Impact of Angiotensin Receptor Blockers on Angiotensin III and Leptin in Rabbits

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Angiotensin III (Ang III) properties include chemotaxis, creation development factors, and chemokines, which are also involved in renal and cardiovascular functions. Angiotensin receptor blockers (ARBs) are commonly used to treat cardiovascular illnesses. One factor that contributes to controlling blood pressure and resting metabolic rate is the protein Leptin (LEP). This study aimed to analyze ARB's (Losartan, Telmisartan, or Candesartan) effects on the levels of Ang III and LEP in rabbits. The characterization of the relationship between the two factors will be studied. The study was conducted on forty Oryctolagus cuniculus male rabbits. They were divided into four groups, randomly. Treated daily for ten days with intraperitoneal Losartan 0.7 mg/kg/day [G I], Telmisartan 0.6 mg/kg/day [G II], Candesartan 0.1 mg/kg/day [G III], and Control [G IV]. Pre- and post-treatment levels of Ang III and LEP, the changes in their histopathological characteristics, and coefficient correlations were analyzed. The three groups exhibited a drastic decrease in serum Ang III and LEP levels compared to the baseline and control. The effects of Losartan, Telmisartan, and Candesartan on the end organs of the liver, kidney, and heart, did not show any alterations. There was a weak positive correlation between the two factors. In conclusion; Losartan, Telmisartan, and Candesartan significantly decreased the activity of the RAS via their effect on Ang III and LEP levels as another mechanism for their efficacy in treating cardiovascular illnesses.

Keywords: Angiotensin III; Candesartan; Losartan; Leptin; Telmisartan.

The Renin Angiotensin system (RAS) is a component of the renal system that is independent of the peripheral RAS. It plays a role in regulating sodium excretion and blood pressure[1]. The entirety of the forerunners of angiotensin (Ang) peptide union is situated inside the proximal renal tubule[2], counting angiotensinogen, renin, and Ang changing over protein messenger ribosome nucleus acid (mRNA)[3]. The Ang III and Ang II convergences are more prominent than could explained solely by the equilibration with the circling focuses[4]. These discoveries recommend that significant impacts are applied by privately produced angiotensins[5].

Furthermore, the two significant receptor subtypes that intercede with activities of RAS, the type 1 Angiotensin receptor (AT1R) and the type 2 Angiotensin receptor (AT2R) are both present in renal proximal tubule cells, predictable with an essential part for rounded as paracrine substances, angiotensins play a role in the regulation of renal capacity[6].

Angiotensin receptor blockers (ARBs) are designed to bind to the Ang II receptor, which has various actions, including vasoconstriction, the production of aldosterone, cytokine, reactive-oxygen-species formation, and mitogenic activity[7].

Angiotensin III is one of the N-terminal angiotensin debasement results of Ang II, which imparts a portion of its properties to Ang II, including chemotaxis and creation development factors and chemokines. Ang III showed comparable capacities to Ang II. The two peptides partake in the CV and renal capacities, animating aldosterone synthesis and diminishing renal bloodstream and renin discharge circulatory strain[8]. ANG III contributes to the development of transcription factors during physiological conditions. Apart from stimulating the production of ANG1 receptors, it also binds to the AT2 receptors, which are known to suppress the expression of certain transcription factors. It has been observed that the tonic inhibition of the AT2 receptors affects the different neuronal populations[9].

Leptin is a hormone that has acquired tremendous advances in the comprehension of CVD, and its primary capacities incorporate; angiogenesis, pulse control, vascular hemostasis, irritation, immune reaction, and metabolic guidelines[10, 11]. Leptin (LEP) contributes to controlling the resting metabolic rate[12] and blood pressure through its actions in the arcuate nucleus[13]. The RAS and AT1R within the brain[14] are likewise engaged with the control of resting metabolic rate and pulse[6], regardless of whether this guideline covers LEP activities is indistinct[15].

This study was performed to find out the impact of ARBs (Losartan, Telmisartan, and Candesartan) on Ang III and LEP in rabbits by the assessment of the following:

1- The effect of Losartan, Telmisartan, and Candesartan on Angiotensin III.
2- The effect of Losartan, Telmisartan, and Candesartan on Leptin.
3- Macroscopic and histopathologic study.
4- Correlation of Angiotensin III with Leptin, and the study parameters.

Study Methods

Setting

The study was carried out at Al-Isra University's Faculty of Pharmacy-experimental animal house, from January 2020 to October 2020. Based on an ethically approved protocol (SRES/21/02/002). All chemicals, reagents, and ELISA kits used in this study with the highest purity, from USA, Canada, and India origin.

Rabbits and housing

The forty male rabbits were housed in a cage and fed and cared for according to the laboratory's conditions in the university's animal house. All the animals in the house are kept in a separate cage. The rabbits were allowed to adjust to their housing conditions a week before the start of the study. They were kept in a standard room and provided with free tap water and rodent chow. The animals were also kept on a 12-hour light cycle and exposed to humidity at a temperature of 25 degrees Celsius.

All the experiments related to the care and use of laboratory animals were carried out according to the NIH's guidelines (NIH-Publications Number 85 to 23, revised-1985).

Experimental design & Study groups

A total of 40 Oryctolagus cuniculus rabbits were divided randomly statistically by Microsoft Excel into four groups, each consisting of 10 rabbits as follows: Oryctolagus cuniculus rabbits were also allowed to eat and drink ad libitum, and they were monitored for their steady weight throughout the experiment. The treated groups were administered drugs I.P. once daily for 10 days. A solution of 1 ml (DMSO was used as a solvent). The forty rabbits were divided randomly into four groups (each 10):
Group I: Losartan treated 0.7 mg/kg/day.
Group II: Telmisartan treated 0.6 mg/kg/day.
Group III: Candesartan treated 0.1 mg/kg/day.
Group IV: Control (vehicle-treated)

Dosage and sample preparation

Losartan, Telmisartan, and Candesartan (from Sigma Aldrich) solution was prepared by reconstitution of Losartan, Telmisartan, and Candesartan powder with DMSO daily under sterile conditions before injection.

