

Evaluation of the Effects of B-Complex Vitamins (Neurobion) on Kidney Function in Albino Mice

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ABSTRACT

Background: B-complex vitamins, particularly in the form of Neurobion (a combination of vitamins B1, B6, and B12), are widely used for their neuroprotective and analgesic properties. However, their potential impact on renal function remains inadequately studied. This study aimed to evaluate the histopathological effects of Neurobion administration on kidney structure in Swiss albino mice (*Mus musculus*). **Methods:** A total of 10 Swiss albino mice were divided into two groups (n = 5 per group). The control group received no treatment, while the experimental group was intraperitoneally injected with Neurobion (0.2 mg/kg) daily for 30 days. The animals were housed in separate cages with continuous monitoring. At the end of the study, the mice were euthanized, and kidney tissues were extracted for histopathological examination. **Results:** Behavioral and physiological changes were observed in the Neurobion-treated group, including reduced water intake, decreased appetite, and weight loss. Histopathological analysis revealed significant renal alterations, including glomerular atrophy, Bowman's space dilation, and tubular necrosis. Degenerative changes were observed in renal tubular epithelial cells, accompanied by swelling, nuclear hypertrophy, and inflammatory cell infiltration. Vascular congestion and thrombosis were also evident, along with thickening of blood vessel walls. Additionally, Periodic Acid-Schiff (PAS) staining demonstrated glycogen accumulation in tubular cell basement membranes and Bowman's capsule, suggesting metabolic alterations in renal tissue. **Conclusion:** The findings suggest that prolonged administration of Neurobion (B1, B6, B12) induces histopathological changes in renal tissue, potentially affecting kidney function. These alterations, including tubular degeneration, glomerular damage, and vascular changes, indicate possible nephrotoxic effects. Further research is necessary to assess the clinical implications and underlying mechanisms of B-vitamin supplementation on renal health.

Keywords: Neurobion, Albino Mice, Kidneys, Bowman's Space.

INTRODUCTION

Human health faces serious risks and can sometimes lead to life-threatening conditions, whether in the short term or long term [1], due to the transfer of pollutants and harmful substances from environmental sources like air water, [2], and soil [3] to be transmitted to humans via the food chain [4]. Exposure, handling or accumulation of various chemicals such as heavy metals [5], dyes [6], inorganic toxins [7], organic acids [8], carcinogenic substances from different resources [9], radioactive elements [10], hardness [11], oil waste [12], medicines [13], drugs [14], chemicals [15], tree leaves [16], pesticides [17], phenols [18], industrial [19] and agricultural residues [20] and others [21], and even essential substances that the human body requires, such as various elements [22] or vitamins [23] may induce toxic effects on the body after a duration that depends on the organism's type, age, gender, and weight. Medications are among the substances that have not been sufficiently highlighted or extensively studied for their effects [24]. The utilization of medications not advised may result in hazardous circumstances and, in some cases, fatality due to their diverse impacts on the functions and operations of the body's organs [25]. Individuals utilize pharmaceuticals to alleviate the symptoms of illnesses; however, certain medications may induce adverse reactions and undesired consequences, thereby impacting public health or posing toxic repercussions [26]. While numerous approaches exist to address and remediate these pollutants, including their removal [27] or exploring alternative eco-friendly utilization methods [28], by converting them into useful materials, such as additives [29], alcohol [30], chemicals [31], pesticides [32], fertilizers [33], catalyst materials [34], nanoo-particles [35], fillers [36], or other methods to dispose of them [37], The degree of pollution

humans encounter from these diverse sources, impacting their health in different manners [38], remains significant, lacking a precise and up-to-date delineation of their consequences [39]. The combination of vitamins B₁, B₆, and B₁₂ combined, as a result of their biochemical synergy, is of particular importance for the metabolism of the nervous system, which justifies their combined use [40]. Moreover, in most patient groups such as the elderly, diabetics, etc., there is a deficiency of all three neurological vitamins [41]. Studies have shown that this combination of neuroprotective B vitamins accelerates regeneration processes in damaged nerve fibers, ultimately promoting the restoration of function and muscle innervation [42]. In an experimental diabetic model in rats, administration of B complex vitamins prevented or mitigated the characteristic nerve damage, such that the deterioration of functional properties was counteracted (anti-neuroprotective effect) [43]. Furthermore, the combination of B₁, B₆, B₁₂ has been shown to have a synergistic effect when combined with NSAIDs in the treatment of pain. The therapeutic supply of Neurobion B₁, B₆, B₁₂ may supplement inadequate dietary vitamin intake and thus ensure the availability of the required amounts of coenzymes [44]. The therapeutic use of this drug in diseases of the nervous system serves, on the one hand, to compensate for the accompanying deficiencies, and on the other hand, to stimulate natural repair mechanisms [45]. Although the use of these medications is safe, it is not without some side effects on many body systems such as the digestive, respiratory, circulatory, and excretory systems, and others [46]. The kidney is one of the important organs exposed to drug interactions that can weaken its structure and functions. The kidneys in vertebrates play a sensitive role in the body, and the failure of this organ means death [47]. The

