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***Original Article***

# Evaluation of the Effect of Alcoholic Extract of *Laurus Nobilis* Leaves on Blood Biochemical Parameters and Histological Changes in the Liver and Kidney among Female Wistar Rats Treated with Depakene (Sodium Valproate)

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**Abstract**

This study aimed to evaluate the effects of *Laurus Nobilis* (Bay leaves) alcoholic extract on glucose, hemoglobin A1c (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea levels; moreover, it was attempted to examine the histological changes induced in the liver and kidney among female albino rats treated with Depakene (Sodium Valproate). The *L. nobilis* leaves were dried in the shade, and they were then ground in mechanical processing. The resulting substance (250 gm) was processed in 70% ethanol for 24 h using a Soxhlet extractor at 45°C. Before being measured, the extract was concentrated in vacuo and stored in a vacuum desiccator until the elimination of all the solvents. In total, 20 female adult Wistar rats (230-250 g) were bred in the Animal House Lab at the University of Kufa, Faculty of Education for Girls, Kufa, Iraq. These animals were randomly divided into four groups (n=5), housed in a typical laboratory setting, and given a standard diet and water. Each animal received the treatments intraperitoneally for 30 days. The experimental groups were designed as follows: group 1 (the control) was given only physiological saline solution; group 2 received alcoholic extract of *L. nobilis* leaves at a dose of 150 mg/kg BW; group 3 received Depakene (Sodium Valproate) at a dose of 500 mg/kg BW; and group 4 received alcoholic extract+Depakene at a dose of 150 mg/kg BW and 500 mg/kg BW. The animals were euthanized following anaesthesia 24 h after the last day of the experiment. Heart blood samples were gathered in gel tubes, the serum was then centrifuged for 15 min at 3000 rpm to measure the biochemical parameter levels, which included glucose, HbA1C, ALT, AST, creatinine, and urea. The liver and kidney organs were removed and placed in a 10% formaldehyde solution instantly. Following fixation, they were processed as usual before being embedded in paraffin for histological analysis. Morphological changes were assessed using hematoxylin and eosin staining techniques. The recorded data showed a major drop ($P$<0.05) in blood glucose and HbA1c levels in group 2 which was given ethanol extract, compared to the other groups. Interestingly, the level of blood glucose and HbA1c levels reduced significantly in group 4, which was given *L. nobilis*+Depakene, compared to the control and the animals treated with only Depakene. Moreover, the results showed a major rise ($P$<0.05) in the liver enzyme among the animals treated with Depakene, compared to other groups. On the other hand, the recorded data showed a substantial drop ($P$<0.05) in creatinine levels in the animals treated with *L. nobilis* leaves extract (group 2) and group 4, compared to group 3 and the control group, respectively. However, no changes were recorded in the case of urea levels among the groups. Finally, the findings of this study showed that the ethanol extract of *L. nobilis* leaves was effectively reduced the adverse effects of Depakene. On the other hand, it had a significant effect on the reduction of blood glucose.

**Keywords:** Depakene drug, Glucose level, *Laurus Nobilis* leaves, Liver and kidney function

## 1. Introduction

Diabetes (Diabetes mellitus) is defined as a condition marked by high blood sugar (hyperglycemia) as a result of insulin insufficiency, either totally or partially, caused by a pancreas gland disorder (1). Some cardiovascular conditions, such as platelet hyperactivity and hyper-aggregability, both linked to increased oxidant generation and irregular cytosolic mobility of $Ca^{2+}$, could result from this rise in blood glucose (2). Permanent and chronic hyperglycemia leads to organ dysfunction and failure, resulting in tissue damage in the long term (2).

According to the World Health Organization, many herbs and plants have been described as having blood sugar-lowering activity when taken orally (3). Most plants contain terpinyl, glycos, alkaloids, flavonoid, and carotenoid; moreover, they are often documented as having an anti-diabetic effect (4). The liver, the body's largest gland, is located mostly in the upper right part of the abdominal cavity, under the diaphragm, and serves as the blood's gatekeeper. The liver extracts toxic contaminants and detoxifies them when blood from the hepatic portal vein flows through it. The liver also extracts nutrients and acts to maintain the blood's consistency. It removes and stores iron and fat-soluble vitamins (A, D, E, and K) from the body. The liver aids in blood cholesterol regulation by producing plasma proteins from amino acids (5). Based on the degree of liver damage (cytolysis), liver cells produced equivalent quantities of enzyme markers linked to hepatocyte damage, such as cytoplasmic alanine and aspartate aminotransferase (alanine aminotransferase [ALT] and AST), into the circulatory system (6). ALT activity, which is observed as an increase in AST, is a specific determinant of hepatocyte cytolysis (7).