The rabbits were Blood collected from the Jugular vein. Then (3ml) of Blood was transferred to a serum (without EDTA) tube. Blood was at once centrifuged at 3000 Revolutions per minute (RPM) for 20 min. The serum was then pipetted to Eppendorf and refrigerated at -70 degrees Celsius. The rabbits' body weight was monitored before and after the duration of the study. The various drugs used in the study, such as Telmisartan, Losartan, or Candesartan, were then given to separate groups of rabbits. They were given prescribed treatment doses according to their conditions.

The rabbits were given a 24-hour extension to their last dose. They were then collected from their Jugular vein and placed into a serum tube, which was centrifuged at 3,000 revolutions per minute. The serum was then separated using a pipette and placed at Eppendorf under the cover of parafilm. After the animals' groups were dissected, their tissues and organs were then dried and placed in a 10% buffered formalin solution.

Rabbit Ang III & Rabbit LEP ELISA Kits

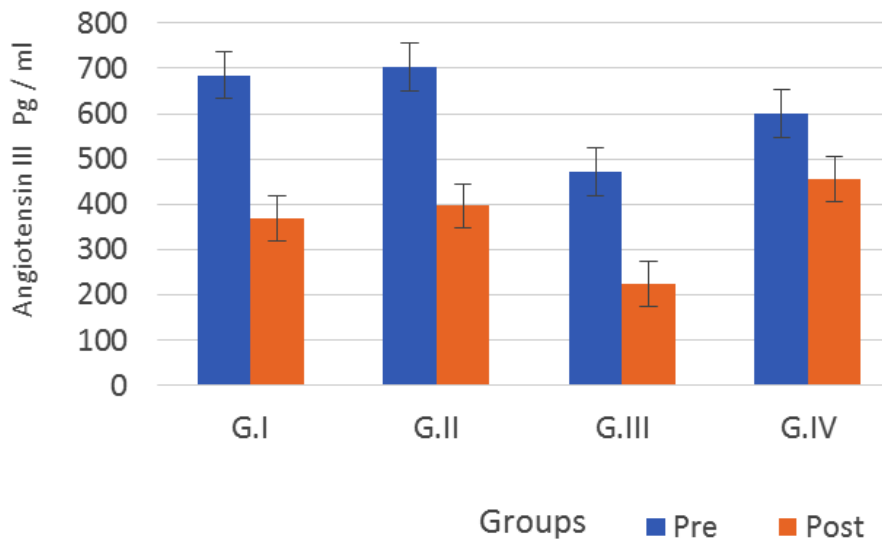For Both Ang III & LEP follow the manufacturer's instructions.

Fig. 1. The serum level of Ang III in tested groups. The serum level of Ang III was decreased significantly (P<0.05) in tested groups; G I: Losartan treated at 0.7 mg /kg/day, G II: Telmisartan treated at 0.6 mg /kg/day, and G III: Candesartan treated at 0.1 mg /kg/day, but the non-significantly decreased in DMSO the control group IV in comparison to baseline (P = 0.0002)
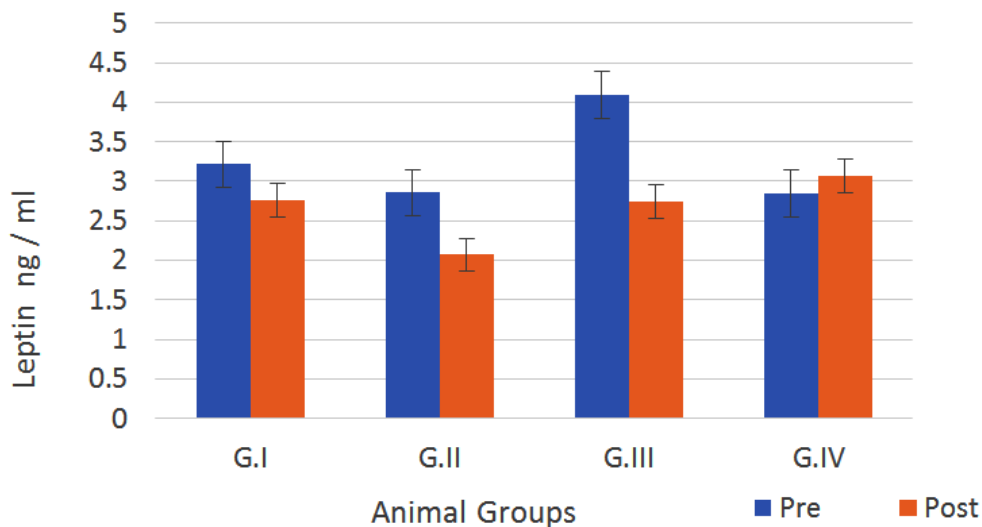

Fig. 2. The serum level of LEP in tested groups. The serum level of LEP was decreased significantly (P<0.05) in tested groups; G I: Losartan treated at 0.7 mg /kg/day, G II: Telmisartan treated at 0.6 mg /kg/day, and G III: Candesartan treated at 0.1 mg /kg/day, but the non-significantly decreased in the DMSO control group IV in comparison to baseline (P = 0.0002)

Macroscopic & Histopathologic Examination

    Organ specimens, such as kidney, liver, and heart, were collected and fixed using immersion in 10% formalin followed by the addition of Xylene, paraffin wax, and a clearing agent. The tissues were then embedded with paraffin wax to form a block. The blocks were then separated using a microtome and placed on slides. They were then stained with eosin and hematoxylin to examine tissue damage, inflammation, and morphological changes.