kidneys perform many essential regulatory and excretory functions, including removing many harmful substances that animals ingest intentionally or unknowingly while eating, drinking, or breathing, maintaining the balance between acids and bases, regulating electrolytes, eliminating nitrogenous wastes, and removing drugs [48]. They are also responsible for the production of essential biochemical substances such as renin, erythropoietin, and the reabsorption of glucose, water, and amino acids [49]. Considering the kidneys' significant role, both in terms of their anatomical position and their multifunctional nature, coupled with the limited existing research on the tissue impact of medications on this crucial organ, this investigation aimed to elucidate the impact extent of Neurobion (B₁, B₆, B₁₂) on kidney tissue structure in adult white mice following a 30-day treatment period.

MATERIALS AND METHODS

Drug Doses & Method of Administration

The current study was planned using the half-lethal dosage (LD₅₀) of Neurobion, which is ranged between 3.51 – 4.00, expressed in milligrams of (Neurobion) per kilogram of rat body weight. To evaluate the drug's harmful effects, dose 0.1 mg/kg were used. The amount of medication administered into the study's mice may be determined using equation 1 [16, 46]:

$$\frac{x}{D} = \frac{W_{mouse}}{1000} \quad (1)$$

Where: x : The amount of drug injected to mice in the experiment, D : The specified dose of Neurobion drug, which is (0.1) mg/kg of body weight, W_{mice} : weight of the mice used in the experiment ranges between (20-25) grams.

Preparation of Animals

Fifteen albino mice aged 3-4 months, weighing 20-25 g, obtained from the animal house at the University of Diyala - College of Education for Pure Sciences - Department of Biology, were prepared and raised in the animal house, by placing them in plastic cages covered with metal mesh covers, measuring 30 × 50 × 20 cm. The cage floors were covered with sawdust, and they were cared for and provided with water and feed. The animal cages were cleaned every day and sterilized with disinfectants. The animals were then placed in

suitable laboratory conditions in terms of ventilation and lighting at a temperature of 23-26 C. Following a week of observation, the animals were divided into two groups: the first one was the control group, which did not get the Neurobion drug, while the other group was administered by 0.1 mg of Neurobion/kg of mice for 30-day period.

Dissection of Animals

The animals of controlled group and mice were dosed for thirty days with Neurobion were dissected, and the kidneys were extracted and prepare to be ready for histological tissues process.

Preparation of Histological Sections

After fixing the samples for 24 hours with a 10% formalin solution, all the procedures needed to prepare tissue slices were carried out, as stated in [50]. The slides were stained with hematoxylin-eosin stain in order to evaluate how the Neurobion medication affected the kidney's histological structure. To perform the requirements of the current investigation through determining the effect of Neurobion on the histological structure of the studied organ, the histological sections of mice's kidneys were prepared by utilizing of glass slides and then photographed using light microscope of different powers equipped with camera of HDCE-20C type. The results obtained from different section of treated kidneys were compared with the sections of controlled group.

Histopathological Changes in Mice Kidneys Injected 0.1 mg/kg Neurobion

The results of microscopic examination of kidney sections in experimental groups injected with 0.1 mg/kg of Neurobion showed that there were many clear effects resulting from treating by drug and that mice dosed with Neurobion at a concentration of 0.1 mg/kg for 30 days suffered from negative effects represented by the occurrence of inflammatory changes in the glomeruli. The obtained results showed an increase in the thickness and density of the basement membrane of the glomeruli and some of the urinary tubules in addition to an enlargement of Bowman's space as a result of damage to the glomerulus by shrinking, as the glomeruli appeared empty or containing glomerular remains and accumulation of glomerular cells, and atrophy of the glomeruli in some sections. Necrosis was also observed in the glomerulus and the glomerular wall area, and the occurrence of cell rupture in the parietal area of the glomerulus.

