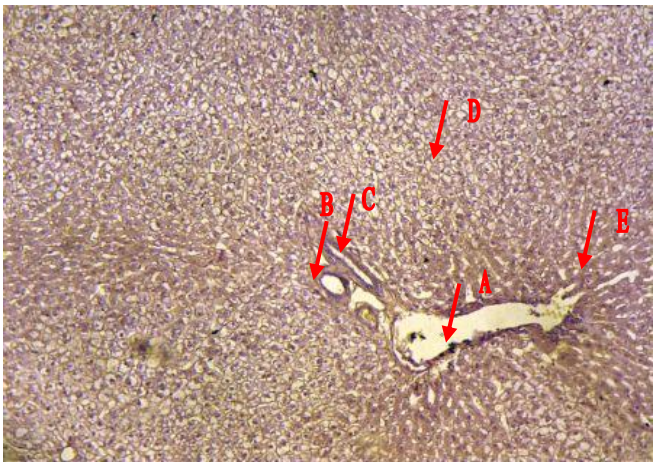


Fig.(16): Cross section of mice liver; central vein (A), hepatic duct (B), hepatic artery (C), hepatocytes (D), sinsoiods (E), PAS,200X.

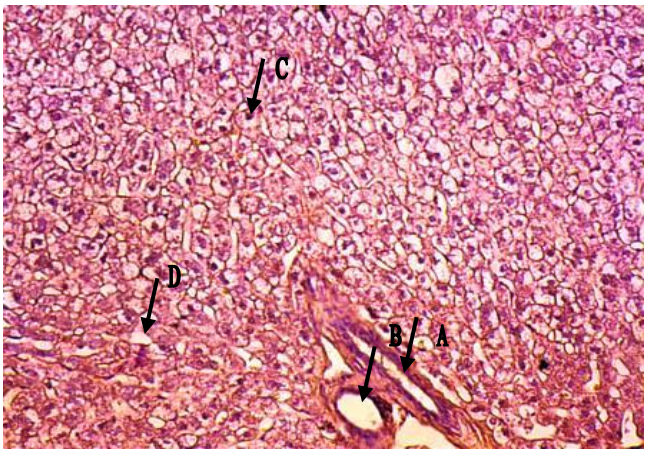


Fig.(17): Cross section of hamster liver; hepatic duct (A), hepatocytes (B), sinsoiods (C), central vein (D), hepatic artery (E), endothelium (F),PAS-AB,200X.

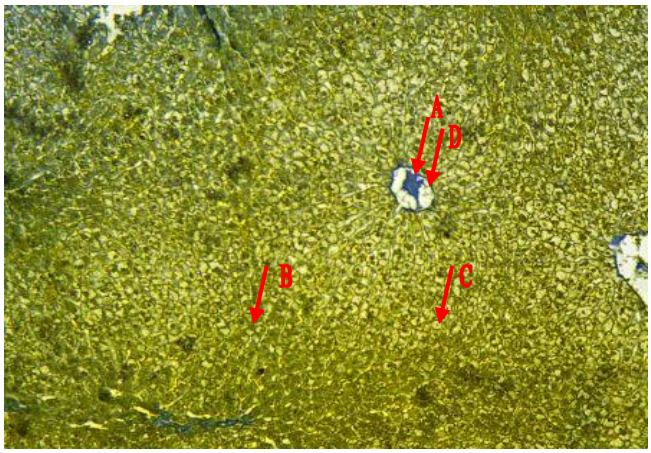


Fig.(18): Cross section of hamster liver; hepatic duct (A), hepatocytes (B), sinsoiods (C), central vein (D), hepatic artery (E), endothelium (F), AB,200X.

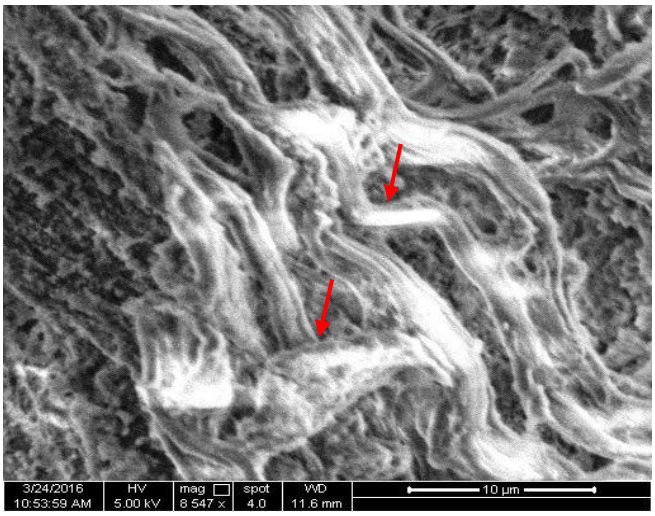


Fig.(19): scanning electron micrograph of the rabbit liver, connective tissue filaments in capsule, X26000.