Statistics analysis

    The data collected during the study were analyzed using the SPSS 24 software. The effect size and sample size calculations were then carried out using the G*Power software. A statistical analysis technique was then used to compare the means of the two groups. A sample-by-sample
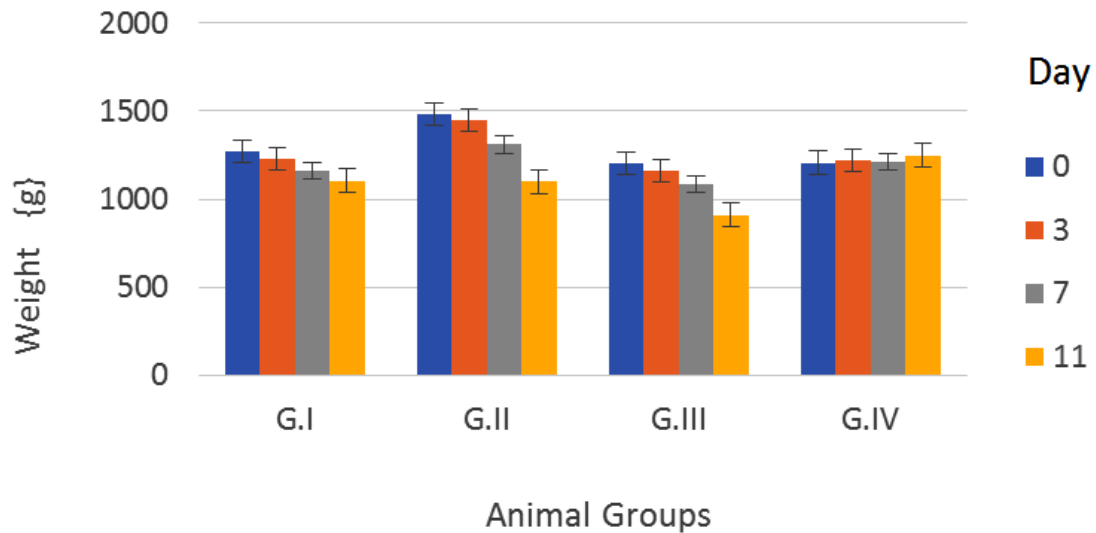

Fig. 3. The average rabbit's weight during the study period. The average weight for groups (I, II, III) showed a non-significant slight decrease while group IV (P=0.06)
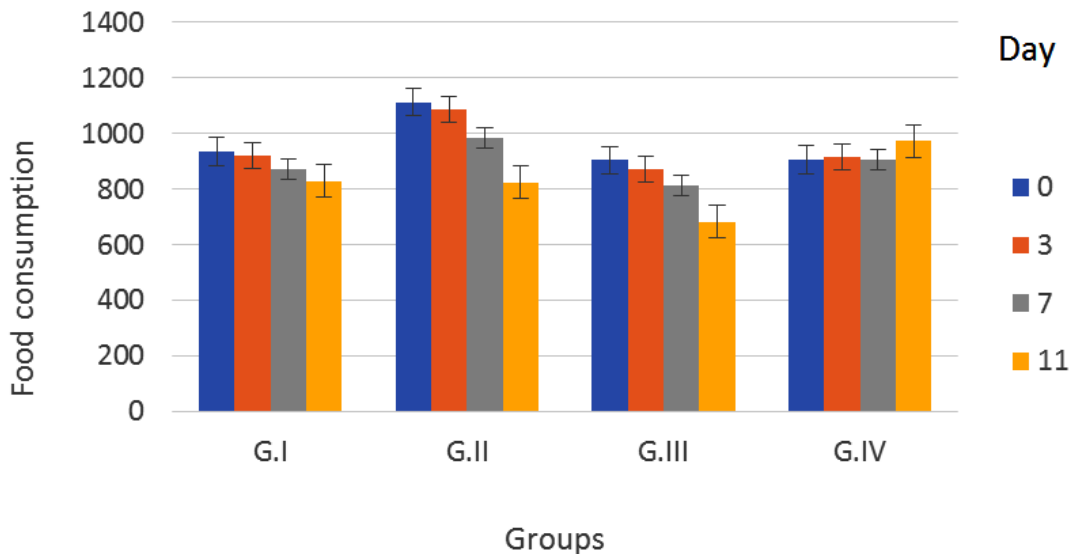

Fig. 4. Rabbit's food consumption in studied groups during the study period. The average for groups (I, II, III) showed a non-significant slight decrease while group IV (P=0.06).

comparison procedure was also carried out to examine the differences in the mean values between the two groups. ANOVA was then performed to analyze the variance in the mean values. The results of the study were then analyzed using the Pearson correlation coefficient. The strength of the correlation was then evaluated to see how significant it was.
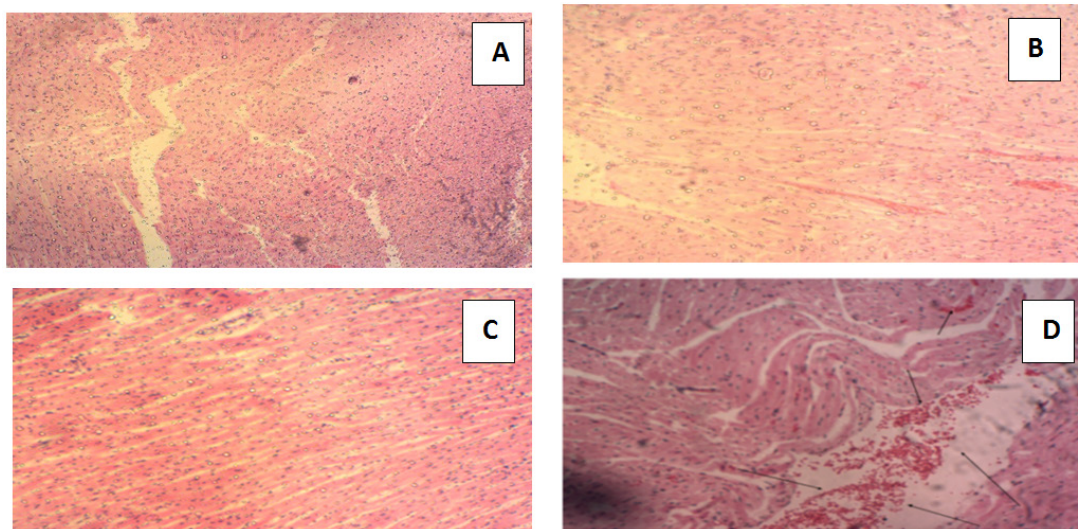