The kidneys are responsible for filtering metabolic waste that the body no longer needs. These compounds include urea (from amino acid metabolism), creatinine (from muscle creatinine), uric acid (from nucleic acids), end products of haemoglobin breakdown (e.g., bilirubin), and hormone metabolites. This waste must be eliminated as rapidly as the body produces them. The kidneys also remove many poisons and other foreign compounds produced by the body or consumed, such as pesticides, pharmaceuticals, and food additives [8]. One of the most helpful markers in the disorder identification of the human excretory system is the level of urea and creatinine in serum and urine (8).

*Laurus Nobilis L.*, commonly known as Bay leaves or simply laurel, is a Mediterranean evergreen plant that is now planted across Southern Europe, Western Asia, Northern Africa, and America for its leaves aroma and as a decorative plant (9). Bay leaves are used to flavour a variety of dishes, particularly in Mediterranean cuisines. Furthermore, the essential oil extracted from these leaves is frequently utilized in the culinary and perfume industries for flavouring meals, soups, and seafood (10). *L. nobilis* leaves and fruits have been utilized in traditional medicine as a general gastrointestinal secretions stimulator, carminative, diaphoretic, and antiseptic since antiquity, as well as for rheumatism, cough, heart disorders, viral infections, diarrhoea, and other ailments (11). Bay leaves' chemical composition has been widely researched. The essential oil, also known as Bay leaves oil, has several volatile compounds that work as an antimicrobial agent (12). Linalool, R-terpinyl acetate, and some monoterpene hydrocarbons, such as -pinene and sabinene, were discovered as well. Sesquiterpene lactones, flavones (apigenin and luteolin), flavonols (kaempferol, myricetin, and quercetin) (13), alkaloids, glycosylated flavonoids, monoterpene, and germacrene alcohols have all been isolated from *L. nobilis* leaves and fruits in earlier phytochemical studies (14). Sesquiterpene lactones are found in roots and leaves, and two different chemical types have been identified, with laurenobiolide and costunolide as significant components, respectively (15). The pharmacological characteristics of sesquiterpene lactones discovered in Bay leaf include the inhibition of nitric oxide generation (anti-inflammatory) (16) and an increase in the glutathione S-transferase activity in the liver (17).

Bay leaves are used in food industries as preservatives for food because of their antimicrobial and insecticidal

properties (18). Moreover, they may help diabetic patients boost their blood glucose metabolism by enhancing capillary activity, lipid metabolism, liver and kidney function, and antioxidant status, among other things (19). Antioxidant, antibacterial, neuroprotective, and anticholinergic functions have also been discovered in laurel extracts. Many chemical substances, such as Terpenoids, Glycosides, Essential Oil, and Anthocyanin are just a few examples that have been discovered to be responsible for *Laurus* effects and have the pharmacological potential for the treatment of a variety of illnesses and disorders, which have been proven to be safe (20, 21).

Depakene (valproic acid) is used as a monotherapy or adjunctive therapy to treat patients with complex partial seizures that occur alone or in combination with other forms of seizures (22). Valproic acid (VPA) is a first-line treatment for generalized and focal epilepsies, including special epilepsies, and is one of the most often used antiepileptic medications in the world (23). It is an antiepileptic medication with a wide range of effects that is generally well-tolerated. In certain patients, serious complications, such as hemorrhagic pancreatitis, coagulopathies, bone marrow suppression, VPA-induced hepatotoxicity (24), and encephalopathy, may develop; however, the prevalence and occurrence of these rare side effects are still unclear (25). Epilepsy is a disorder of the central nervous system marked by uncontrolled nerve cell activation, and in certain cases, convulsive seizures with or without loss of consciousness. VPA is a widely used antiepileptic medication that is extremely toxic to the liver (26). Depakene, a branched short-chain fatty acid, is a common antiepileptic and mood stabilizer. Gamma Amino Butyrate trans-aminobutyrate and ion channels inhibition has been linked to antiepileptic properties. VPA was recently identified as a Histone Deacetylase Inhibitor, which acts by suppressing histone deacetylation, thereby rendering transcription sites more accessible at the level of gene transcription (25).