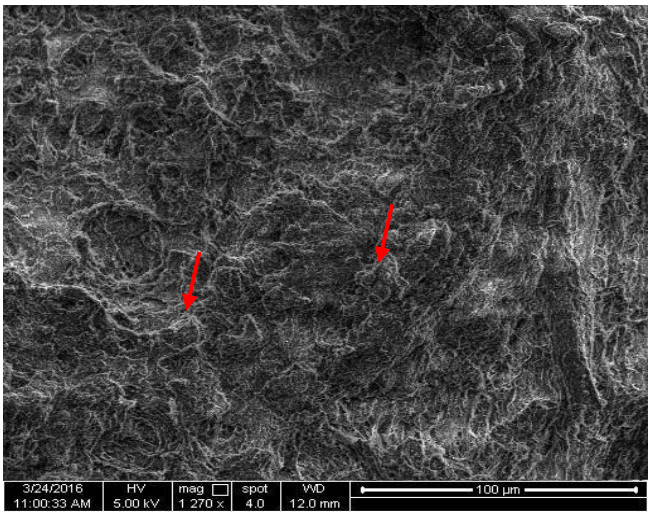


Fig.(20): scanning electron micrograph of the cytoplasm of liver cells in hare, X35000.

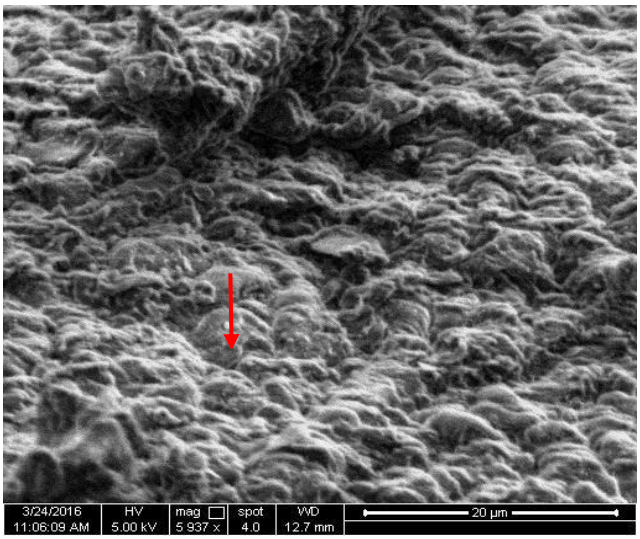


Fig.(21): scanning electron micrograph of the liver cells in hamster, X60000

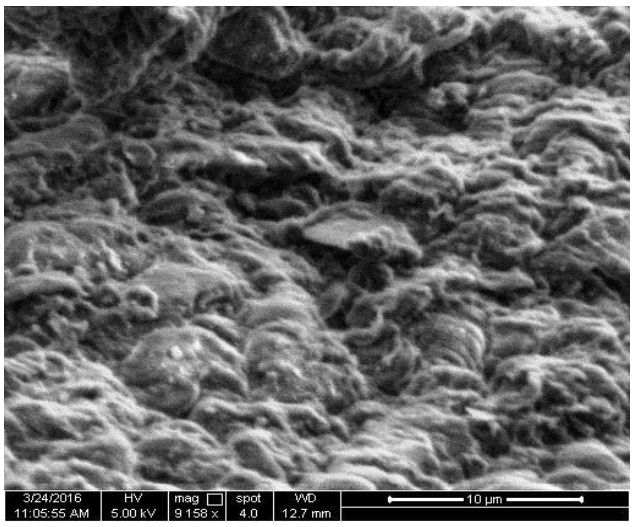


Fig.(22): scanning electron micrograph of the cytoplasm of liver cells in mice, X 26000

CONCLUSION: The structure of kidneys and liver differs by species, while not differ in structure of adrenal glands, the levels of minerals and enzymes differ among research animals, and the level of blood urea nitrogen is easily altered by physiological or environmental conditions. Concentrating efficiency in dehydration appears to be related to solute concentrations, primarily sodium and urea, in the inner medulla and papilla of the kidney. The serum urea level was likewise highest in cape hare animals, which is likely to be the cause for the high concentration in the cortex, as cortical tissue is thought to be in osmotic and chemical balance with the blood in general. This discovery indicated that the collagen framework of glomeruli is related to renal efficiency, regulated metabolism, and ion balance in animals. There was no consistent fluctuation in potassium, sodium, urea, or ammonia levels across animals.