Fig. 5. A. The heart section of the treated animals' control (DMSO) group [G IV]. G IV was normal with no necrosis and no inflammation. B. Heart section of treated animals group Losartan (G I). G I was normal no necrosis and no inflammation. C. The heart section of treated animals' group Telmisartan (G II). G II was normal, with no necrosis and no inflammation. D. Heart section of treated animals group Candesartan (G III) was seen to have congested blood vascular but no necrosis and no inflammation.
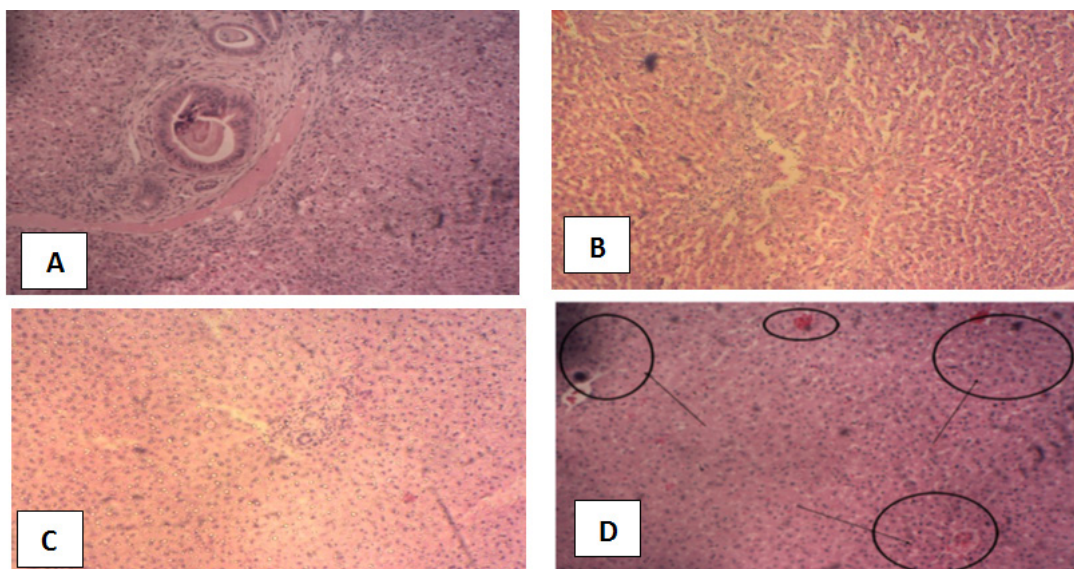

Fig. 6. A. Section of the liver in tested control (DMSO) group [G IV]. G IV was normal with no necrosis and no inflammation. B. Section of the liver in tested group Losartan (G I). G I was normal no necrosis and no inflammation. C. Section of the liver in tested group Telmisartan (G II). G II was normal, with no necrosis and no inflammation. D. Section of the liver in tested group Candesartan (G III) was observed to have congested blood vascular but no necrosis and no inflammation.

RESULTS

The effect of Losartan, Telmisartan, and Candesartan on Ang III

The serum level of Ang III was decreased significantly (P<0.05) in tested groups; G I: Losartan treated at 0.7 mg /kg/day, G II: Telmisartan treated at 0.6 mg /kg/day, and G III: Candesartan treated 0.1 mg /kg/day, highly significant difference compared to baseline and control with 95% CI (P=0.0002). However, a non-significant decrease in the control group IV compared to the baseline, as shown in Figure 1.

The effect of Losartan, Telmisartan, and Candesartan on LEP

The serum level of LEP was decreased significantly (P<0.05) in tested groups; G I and II while G III showed a highly significant difference compared to pre-values with 95% CI (P=0.0002). However, a non-significant difference in the control group IV compared to the baseline, as shown in Figure 2.

Macroscopic and histopathologic study
The ARBs (Losartan, Telmisartan, and Candesartan) efficacy on the rabbit's weight

The rabbit's weight was measured (0, 3, 7, 11) days for all tested groups during the study period starting from day zero to day 11. The average weight for the groups (I, II, III) showed a non-significant slight decrease while group IV showed no difference in the rabbit's weight compared to baseline, as in Figure 3.

The ARBs (Losartan, Telmisartan, and Candesartan) efficacy on the food consumption

The food consumption for animals was measured daily during the study period. The daily average food intake for the treated groups (I, II, III) was a non-significant slight decrease, while group IV showed no difference in food intake. Both are non-significant (P=0.06), as presented in Figure 4.