Therefore, this study aimed to evaluate the effects of *L. nobilis* alcoholic extract on glucose, hemoglobin A1c (HbA1c), ALT, AST, creatinine, and urea levels; moreover, it was attempted to examine the histological changes induced in the liver and kidney among female albino rats treated with Depakene (Sodium Valproate).

## 2. Materials and Methods

The *L. nobilis* leaves were dried in the shade, and they were then ground in mechanical processing. The resulting substance (250 gm) was processed in 70% ethanol for 24 h using a Soxhlet extractor at 45˚C.

Before being measured, the extract was concentrated in vacuo and stored in a vacuum desiccator until the elimination of all the solvents (27). In total, 20 female adult Wistar rats (230-250 g) were bred in the Animal House Laboratory at the University of Kufa, Faculty of Education for Girls, Kufa, Iraq. These animals were randomly divided into four groups (n=5), housed in a typical laboratory setting, and given a standard diet and water. Each animal received the treatments intraperitoneally for 30 days.

Group 1 **(**the control) was given only physiological saline solution; group 2 received an alcoholic extract of *L. nobilis* leaves at a dose of 150 mg/kg BW; group 3 received Depakene (Sodium Valproate) at a dose of 500 mg/kg BW; and group 4 received alcoholic extract+Depakene at a dose of 150 mg/kg BW and 500 mg/kg BW. The animals were euthanized following anaesthesia 24 h after the last day of the experiment. Heart blood samples were gathered in gel tubes, and the serum was then centrifuged for 15 min at 3000 rpm to measure the biochemical parameter levels, which included glucose, HbA1C, ALT, AST, creatinine, and urea. The liver and kidney organs were removed and placed in a 10% formaldehyde solution instantly. Following fixation, they were processed as usual before being embedded in paraffin for histological analysis. Morphological changes were assessed using hematoxylin and eosin staining techniques.

### 2.1. Statistical Analysis

The recorded data were examined using one-way analysis of variance, and group variations were

determined using Duncan multiple range tests. Data were presented as mean±SEM, with distinct letters indicating a significant difference ($P$<0.05).

## 3. Results

The recorded data showed a major drop ($P$<0.05) in blood glucose and HbA1c levels (Table 1) in group 2, which was given ethanol extract, compared to the other groups. Interestingly, the level of blood glucose and HbA1c levels reduced significantly in group 4, which was given *L. nobilis*+Depakene, compared to the control and treated animals with only Depakene (Table 1).

**Table 1.** Effects of ethanol extract of *Laurus nobilis* (Bay leaf) on glucose and HbA1c levels in female albino rats treated with Depakene

| Parameters / Groups | HbA1c mg/dl | Glucose mg/dl |
|---|---|---|
| Control | 6.73±0.21[a] | 104.00±6.00b |
| Ethanol extract | 5.36±0.15[b] | 88.00±2.64[c] |
| Drug | 7.10±0.10[a] | 116.00±2.64[a] |
| Extract+Drug | 6.76±0.49[a] | 97.00±3.00[b] |

Means±SD
Changes a, b, c are substantial ($P$<0.05) as compression between columns

The results revealed a substantial increase ($P$<0.05) in the AST concentration in animals treated with Depakene, compared to the other groups, as well as a significant increase in ALT in *L. nobilis*+Depakene treated group, compared to the control and the group treated with *L. nobilis* extract (Table 2). However, the ethanol extract group showed a major decrease in the AST and ALT concentrations, which was not significant, compared to the control group.

**Table 2.** Effects of ethanol extract of *L. nobilis* (Bay leaf) on the AST and ALT levels in female albino rats treated with Depakene

| Parameters / Groups | ALT IU | AST IU |
|---|---|---|
| Control | 42.00±2.64[b] | 144.67±1.52[c] |
| Ethanol extract | 42.00±3.61[b] | 144.00±3.00[c] |
| Drug | 86.67±6.65[a] | 175.67±3.05[a] |
| Extract+Drug | 67.00±3.00[a] | 155.67±4.04[b] |

Mean±SD
Changes a, b, c are major ($P$<0.05) as compression between columns

The recorded data showed that creatinine levels in groups 2, 3, and 4 increased considerably ($P$<0.05) in contrast to the control group. However, the drug's efficacy was reduced in the group treated with *L. nobilis*+Depakene (Table 3). In addition, the findings showed an increase in urea levels; however, the difference was not significant across all groups.