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Availability of data and materials
Data of the current study area available with reasonable request.

Competing interest
There are no conflict of interest between the authors.

Ethical consideration
The current research has followed the accepted principles of ethical conduct by the college of veterinary medicine, University of Al-Muthanna, Iraq, during December 2022 to August 2023.

Author contributions
All authors contributed equally.

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REFERENCES

1. Struillou, X.; Boutigny, H.; Soueidan, A.; Layrolle, P. (2010). Experimental animal models in periodontology: A review. Open Dent. J., 4, 37–47.
2. Mapara M, Thomas BS, Bhat KM. (2012). Rabbit as an animal model for experimental research. Dent Res J. 2012;9(1):111-118. DOI: 10.4103/1735-3327.92960.
3. Bosze Z, Houdebine LM. (2006). Application of rabbits in biomedical research: A review. World Rabbit Sci.;14:1-14. DOI: 10.4995/WRS.2006.712.
4. Padilla-Carlin, D.J.; McMurray, D.N.; Hickey, A.J. (2008).The guinea pig as a model of infectious diseases. Comp. Med., 58, 324–340.
5. Suhett, W.G.; Gerez, J.; M.S. Hohmann, L. Staurengo-Ferrari, W.A. Verri, F.H.O. Pinho, L.D. de Barros, S.T. Cardim, K.M.C. Flaiban, Ana Paula F.R.L. (2023). Bracarense, Exploring porcine kidney explants as a model for the study of nephrotoxins and the therapeutic potential of phytic acid, Environmental Toxicology and Pharmacology,Volume 102,2023,104241,https://doi.org/10.1016/j.etap.104241.

6. Alvira-Iraizoz F, Gillard BT, and Lin P (2021). Multiomic analysis of the Arabian camel (*Camelus dromedarius*) kidney reveals a role for cholesterol in water conservation. *Commun Biol* 4, 779 (2021). https://doi.org/10.1038/s42003-021-02327-3.
7. Stieger-Vanegas SM and McKenzie E (2021). Imaging of the urinary and reproductive tract in small ruminants. Vet Clin Food Anim Pract. 2021;37(1):75-92. DOI: 10.1016/j.cvfa.2020.10.002.
8. Marques-Sampaio BP, Pereira-Sampaio MA, Henry RW, Favorito LA, and Sampaio FJ (2007). Dog kidney: Anatomical relationships between intrarenal arteries and kidney collecting system. Anat Rec.;290(8):1017-22. DOI: 10.1002/ar.20567.
9. Dutta P, Hakimi S, Layton AT. (2024). How the kidney regulates magnesium: a modelling study. R Soc Open Sci.; 11(3):231484. doi: 10.1098/rsos.231484.
10. Finco DR, Cooper TL. (2000). Soy protein increases glomerular filtration rate in dogs with normal or reduced renal function. J Nutr. 2000 Apr;130(4):745-8. doi: 10.1093/jn/130.4.745.
11. Rossi, G.; Liu, K.-F.; Kershaw, H.; Riddell, D.; Hyndman, T.H.; Monks, D.; Musk, G.C. (2023). Biological Variation in Biochemistry Analytes in Laboratory Guinea Pigs (*Cavia porcellus*). *Vet. Sci.*, *10*, 621. https://doi.org/10.3390/vetsci10100621.
12. Kalita A and Kalita PC (2014). Urinary system of mizo local pig (zovawk): A gross morphological and morphological study. Euro. J. Bio. Parm. Sci. 1(3): 458-464.
13. Elsheikh, E.M. (2023): Histogenesis of the rabbit liver (pars hepatica) with particular reference to the portal area, Iraqi Journal of Veterinary Sciences, 37 (1): 177-182.
14. Al-Hamdany, M.Z. (2019): Comparative anatomical, histological, and histochemical study of liver in human and domestic rabbit, Iraqi Journal of Veterinary Sciences, 33 (2): 437-446.
15. Al-Mahmood S. (2020). Improving light microscopic detection of collagen by trichrome stain modification. Iraqi J Vet Sci.;34(2):273-281. Doi:10.33899/ijvs.2019.126176.1256.
16. Yousif N H, Hadi H D, Jihad H M. (2023). Histological study of liver in guinea pig *Cavia porcellus* (Linnaeus , 1758) in Iraq. Revis Bionatura;8 (3) 80. doi.org /10.21931/ RB/ 2023.08.03.80.
17. Yousif, N. H. (2022). Histological study of liver for squirrel (*Sciurus anomalus*) (Güldenstädt, 1785) in Iraq. Biological and Pharmaceutical Sciences, 20(1), 091-094.
18. Abdulrazzaq BK, Hazem KA, Hassaneen AS, Sameer A, and Nabeel A (2023). Anatomical and histological study of the kidney of the one-humped camel. Iraqi Journal of Veterinary Sciences, 37, Supplement I-IV, 2023, 73-79. doi: 10.33899/ijvs. 2023. 1372310. 2655.
19. Kaewmong P, Jongjit P, Boonkasemsanti AK, Kongtueng P, Matchimakul P, Tangphokhanon W, Pirintr J, Piboon P, Umsumarng S, Nganvongpanit K, and Pongkan W (2023). Histological study of seventeen organs from dugong (*Dugong dugon*) *PeerJ* 11:e15859 https://doi.org/10.7717/peerj.15859
20. Zhou Z, Xu MJ, Gao B. (2016). Hepatocytes: a key cell type for innate immunity. Cell Mol Immunol;13:301-315.
21. Salman, A.K.; Zai Hasan, Z.Y.M. and Al-Kennany, E (2024): Experimentally Histological Assessment for the Effect of Resveratrol in the Liver of Mice, Annual Research & Review in Biology, 39(3):7-17. DOI: 10.9734/arrb/2024/v39i32061.