The Microscopic feature
Histopathology of the heart

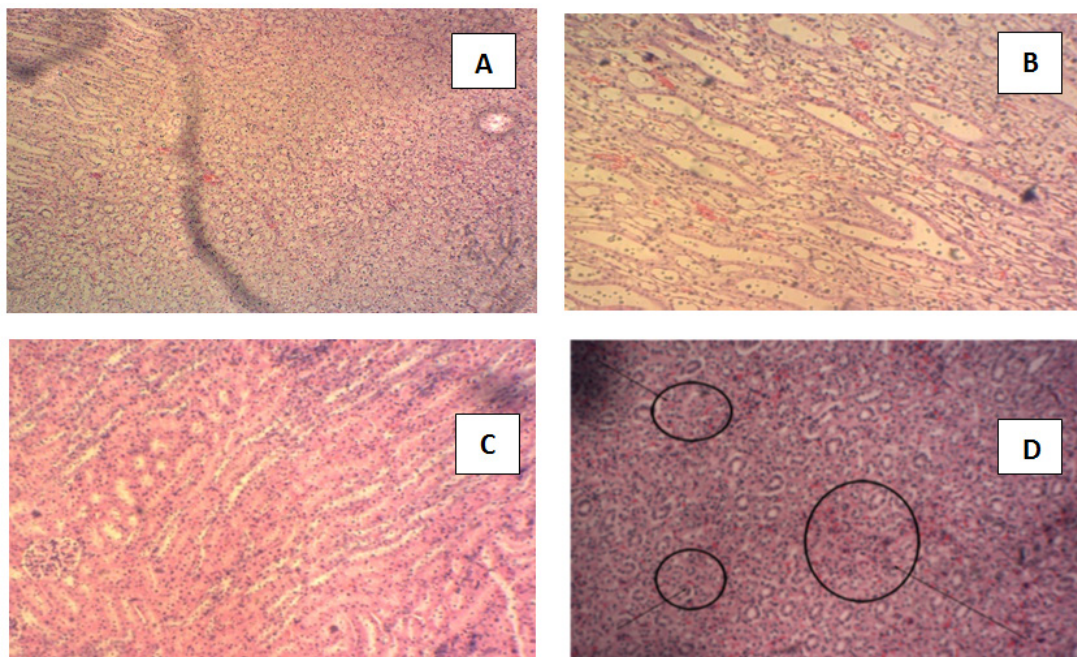Histopathological studies of the groups' heart sections; G (I, II, IV) were normal, as shown

Fig. 7. A. Normal Kidney section in tested control (DMSO) group [G IV]. G IV was normal with no necrosis and no inflammation. B. Normal Kidney section in tested group Losartan (G I). G I was normal no necrosis and no inflammation. C. Normal Kidney section in tested group Telmisartan (G II). G II was normal, with no necrosis and no inflammation. D. Section of the kidney in tested group Candesartan (G III) was seen to have congested blood vascular but no necrosis and no inflammation.

in Figures (5A-C). While there was congestion blood vascular in G III shown in Figure 5D for all groups, there was no necrosis and no inflammation.

Histopathology of the Liver

Histopathological studies of liver sections of the studied groups; G I, II, and IV were normal

shown in Figures (6 A-C) except for G III; there was congested blood vascular shown in Figure 6 D. All groups showed no necrosis or inflammation.

Histopathology of the Kidney

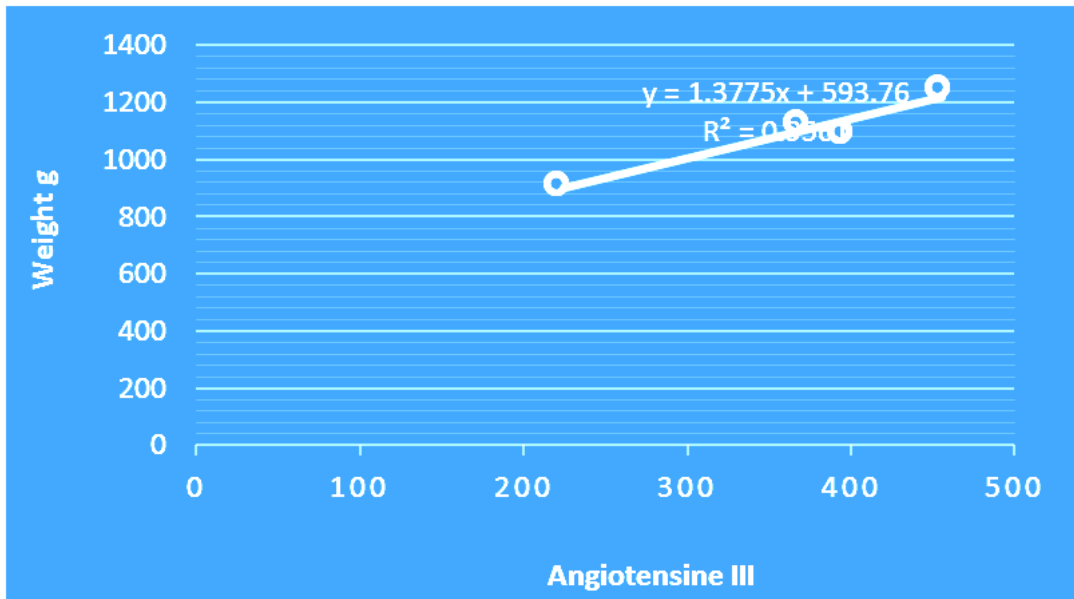Histopathological studies of the kidney sections of the studied groups; G (I, II, and IV)