The findings of the histological study showed that the control group (Figure 1) and those treated with the alcoholic extract of *L. nobilis* leaves (Figure 2) had no differences in the histological structure of the liver. The histological section was normal in terms of the structure of the polygonal liver cells, hepatic cords were structured in a common radial shape, and the nucleus took on a normal shape. In addition, the histological sections noticed the existence of Binucleated cells. In the third group, which was treated with Depakene, there was a change in the histological structure, which included liver cell vacuolation, inflammatory cell infiltration, congestion, central venous haemorrhage, and hepatic cord irregularity (Figures 3 and 4). In the fourth group treated with ethanol *L. nobilis* extract+Depakene, there was a partial improvement in liver cells, with the radial form of cell structure recovery and a decrease in congestion and lymphocyte infiltration (Figure 5).

The findings of the histological study of all the treated groups in figures 6, 7, 8, and 9 showed the presence of renal glomerulus, which took on a regular form and contained the nuclei in their specified spots. The glomerular wall was not destroyed, and both the proximal and distal convoluted renal tubules were clear.

**Table 3.** Effect of *L. nobilis* (Bay leaf) ethanol extract on creatinine and urea levels in female albino rats treated with Depakene

| Parameters / Groups | Urea mg/dl | Creatinine mg/dl |
|---|---|---|
| Control | 42.33±4.50[a] | 0.24±0.06[b] |
| Ethanol extract | 45.67±1.53[a] | 0.26±0.02[b] |
| Drug | 48.00±7.21[a] | 0.41±0.08[a] |
| Extract+Drug | 46.67±3.51[a] | 0.34±0.03[ab] |

Mean±SD
Changes a and b are considerable ($P$<0.05) as compression between columns

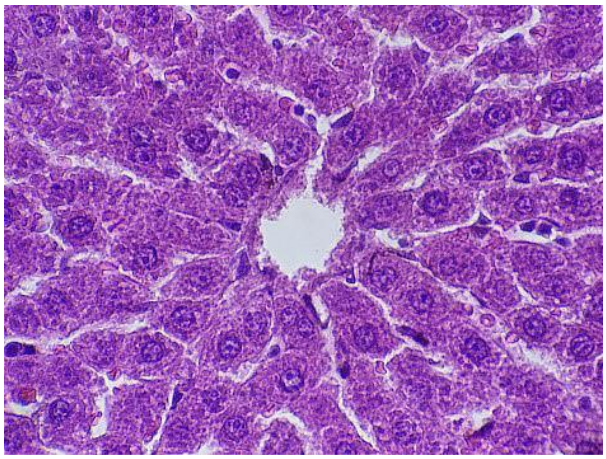

**Figure 1.** Image of central venous and hepatocyte arrangement in the liver tissue of a female rat treated with normal saline (control). (Hematoxylin and Eosin staining 10×)

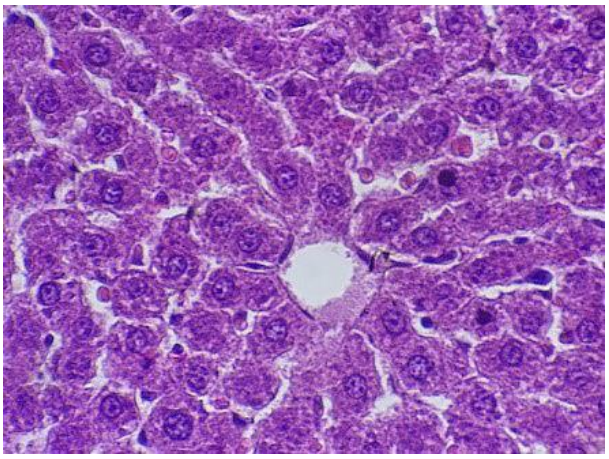

**Figure 2.** Image of central venous and hepatocyte arrangement in the liver tissue of a female rat treated with an ethanol extract of *Laurus nobilis* leaves. (Hematoxylin and Eosin staining 10×)

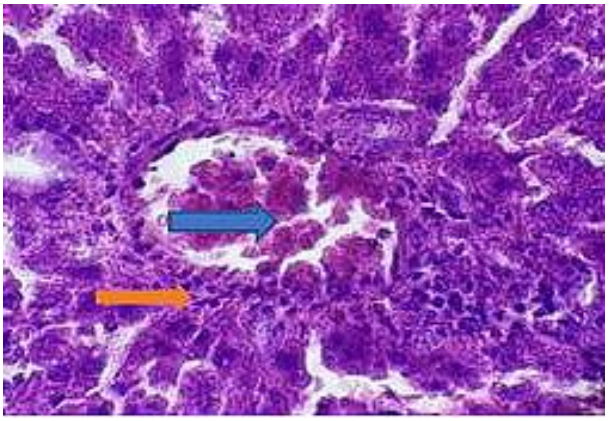

**Figure 3.** Image of liver tissue of a female rat treated with Depakene (Sodium Valproate), showing bleeding in central venous (Blue Arrow) and infiltration of lymphocyte (Orang Arrow). (Hematoxylin and Eosin staining 40×)