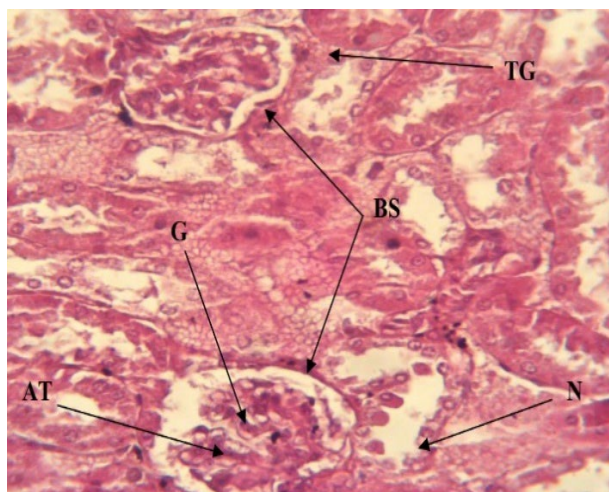


FIGURE 1: Cross section of the kidney of Mice of the Experimental Group Treated with 0.1 mg/kg Neurobion for 30 days, showing the Occurrence of Inflammatory Changes in the Glomeruli within the kidney. G Glomerulus, BS Bowman's space, TG Glomerular Basement Membrane Thickness and Density, AT Glomerular Atrophy, N Necrosis, Stained (H&E 40×).



FIGURE 2: Cross section of the kidney of Mice of the Experimental Group Treated with 0.1 mg/kg Neurobion for 30 days, showing the Occurrence of Inflammatory changes in the Glomeruli inside the kidney. BS Bowman's Space, TG Glomerular Basement Membrane Thickness and Density, CO Vascular Congestion, DT some Cell Destruction, V Cell Vacuolization in the Parietal Region of the Glomerulus, Stained (H&E 40×).

Hemorrhage was also observed inside the glomerulus, Figures 1 and 2, while in other sections, an enlargement of the glomerulus was

observed, which led to a reduction in Bowman's space, as shown in Figure 3.

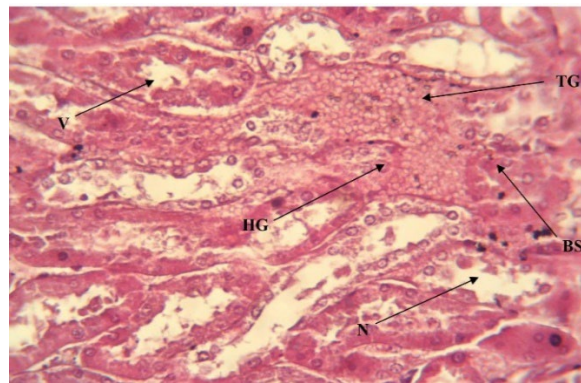


FIGURE 3: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 mg/kg Neurobion for 30 days, showing HG Enlarged Glomerulus, BS reduced Bowman's Space, TG Thickness and Density of the Basement Membrane of The Glomerulus, N Necrosis, V Vacuolization, Stained (H&E 40×).

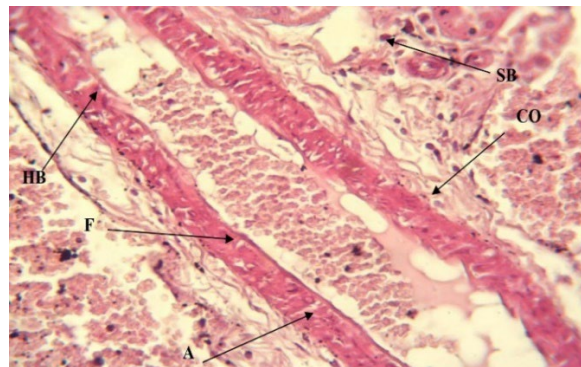


FIGURE 4: Cross Section of the kidney of Mice in The Experimental Group Treated with 0.1 mg/kg Neurobion for 30 days, showing the Occurrence of Blood Congestion of the Blood Vessels inside the kidney. SB: Separation of Some Tubule cells from the Basement Membrane, F: Perivascular Fibrosis, CO: Vascular Congestion, HB: Hypertrophy of the Blood Vessel Wall, A: Infiltration of Inflammatory Cells, Stained (H&E 40×).