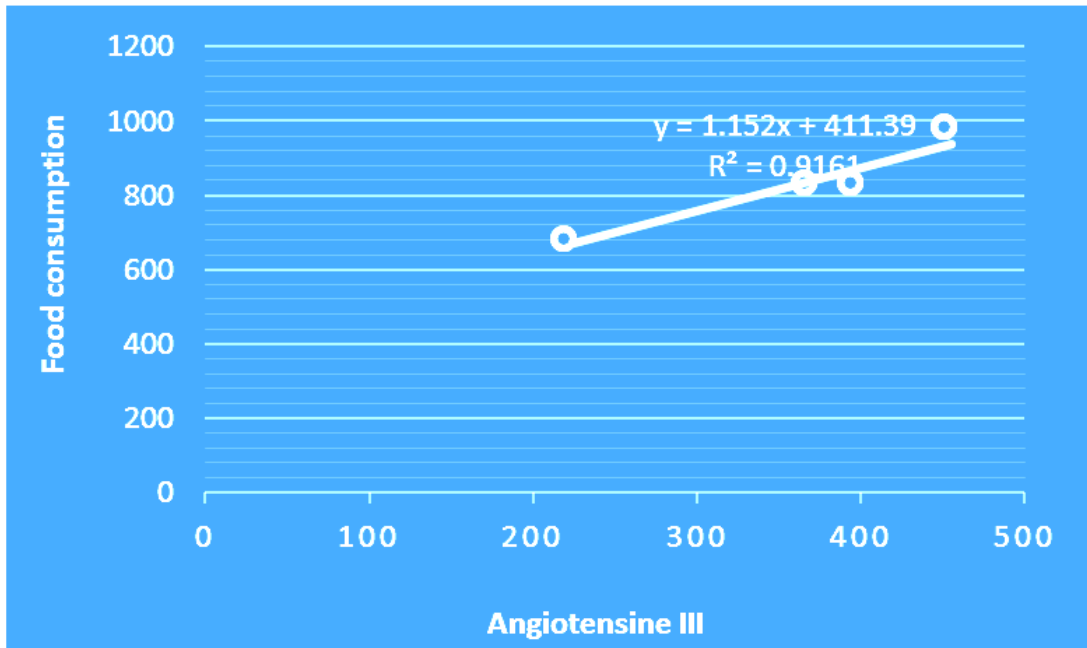**Fig. 8A.** The correlation analysis between serum Ang III and weight.

Fig. 8B. The correlation analysis between serum Ang III and food consumption.

were normal shown in Figures (7 A-C), with no pathological changes (necrosis or inflammation), G III showed congestion blood vascular only shown in Figure 7 D.

Correlation study

Pearson's correlation coefficient (r) was used to show the correlation of serum biomarker Ang III and LEP levels to the studied parameters. Body weight and food consumption of Oryctolagus cuniculus rabbits for all tested groups were assessed and analyzed according to their r with significance, and the correlation of Ang III to LEP.

Analysis of Ang III correlation to weight and food consumption

All samples were measured within the same experiment in studying the correlation. Analysis of serum Ang III to weight correlation showed a highly significant strong positive
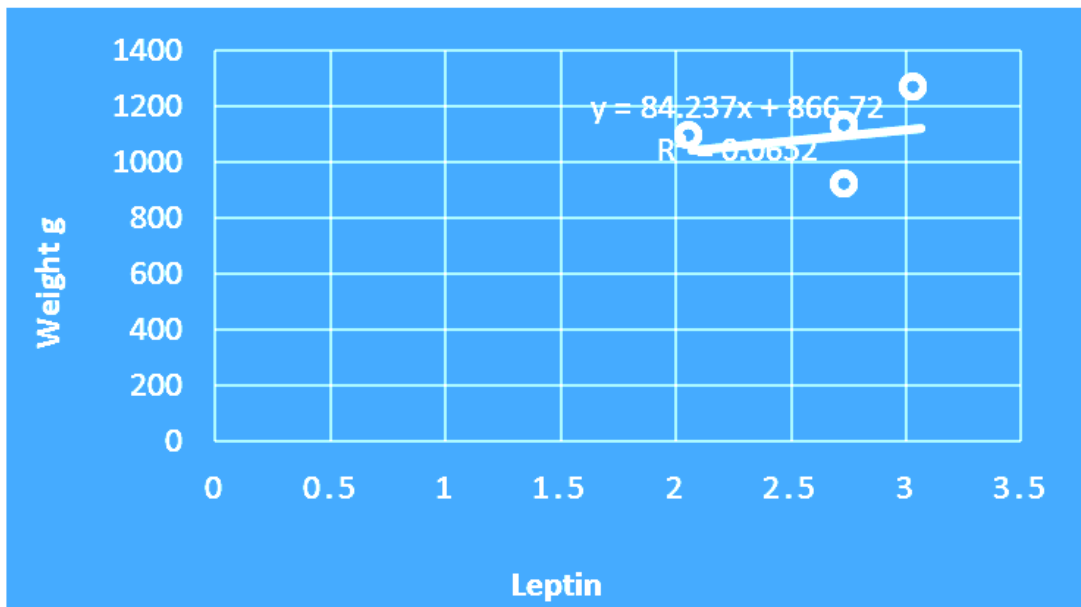

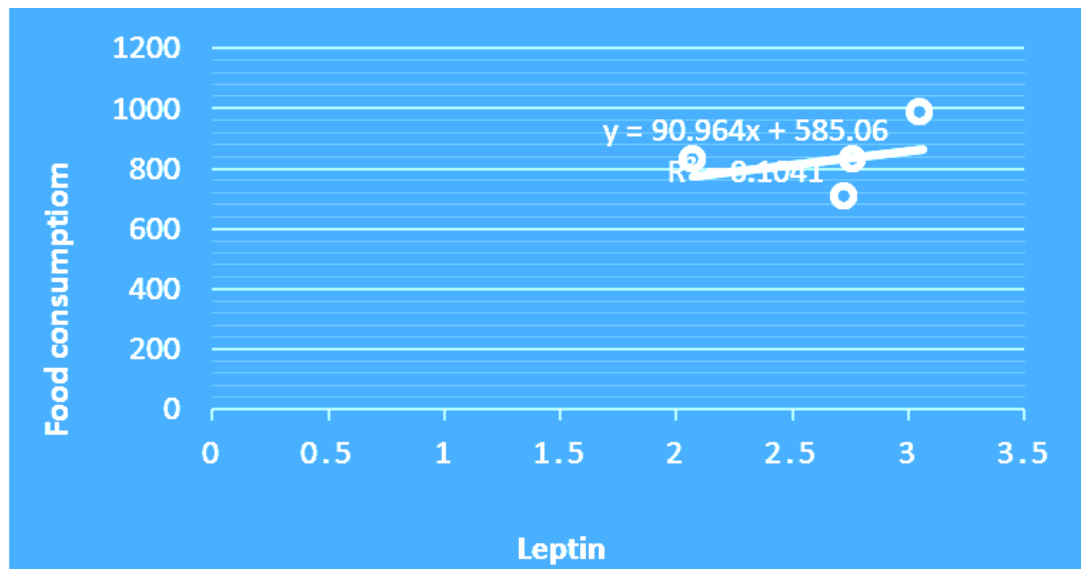
Fig. 9A. The correlation analysis of LEP and weight.