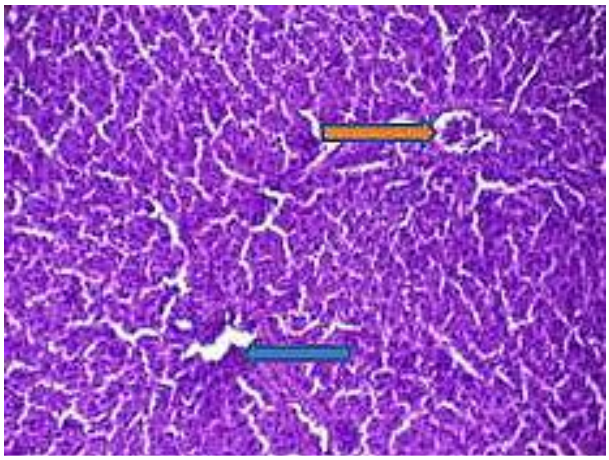

**Figure 4.** Image of liver tissue of a female rat treated with Depakene (Sodium Valproate), showing bleeding (Orang Arrow) and infiltration of lymphocyte (Blue Arrow). (Hematoxylin and Eosin staining 10×)

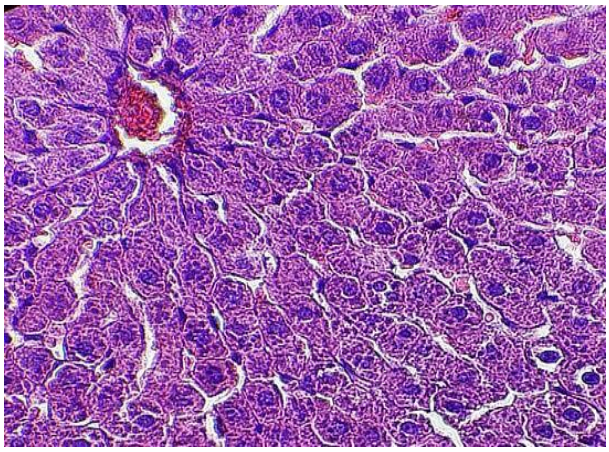

**Figure 5.** Image of central venous and hepatocyte arrangement in the liver tissue of a female rat treated with ethanol extract of *Laurus nobilis* leaves and Depakene (Sodium Valproate). (Hematoxylin and Eosin staining 10×)

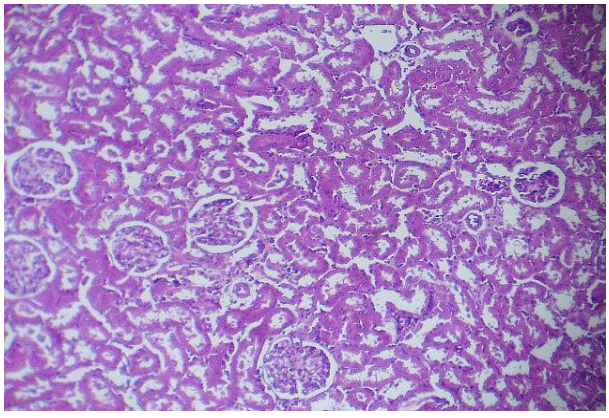

**Figure 6.** Image of normal glomerulus and renal tubule in the kidney tissue of a female rat treated with normal saline (control). (Hematoxylin and Eosin staining 10×)

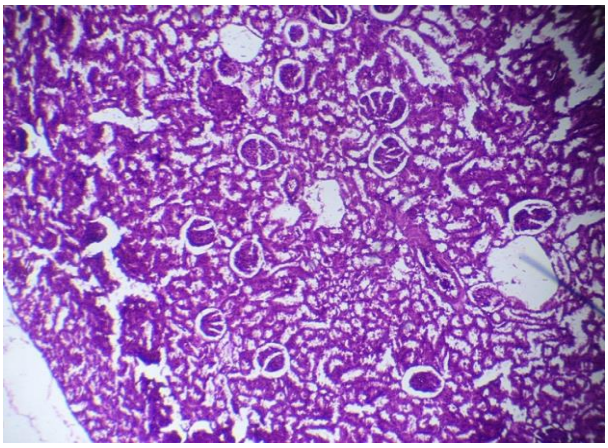

**Figure 7.** Image of normal glomerulus and renal tubule in a female rat kidney tissue treated with ethanol extract of *Laurus nobilis* leaves (Hematoxylin and Eosin staining 10×)