The results of the current study also showed the occurrence of congestion of blood vessels, bleeding of blood vessels, and the formation of a blood clot inside the blood vessels, as shown in Figure 4. The results also showed the presence of several inflammatory foci of varying sizes in different areas of the renal parenchyma around the vessels, glomerulus, and renal tubules, and the occurrence of degenerative changes in the renal tubules resulting from the enlargement and separation of some epithelial cells lining the renal tubules from the basement membrane and their accumulation in the cavity, causing high degeneration in the renal tubules, represented by the death of the epithelial cells lining the renal tubules, which are

characterized by the loss of nuclei, the decrease in the size of their cells, and the enlargement of some of them, as well as the damage and detached cells from the basement membrane, as in Figure 5 and 6. Figure 7 showed thickening of the nuclei in the renal tubules and degeneration of the epithelial cells in the renal tubules, as well as necrosis and rupture between some of the proximal and distal tubules, decreased cell size, damage to the lining epithelial cells in most tissue sections and their separation from the detached basement membrane and the appearance of edema between the cells. While the results of examining the sections showed the appearance of fibrosis around the blood vessels and sometimes near the renal corpuscle, as in Figure 8.

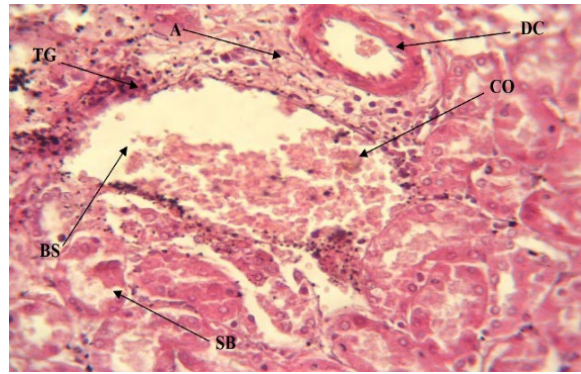


FIGURE 5: Cross Section of the kidney of Mice of the Experimental Group Treated with 0.1 mg/kg Neurobion for 30 days, A shows Infiltration of Inflammatory Cells inside the kidney, SB Detachment of some Tubule Cells from the Basement Membrane, TG thickness of the Basement Membrane of the Glomerulus, CO Vascular Congestion, BS Bowman's Space, Stained (H&E 40×).

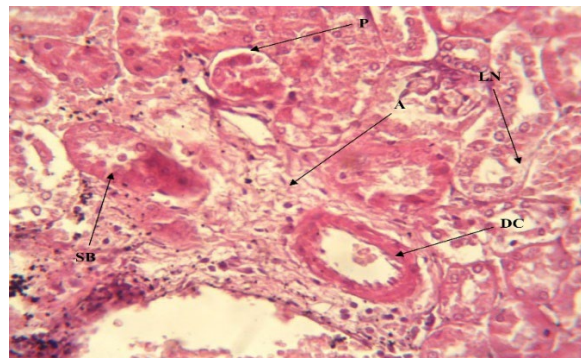


FIGURE 6: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 mg/kg of Neurobion for 30 days, showing the Infiltration of Inflammatory Cells Inside the Kidney, SB Detachment of some Tubule Cells from the Basement Membrane, DC Cell Size Decrease, An Infiltration of Inflammatory Cells, LN Loss of Nuclei, P Thickening of Nuclei Stained (H&E 40×).

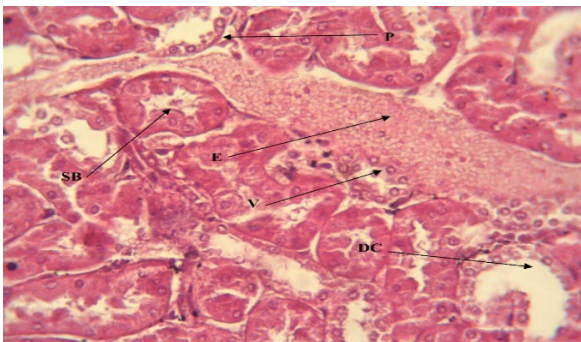


FIGURE 7: Cross Section of the Kidney of the Experimental Group of Mice Treated with 0.1 mg/kg of Neurobion for 30 days, P Noted Thickening of the Nuclei in the Renal Tubules within the Kidney, SB Detachment of some Tubule Cells from the Basement Membrane, F Fibrosis around the Vessels, DC Cell Size Decrease, E Edema, V Vacuolization, Stained (H&E 40×).