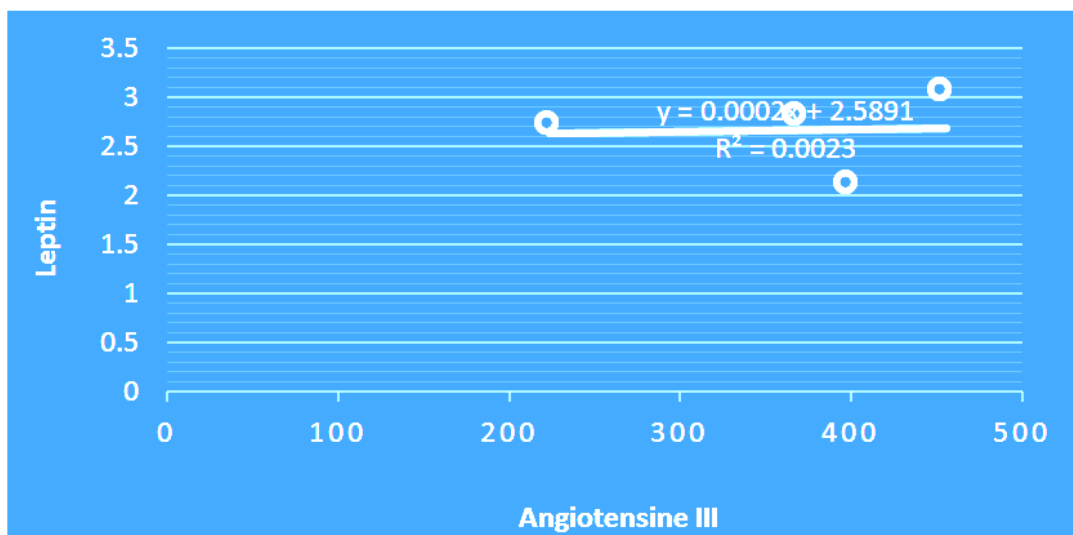**Fig. 9B.** The correlation analysis of LEP and food consumption.

Fig. 10. The correlation analysis between serum Ang III and LEP.

correlation between rabbit's weight post-treatment & serum Ang III conc.; r = 0.9778, P = 0.0001, as shown in Figure 8 A.

Concerning the correlation of serum Ang III to food consumption, a highly significant strong positive-correlation between both parameters, r = 0.957, p = 0.002, as shown in Figure 8 B.

Analysis of LEP correlation to weight and food consumption

A weak, non-significant positive-correlation of serum LEP to weight, r= 0.255, P= 0.06. The same was observed in the correlation of serum LEP to food consumption. There is a weak, non-significant positive-correlation, r = 0.322, p = 0.07, Figures (9 A & B) respectively.

Correlation analysis of Ang III and LEP

Pearsons-correlation analysis showed weak non-significant positive-correlation between the tested groups' serum Ang III & LEP levels, correlation coefficient (r = 0.047, P= 0.06), as shown in Figure 10.

DISCUSSION

Ang III similarly mediates several physiological functions to Ang II by activation of AT1Rs. It is important to note that the heptapeptide Ang III is produced from Ang II by the enzyme APA. Thus, the two peptides are remarkably similar (one amino acid difference) in structure[16]. The Ang III actions are the result of the interactions between the AT1R and the AT2R. These actions have fueled the debate about the physiological relevance of the RAS's peptides. Some studies suggest that Ang III can be the true agent of the RAS in controlling blood pressure[17, 18]. also vasopressin release[19]. Amastatin [selective APA inhibitor] was added to block the AR, but this inhibitor was not able to prevent the Ang III response[20]. The effects of Ang III on different conditions, including kidney function and blood pressure, were similar to those of Ang II[21].

This study is the first to assess the effect of ARBs (Losartan, Telmisartan, or Candesartan) on Ang III and LEP. ARBs can be made by blocking the AngII-AT1R interaction, which some believe acts as a mediator between Ang II and its negative effects on the CV[22]. Compared to ACEIs, ARBs are more efficient at restricting the RAS blockade. This mechanism is independent of the formation pathway of AngII[23].

Forty Oryctolagus cuniculus male rabbits were utilized to evaluate the efficacy of Losartan, Telmisartan, and Candesartan as ARBs drugs on Ang III and LEP. It was noticed that the serum Ang III decreased in the groups G I: Losartan treated at 0.7 mg /kg/day, G II: Telmisartan treated at 0.6 mg /kg/day, and G III: Candesartan treated at 0.1 mg / kg/day, but non-decreased in the control (DMSO) group IV. However, the decrease was somewhat

noticeable in Group III, and Ang II and Ang III could stimulate aldosterone release. However, this is one of the factors that lead to CVD. ARBs are well-known for treating CVD[24]. The present study is consistent with the earlier studies in that Losartan and Telmisartan act more on the AT1R and Candesartan act on the AT1R and AT2R[25]. Thus, it was explained that the level of Ang III reduced significantly in group three in comparison to groups one and two.