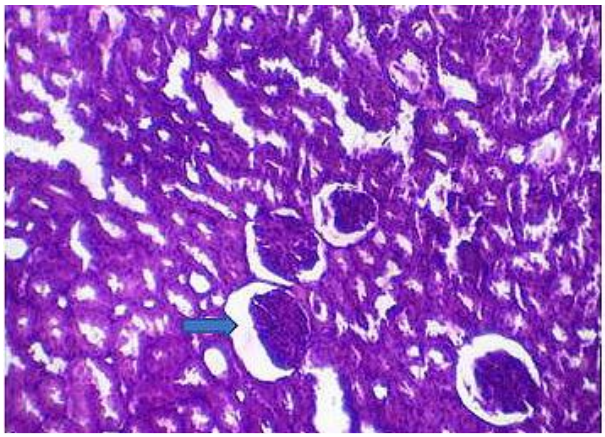

**Figure 8.** Shrinkage in the size of the glomerulus in the kidney tissue of a female rat treated with Depakene (Sodium Valproate). (Hematoxylin and Eosin staining 10×)

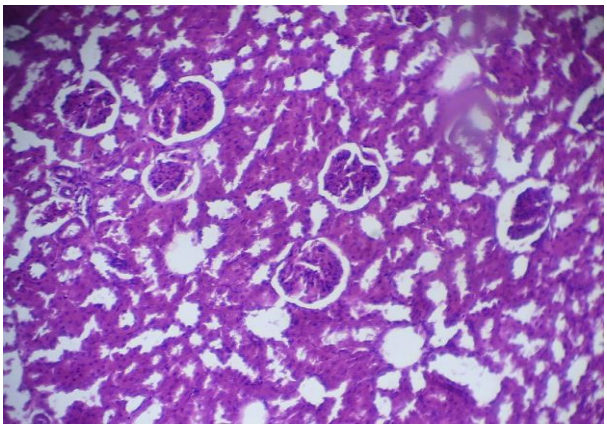

**Figure 9.** Image of normal glomerulus and renal tubule in a female rat kidney tissue treated with ethanol extract of *Laurus nobilis* leaves and Depakene. (Hematoxylin and Eosin staining 10×)

## 4. Discussion

The plant *Laurus* is a member of the Lauraceae family and has a wide range of physiological properties. Antimicrobial, antifungal, antioxidant, and other qualities of laurel oil and its components make it a decent and useful element for use in medicines and cosmetics. Turbines, anthocyanins, coumarins, and other chemical substances were also found in it (11). The results showed that giving the animals an alcoholic extract of Bay leaves resulted in a significant drop in glucose and HbA1C levels, compared to the rat groups given the Depakene drug. The flavonoids in the extract are responsible for the extract's effects. The structure of flavonoids of a plant includes 12 components which are glycosides of quercetin and kaempferol; therefore, the compound has a direct effect on decreasing the level of blood glucose (13).

This study confirms the findings of Mohammed, Omer (20), who discovered that giving *L. nobilis* leaf extracts to rats at a safe dosage level meaningfully reduced streptozotocin-induced diabetes. According to our findings, which show that *L. nobilis* leaf extracts have beneficial impacts on blood sugar levels and pancreatic islet regeneration, an additional preclinical investigation into the efficacy of *L. nobilis* therapy might suggest its suitability as a possible treatment in diabetic patients. According to Alchalabi, Majeed (28), there was a reduction in fasting blood glucose levels, an increase in fasting insulin levels, as well as a significant decrease in ALT, AST, alkaline phosphatase, blood urea, and serum creatinine levels after treatment with 200 mg kg B.W alcoholic extract of Bay leaves for 30 days.

The extract is distinguished by the presence of antioxidants that help to lower glucose levels. Proteins, free sugars, organic acids, and tocopherols, as well as antioxidant activity, such as scavenging, decreasing strength, lipid peroxidation inhibition, and glucose reduction, are found in *L. nobilis* (29). Since the high effectiveness of liver enzymes (ALT, AST) in the blood is the best indicator of liver damage, their high levels in the blood can be used to predict inflammatory

changes in the liver (6). The findings of the current study showed a significant increase in both enzyme levels in animal groups treated with the drug.