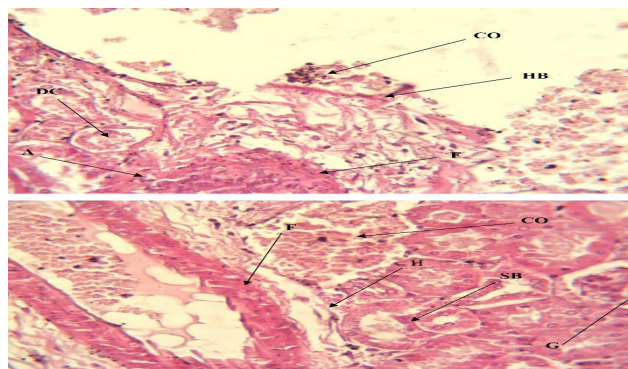


FIGURE 8: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 mg/kg of Neurobion for 30 days, showing the Appearance of Fibrosis around the Vessels Inside the Kidney, SB Separation of Some Tubule Cells from the Basement Membrane, F Perivascular Fibrosis, CO Vascular Congestion, DC Cell Size Reduction, HB Vascular Wall Hypertrophy, An Inflammatory Cell Infiltration, H Hemorrhage, G Glomerulus, Stained (H&E 40×).

The tissue sections passing through the pulp area showed some pathological tissue changes such as the presence of infiltration of inflammatory cells, the presence of necrosis and rupture of the cells forming the collecting tubules and other tubules present within this area, and swelling of some cells was also observed, which indicates the occurrence of hydropic degeneration and thickening of the nuclei of others, Figures 9 and 10. Histological sections of mice in the experimental group treated with 0.1 mg/kg of Neurobion for 30 days showed necrosis of the glomeruli, enlargement of the capsule space, shrinkage of the glomeruli, and clear atrophy of many glomeruli, as well as damaged exterior of Bowman's capsule, which is characterized

by loss of the outer squamous layer of the capsule, and detached epithelial cells of the visceral layer from the basement membrane, as shown in Figures 11 and 12. The study also showed that the drug had a harmful effect on the proximal tubules, as swelling of the cells (swelling cells) was observed, and there was expansion in the lumen of some renal tubules, and the appearance of edema, rupture, and severe necrosis between the tubules, as shown in Figure 13, with the continued infiltration of inflammatory cells, which was observed in all tissue sections, with the severity of the infiltration being noted, as the accumulation of inflammatory cells that appeared between the renal tubules and the glomerulus increased, as shown in Figure 14.

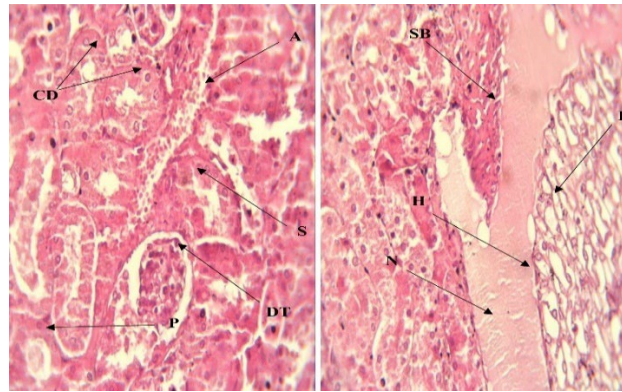


FIGURE 9: Cross Section of the Kidney of the Experimental Group of Mice Treated with 0.1 Mg/Kg of Neurobion for 30 Days, Showing Degenerative Changes in the Medullary Area within the Kidney, S Swelling of Some Tubule Cells, SB Separation of Some Tubule Cells from the Basement Membrane, P Thickening Of Cell Nuclei, V Rupture Of Cells, DT Destruction of Some Cells, N Cell Necrosis, CD Collecting Tubules, Stained (H&E 40×).