22. AL-Aamery, Rana Alaa, et al. (2020). Morphological description and comparative histological study of the liver in two iraqi mammals: weasel (*herpestes javanicus*) and eastern gray squirrel (*sciurus carolinensis*). Biochem. Cell. Arch. 2020 , 20(1): 167-170.
23. Rogers A B and Dintzis R Z . (2018). Hepatobiliary system in Comparative anatomy and histology: a mouse, rat, and human atlas (eds: Treuting P M, Dintzis S M and Montine K S) 2nd edn., Academic press an imprint of Elsevier, London,U.K.: 229-240.
24. Katsoulos P D, Athanasiou LV, Dedousi A, Psalla D, Marouda C, Papchianou M, Tsitsos A, and Boscos C (2020). Morphometrical study of bovine kidneys with and without mild histological lesions. Morphologie.;104(346):169-173. doi:10.1016/j.morpho.2020.01.001.
25. Marco A, Sampaio B, Beatriz P, Sampaio D, Henry R, Favorito M, and Francisco S (2009). The dog kidney as expermental model in endourology: anatomic contribution. J. endourology. 23(6): 989-993.
26. Genzer, S.C.; Huynh, T.; Coleman-Mccray, J.D.; Harmon, J.R.; Welch, S.R.; Spengler, J.R. (2019). Hematology and Clinical Chemistry Reference Intervals for Inbred Strain 13/n Guinea Pigs (*Cavia porcellus*). *J. Am. Assoc. Lab. Anim. Sci.*, *58*, 293–303.
27. Spittler, A.P.; Afzali, M.F.; Bork, S.B.; Burton, L.H.; Radakovich, L.B.; Seebart, C.A.; Moore, A.R.; Santangelo, K.S. (2021). Age- and sex-associated differences in hematology and biochemistry parameters of Dunkin Hartley guinea pigs (Cavia porcellus). *PLoS ONE* , *16*, e0253794.
28. Goel, H.; Printz RL, Shiota C, Estes SK, Pannala V, Abdul, M, Shiota M, Wallqvist, A. (2023). Assessing kidney injury induced by mercuric chloride in guinea pigs with in vivo and in vitro experiments. Int J Mol Sci. 24(8):7434. doi: 10.3390/ijms24087434.
29. Yang, I.S.; Jang, I. and Yang J.O. (2023). CanISO: a database of genomic and transcriptomic variations in domestic dog (*Canis lupus familiaris*). *BMC Genomics* 24, 613. https: //doi. org/10.1186/s12864-023-09655-0.
30. Mahmood MB. (2022). A comparison between ketamine-xylazine and ketamine-midazolam or all of them to induce balance anesthesia in rabbits. Iraqi J Vet Sci.; 36(2): 499-506. DOI:10.33899/ijvs.2021.130618.1852.
31. Suvarna SK, Layton C, and Bancroft J D (2018). Bancroft's theory and practice of histological techniques, 8th ed. Churchill Livingstone Elsevier Philadelphia, Pp: 176 -725.
32. Rajathi S, Ramesh G, Kannan TA, Dharani P, Gnanadevi R. Liver in guinea pig: Scanning and transmission electron microscopic studies. Microsc Res Tech. 2022 Oct;85(10):3391-3396. doi: 10.1002/jemt.24194.
33. Al-Rawi KM and Kalaf-Allah IS (1980). Design and Analysis Agriculture Experiments. Dar-Al Kutub-Mosul, Iraq, Pp: 65, 95-107.
34. Abass,T. A. (2017). Anatomical and histological study of adrenal gland in new natal and adult guinea pig (Caviaporcellus). *Kufa Journal for Veterinary Medical Sciences*, *8*(1):181-192.
35. Ekele, I; Uchenna, N and Ibe C.S. (2014). The Kidney and adrenal gland of the African Palm Squirrel *(Epixerus ebii*): A Microanatomical Observation.Rev. Fac. Cs. Vets. 55(2):60-67.