This significant increase may explain why the drug at 500mg/kg for a month increased lipid peroxidation (the process of unsaturated fatty acid decomposition in cell membranes by the chain reactions of self-stimulation of free radicals) in the blood and tissues of animals treated with it. As the process outputs, free radicals, penetrate cell membranes and attack the DNA directly resulting in the apoptosis phenomenon (30). The increase in these enzymes can also be attributed to the breakdown and damage to liver tissue caused by excessive drug exposure, which results in the drug being released from the cytosol of the liver into the bloodstream, increasing its concentrations (6). The changes shown in the liver tissue support the results of this study (as in Figures 4 and 5). The treatment of animals with the extract and medication resulted in a significant decrease in the liver enzyme levels. The explanation for this is that the extract contains phytochemicals that shield the liver from the drug's adverse effects. This is more likely attributed to the existence and joint activity of extract phytocomponents of flavonoid and nonflavonoid origins, such as terpenes and terpenoids, which have antioxidant properties (12, 31). According to several pieces of research, combining CCl4 with *L. nobilis* leaves extract decreased mortality and normalized the main indicators of liver damage and De-Ritis ratio. The current study showed that *L. nobilis* extract can cure metabolic and histological abnormalities in hepatocytes caused by CCl4 toxicity (32).

According to Kaurinovic, Popovic (33), Bay leaf extracts showed a variety of activities with the biochemical parameters studied. The findings attained for the values of the studied systems (GSH, GSHPx, LPx, Px, CAT, and XOD) following treatment with laurel extracts in combination with CCl4, suggest that the extracts have a protecting effect, which is more noticeable in the liver than the serum parameters. The liver's abundance of enzymatic systems, which may be included in the pathways of the antioxidant mechanism, might be an explanation. The differences in the activity observed in various extracts are most likely attributable to different amounts of flavonoids and other antioxidant components in them.

These findings encouraged us to explore if orally administering dried Bay leaves is linked to improved antioxidant synthesis in both plasma and lenses of rabbits on a fat-rich diet (34). According to our knowledge, this is the first study on the *L. nobilis* impact on cataract prevention. Patrakar, Mansuriya (35) also studied *L. nobilis* ethanol extract antioxidant characteristics. Researchers examined antioxidant features, decreasing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities to define extracts' overall antioxidant ability. Extracts showed high total antioxidant activity in a linoleic acid emulsion.

The findings of the study revealed a significant increase in creatinine levels in the Depakene-given animals in contrast to the control group, which might be attributed to the nephron's failure to fulfill its work and kidney tissues. The renal glomerulus shrinkage in size (Figure 9) may impair liver function, according to the histological analysis. The drug and extract treatment caused a significant decrease in creatinine and urea levels in the animals. This may be attributed to the antioxidant content of the extract, which led to an increase in protein synthesis and a decrease in catabolism (32).

According to Ravindran, Murugaiyah (36), it was found that therapy with a methanol extract of *L. nobilis* at dosages of 200 mg/kg and 400 mg/kg restored kidney indicators to normal. As a consequence, *L. nobilis* methanolic extract (200 mg/kg and 400 mg/kg) is thought to protect the kidney's functional capability against paracetamol toxicity. When oxygen reactive species are present in low to moderate amounts, they

are required for cellular structure development and may also operate as a defense mechanism for the host. Several pieces of data show that dietary modifications might lower the risk of cataracts by inhibiting the oxidation process that produces free radicals (37). Low levels of glucose, ALT, AST, creatinine, and urea were found in the group treated with oral gavage small dose (250 mg/Kg/BW) and big dose (500 mg/Kg/BW) of Bay leaf daily for three weeks (19).

Minor biochemical dysfunctions due to the long-term Sodium Valproate treatment are reversible, suggesting that Sodium Valproate has no major dose- or time-related negative effects on renal functioning. The metabolic and histological features of rat renal tissue matched those of human renal tissue. Kidney failure is not predicted in people, as no clinically statistically significant renal side effects were identified in this study (38). Finally, the findings of this study showed that the ethanol extract of *L. nobilis* leaves was effectively reduced the adverse effects of Depakene. On the other hand, it had significant effects on the reduction of blood glucose.