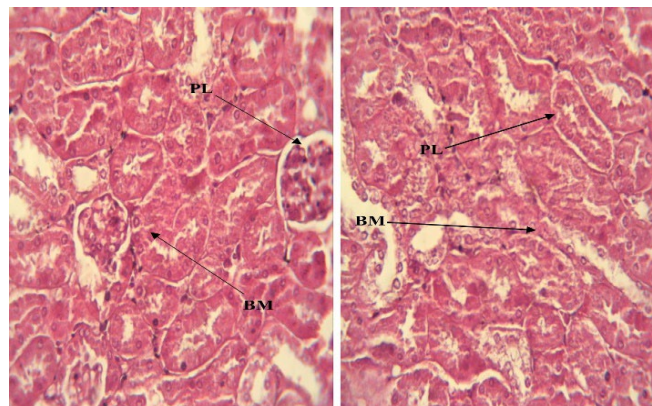


FIGURE 10: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg of Neurobion for 30 Days, Showing the Destruction of Some of the Basement Membranes (BM) and the Parietal Layer (PL) of Bowman's Capsule, Stained (H&E 40×).

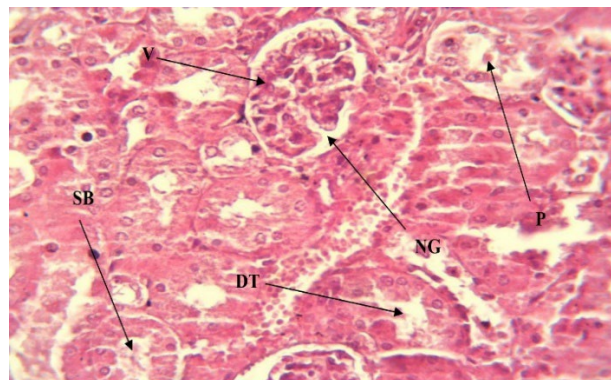


FIGURE 11: Cross Section of the Kidney of the Experimental Group of Mice Treated with 0.1 Mg/Kg of Neurobion for 30 Days, Showing the Occurrence of Severe Degenerative Changes Inside the Kidney, SB Separation of Some Tubule Cells from the Basement Membrane, P Thickening of Cell Nuclei, V Rupture of Cells, DT Destruction of Some Cells, NG Necrosis of the Glomerulus, E Edema, NB Necrosis of the Parietal Area of Bowman's Space, DN Death or Decomposition of Nuclei, Stained (H&E 40×).

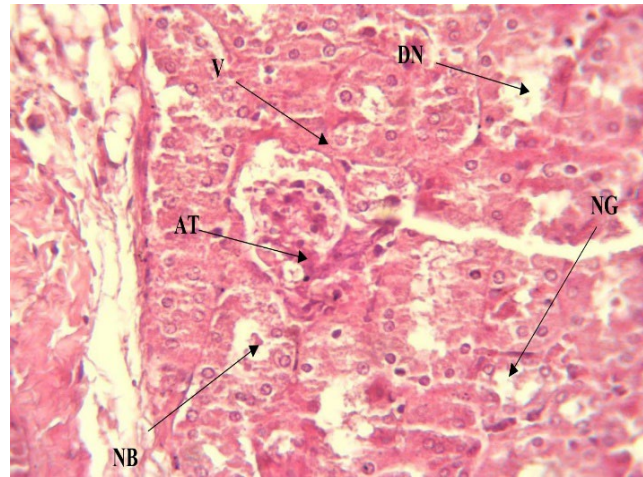


FIGURE 12: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg Neurobion for 30 Days, Showing Severe Degenerative Changes within the Kidney, aT Renal Shrinkage, V Cell Rupture, NG Glomerular Necrosis, NB Necrosis of the Parietal Area of Bowman's Space, DN Nuclei Death or Degeneration, Stained (H&E 40×).

In addition to what was mentioned above, the results showed the presence of fibrosis, which appeared in larger areas than the previous groups, as it was clear in most sections and was concentrated in the cortex area, in addition to its presence in the medulla area to a lesser

extent, see Figure 15. As for the medullary region of the kidney, the damage appeared clearly in the collecting tubules, which included necrosis and rupture of the cells that make them up, with thickening of the nuclei and dissolution of some of them.