36. Sarwar, G; Sughar, P. and Aslam, M. S. (2008). Comparison of the effects of experimentally induced hypothyroidism in adrenal gland morphology in pre- postnatally treated albino rats .Pakistan Journal of Pharmacology. 25 (1):13-18.
37. Yılmaz, S and Girgin, A.(2005). Light and electron microscopic observations on the structure of the porcupine (*Hystrix cristata*) adrenal gland*Vet*. arhiv*75*(3): 265-272.
38. Seema S, Rakesh M, Sanjeev J, Geeta BR, and Vikas K (2017). Histological Study on capsule of the kidney in large white Yorkshire Pig (*Susscorfa*); Indian Journal of Veterinary Anatomy 28(2):29-30.
39. Al-jebori J, Al badri AM, and Jassim BA (2014). Study the anatomical and histomorphloogical description of the kidney in adult white rabbits female (New Zealand strain). World Jour. Pharm. Pharmaceu. scien. 3(6): 40-51.
40. Baragooth AF, Ghazi HA, and Abdzaid K (2014). Histological study to the nephrons of the kidney in dogs (*Canis familiaris*) in middle of Iraq. Kufa Jurnal for Veterinary Medical Science, 5(1):98-103.
41. Shil SK, Ferdows S, Sutradhar BC, and Das B C (2014). Topographic anatomy of visceral organs of a spotted deer (*Axis axis*). Res J Vet Pract.;2(4):55-7. DOI:10.14737 /journal. rjvp/ 2014/ 2 .4.55.57.
42. Abdalla M (2020). Anatomical features in the kidney involved in water conservation through urine concentration in dromedaries (*Camelus dromedarius*). Heliyon; 6(1):e03139. DOI: 10.1016/j.heliyon.2019.e03139.
43. Madrazo-Ibarra A and Vaitla A (2020). Histology, Nephron. National Library of Medicine, National Institutes of Health pp:2-3.
44. Maurya H, Kumar T, and Kumar S (2018). Anatomical and physiological similarities of kidney in different experimental animals used for basic studies. J Clin Exp Nephrol.;3(09). DOI: 10.21767/2472-5056.100060.
45. Nawata MC and Pannabecker T L (2018). Mammalian urine concentration: a review of renal medullary architecture and membrane transporters. J. Comp. Physiol. B. ;188(6):899–918.
46. Marc-André DA, Bédard A, and Marilyn ED (2011). Clinical significance of renal pelvic dilatation on ultrasound in dogs and cats. *Ultrasound.* 2011;52(1):88–94.
47. Dyce KM, Sack WO, and Wensing CJG (2010). Textbook of veterinary anatomy.4rd ed. W. B. Saunders company. Philadelphia, Pp: 554 - 694.
48. Mukoyama M and Nakao K (2005). Hormones of the kidney. In: Melmed S, Conn PM, editors. Endocrinology. USA: Springer: 353-65 .
49. Pereira-Sampaio MA, Favorito LA, and Sampaio FJ (2004). Pig kidney: Anatomical relationships between the intrarenal arteries and the kidney collecting system. Applied study for urological research and surgical training. J Urol.;172(5):2077-81. DOI: 10.1097/01.ju.0000138085.19352.b5.