Although Ang-I is biologically inactive, its metabolites, Ang II, and Ang III, are known to mediate dipsogenic and pressor effects through the AT1 and AT2 receptor categories. Both compounds are full agonists of the two receptor types[26, 27]. A proposed counter-regulator axis between the Ang II-Ang III and AT1 receptors has been presented, which includes ACE2 and Ang (1-7). It is believed that this system promotes various actions, such as antiproliferative, vasodilation, antifibrosis, and antihypertrophic[28, 29]. Albeit insufficient information has been amassed on Ang III and AT2R, it has been written down that Ang III intervenes natriuretic reaction through AT2R under foundational AT1R barricade[30, 31].

LEP, the cloning of the ob-gene product was carried out in 1994[32]. It was shown that the LEP participated in regulating the body's weight control[33]. LEP was regarded as a pioneering discovery because it was able to show how an adipose signal can be used to control the energy balance. Many of its activities are related to the impacts of the brain, crossing the BBB through receptor-mediated endocytosis[5] It is suspected that elevated LEP levels in obese patients lead to low-grade systemic inflammation, rendering obese individuals more prone to CVD[34].

The level of serum LEP decreased in the groups (I, II, III), and group III decreased slightly more.

Prior treatment with losartan, an angiotensin receptor type 1 antagonist, prevented the pressor response. It was shown that blocking APN's action on the metabolism of Ang III leads to a rise in its endogenous levels, which can trigger an increase in arterial blood pressure. The findings support the notion that the brain RAS produces a major effector Ang III, which exerts a tonic control of blood pressure. The APA, which forms the brain's Ang III is regarded as a potential therapeutic target[35].

The effects of leptin on the development of conditions such as stretching are known to be mediated by the increase in oxygen species. They can also be triggered by the activation of the endothelin and angiotensin systems. Other studies suggest that the effects of leptin are mediated by the increase in nitric oxide production in vascular smooth muscle (VSMC). It can also impair the angiotensin II's vasoconstriction and proliferative actions[36]. ARBs would lead to a decrease in the Ang II, which leads to the expansion of vasodilation and limits the sympathetic neighbor's activity, thus leading to a decrease in the level of LEP[37, 38], the present study is consistent with the earlier studies.

The empirical analysis of microscopical and histopathological appearance showed that the body-weight of the rabbit's loads was estimated daily for the four tried gatherings during the investigation period. The standard load for the gatherings was diminished within 10 days and showed a non-significant slight decrease while group IV showed no difference.

Studying the efficacy of Losartan, Telmisartan, and Candesartan on the rabbit's food consumption, the standard dietary admission showed a non-significant slight decrease while group IV showed no difference, where food admission was altogether decreased during the examination time frame.

The empirical analysis of histopathological appearance was heart, liver, and kidney tissues. All these changes were nonspecific (reactive changes). Precedent studies mentioned that specific heart, liver, gastrointestinal, and kidney changes were found[39]. No general specific changes that occur in these organs were our explanation for such a specific change. However, in this study, all the changes were nonspecific.

Concerning the Correlation study of serum Ang III and LEP levels the studied parameters were assessed and analyzed according to their positive and negative correlation coefficient with significance. The correlation of serum Ang III to weight, and food consumption shows a highly significant strong positive-correlation amidst rabbit's body-weights & food consumption post-treatment and serum Ang III concentrations.

The increase in weight and food is through an increase in adipocytes and their synthesis. These are activated by several factors, the most important of which is the RAS by activating the increase of the Ang II & the ACE2-Ang_(1–7)-Mas[37, 40-42]. The present study is consistent with previous studies in that LEP does not have a significant role in regulating food and weight but rather is one of several factors affecting them.

A decrease in LEP level leads to the suppression of activates STAT 3 specifically and exclusively in the hypothalamus[43, 44].

The correlation of serum LEP to weight, and food consumption showed a weak; non-significant positive -correlation amidst rabbit body-weights and food consumption post treatment & serum LEP concentrations. Also, a weak non-significant positive-correlation amidst serum Ang III & LEP levels in the tested groups of this study. The coefficient correlation may be changed to a highly significant strong positive if the no. of samples increases.

The elevated levels of angiotensin II are influenced by the presence of renin-angiotensin-aldosterone system (RAAS) components in which the adipose tissue is its source. These components are involved in regulating adipocyte functions. The interaction between RAAS components and adipokines has been shown to contribute to the development of heart failure[45]. Angiotensin II and angiotensin III binding to the AT2R have effects that generally counteract its classical actions, producing vasodilation and Natriuresis[46]. It increases the expression and the release of proinflammatory cytokines[37, 40-42], and increments LEP quality articulation and discharge. Accordingly, the hindrance of Ang II by ARBs may bring about decreased LEP[38]. The ARBs Losartan, Telmisartan, and Candesartan lowered the level of Ang III and LEP levels, as it lowered the level of Ang II, which suppresses the activity of the RAS that reduced the level of Ang III and reduced the level of adipocytes reduced the level of LEP.

CONCLUSION

The study's findings show that the serum levels of Ang III and LEP were significantly decreased in rabbits that were given ARBs (Losartan, Telmisartan, or Candesartan) in comparison to baseline and controls related to the effects of ARBs on the levels of Ang II and the adipocytes that were shown to restrain the RAS activity. The histological assessment of the kidney, liver, and heart revealed that the use of Losartan, Telmisartan, or Candesartan, confirmed non-specific changes in the histopathological profile. A non-significant weak positive-correlation coefficient was observed between Ang III & LEP.

Future work

Suggestions for this study to find out:
• Assessment of the Ang III and LEP levels on large no. of animals.
• Clinical study to assess the Ang III and LEP levels in humans.

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