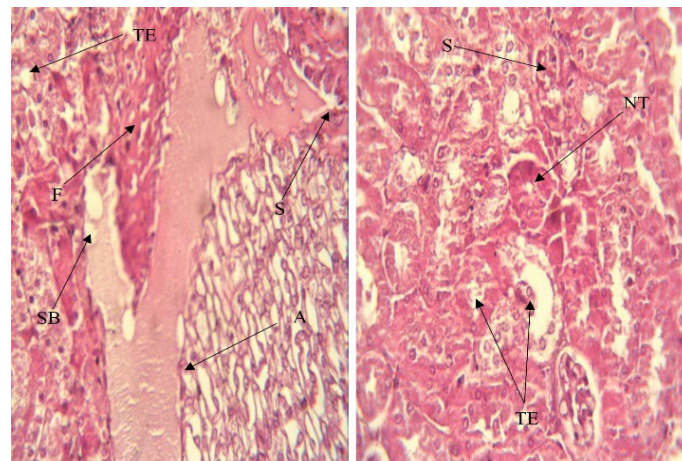


FIGURE 13: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg Neurobion for 30 Days, Showing Acute Degenerative Changes within the Kidney, TE Tubular Dilatation, NT Tubular Necrosis, S Swelling, SB Cell Detachment, F Fibrosis, A Infiltration, Stained (H&E 40×).

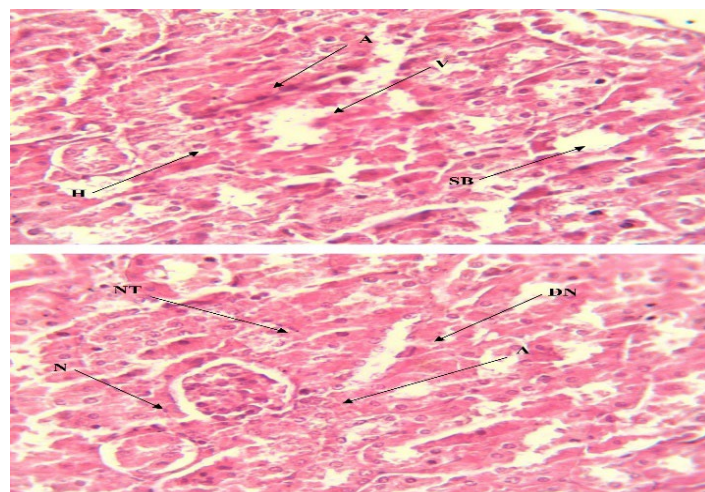


FIGURE 14: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg of Neurobion for 30 Days, Showing Acute Degenerative Changes within the Kidney, An Acute Infiltration of Inflammatory Cells, V Rupture, H Hemorrhage, N Necrosis, SB Cell Separation, NT Tubular Necrosis, DN Death or Decomposition, Stained (H&E 40×).

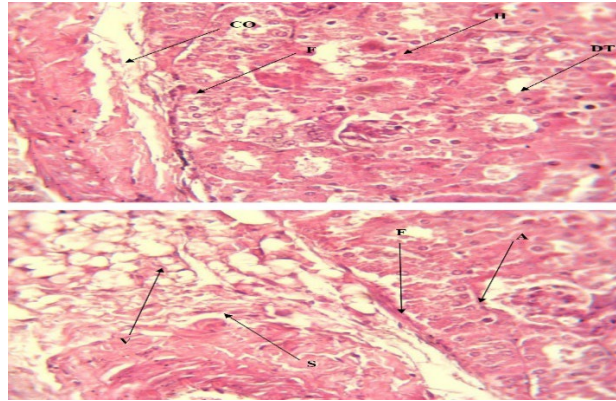


FIGURE 15: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg of Neurobion for 30 Days, note: Fibrosis F, Infiltration A, Detachment of Cells from their Basement Membrane DT, Swelling of Cells S, Vascular Congestion CO, Hemorrhage H, Vacuolation V, Stained (H&E 40×).

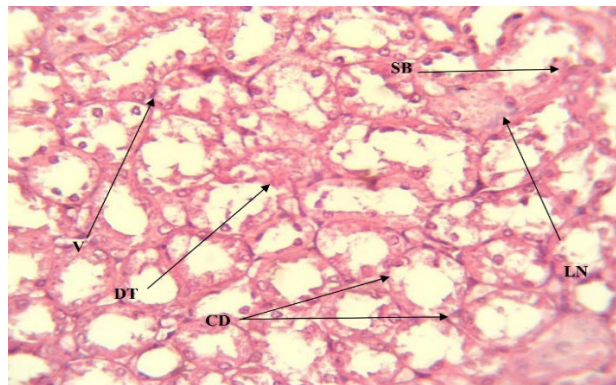


FIGURE 16: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg of Neurobion for 30 Days, Showing Necrosis N in Collecting Tubule Cells CD, Cell Detachment DT, Anuclearity LN, Basement Membrane Detachment SB, Hemorrhage H, Vacuolation V, Stained (H&E 40×).

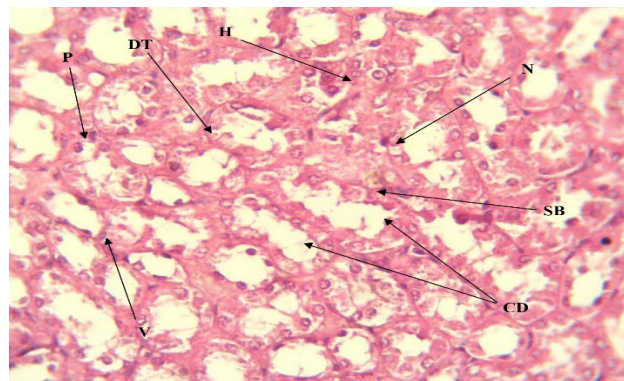


FIGURE 17: Cross Section of the Kidney of Mice of the Experimental Group Treated with 0.1 Mg/Kg Neurobion for 30 Days, Showing Necrosis N in Collecting Tubule Cells CD, Cell Separation DT, Anuclear Aplasia LN, Cell Thickening P, Basement Membrane Separation SB, Hemorrhage H, Vacuolization V, Stained (H&E 40×).

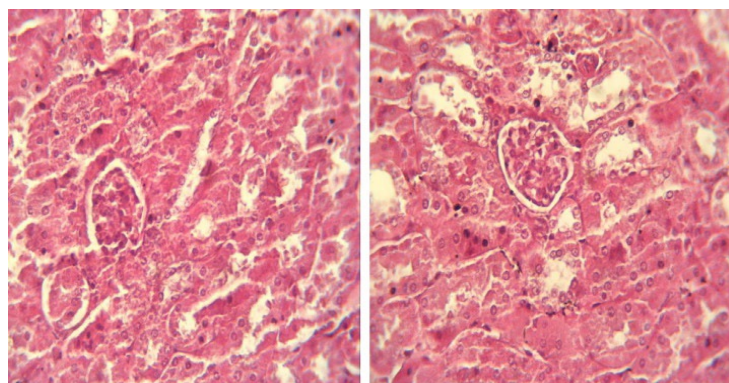


FIGURE 18: Cross Section of the Kidney of Mice from the Experimental Group Treated with 0.1 Mg/Kg Neurobion For 30 Days Showing Accumulation of Glycogen Between Tubules as Well as in Bowman's Space, Destruction of the Basement Membranes of Tubules, Stained (H&E 40×).

There was also severe degeneration, death of the epithelial lining cells of the tubules and collecting ducts, loss of nucleus, and detached cells from their basement membrane, and damage to the cells that were characterized by their small size, vacuolation, and the presence of shedding of the cells of the epithelial layer of the tubules and their accumulation inside the cavity and their partial or complete disappearance, leaving only their basement membrane, as shown in Figures 16 and 17. Figure 18 showed the absence of glycogen in it, while some sections showed the presence of deposits of these materials between the tubules and Bowman's space. Also, the clear destruction of the basement membrane of the tubules was noted.

Conclusions

The current study reached a number of conclusions, the first of which is that exposure to Neurobion B1, B6, B12 led to a change in the

weight rate of mice in addition to behavioral and clinical changes in the animals. In addition, Neurobion B1, B6, B12 has clear effects that appeared in the kidney tissue of the adult white mouse. The effects of the drug were characterized by clear damage to the cortex area represented by the expansion of the capsular space as a result of the degeneration of the glomerular cells, which led to shrinkage and atrophy of the glomeruli, and damage to the glomerular cells either due to the loss of nuclei or the small size of the cells or their separation from the basement membrane, which led to cell death, and damage to the external appearance of Bowman's capsule as a result of the death of the epithelial cells of the parietal layer. Also, treatment with Neurobion B1, B6, B12 for a month caused severe histopathological damage to the kidney tissue represented by congestion and bleeding, and the drug has an effect on the average diameter of the glomeruli and Bowman's space in the adult white mouse.

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