## Authors' Contribution

Study concept and design: S. M. J. A.
Acquisition of data: Z. S. M. A.
Analysis and interpretation of data: Z. S. M. A.
Drafting of the manuscript: S. M. J. A.
Critical revision of the manuscript for important intellectual content: Z. S. M. A.
Statistical analysis: J. A. M. and B. C. J.
Administrative, technical, and material support: Z. S. M. A.

## Ethics

The study design was approved by the ethics committee of University of Kufa, Kufa, Iraq.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013;36(1):67-74.
2. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci U S A. 2006;103(8):2653-8.
3. Nandhini LP, Kamalanathan S, Sahoo J. Definition, Diagnostic Criteria, Screening, Diagnosis, and Classification of Diabetes and Categories of Glucose Intolerance. In: Rodriguez-Saldana J, editor. The Diabetes Textbook: Clinical Principles, Patient Management and Public Health Issues. Cham: Springer International Publishing; 2019. p. 71-85.
4. Rahimi M. A review: antidiabetic medicinal plants used for diabetes mellitus. Bull Environ Pharmacol Life Scienes. 2015;4(2):163-80.
5. Guyton A, Hall J. Textbook of medical physiology, 11th. Elsevier Inc.; 2006.
6. Singh A, Sharma O. Clinical Biochemistry of Hepatotoxicity. J Clinic Toxicol. 2011:1-19.
7. Yakubu MT, Adebayo OJ, Egwim EC, Owoyele VB. Increased liver alkaline phosphatase and aminotransferase activities following administration of ethanolic extract of Khaya senegalensis stem bark to rats. Biokemistri. 2005;17:27-32.
8. Guyton AC, Hall JE. Textbook of Medical physiology 14th, editor: Elsevier Inc; 2021.
9. Qutob FT, . Medical plants:Planting and Component.1981.
10. Al-Janabi B. Herbs and Seasoning.1988.
11. Chahal K, Kaur M, Bhardwaj U, Singla N, Kaur A, Kaur M, et al. A review on chemistry and biological activities of Laurus nobilis L. essential oil. J pharmacogn. phytochem. 2017;6(4):1153-61.
12. Yahyaa M, Berim A, Isaacson T, Marzouk S, Bar E, Davidovich-Rikanati R, et al. Isolation and Functional Characterization of Carotenoid Cleavage Dioxygenase-1 from Laurus nobilis L. (Bay Laurel) Fruits. J Agric Food Chem. 2015;63(37):8275-82.
13. Elena T, Zhilyakova* OO, Novikov DI, Pisarev AY, Malyutina NN, Boyko. Studying The Polyphenolic Structure Of Laurus Nobilis L. Leaves. Indo Am J Pharm Sci. 2017;04:3066–74.
14. Mansour O, Darwish M, Ismail G, Douba ZA-A, Ismaeel A, Eldair K. Review Study on the Physiological Properties and Chemical Composition of the Laurus nobilis. 2018;5.

15. TADA H, TAKEDA K, Bulletin P. Sesquiterpenes of lauraceae plants. IV. Germacranolides from Laurus nobilis L. Chem Pharm Bull 1976;24(4):667-71.
16. Matsuda H, Kagerura T, Toguchida I, Ueda H, Morikawa T, Yoshikawa M. Inhibitory effects of sesquiterpenes from bay leaf on nitric oxide production in lipopolysaccharide-activated macrophages: Structure requirement and role of heat shock protein induction. Life Sci. 2000;66(22):2151-7.
17. Santos FA, Rao VSN. Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phytother Res. 2000;14(4):240-4.
18. Silva N, Fernandes Júnior A. Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis.2010;16(3):402-13.
19. Jian Ik, Alzobidy AMH. Study the effect of bay leaf extraction the some biochemical parameters in diabetic male rat Iinduced by alloxan. Bas J Vet Res. 2018;17(1).
20. Mohammed RR, Omer AK, Yener Z, Uyar A, Ahmed AK. Biomedical effects of Laurus nobilis L. leaf extract on vital organs in streptozotocin-induced diabetic rats: Experimental research. Ann Med Surg. 2021;61:188-97.
21. Rishan AM, Aziz RA, Al-Temimi SS. Determine the chemical content of Bay (Laurus nobilis L.) leaves extract and its effectivenss against some bacterial species: Determine the chemical content of Bay (Laurus nobilis L.) leaves extract and its effectivenss against some bacterial species. Iraq J Mark Res Consum Protect. 2016;8(1):281-301.
22. Fagundes SBR. Ácido Valpróico:: revisão. Revista Neurociências. 2008;16(2):130-6.
23. Gayam V, Mandal AK, Khalid M, Shrestha B, Garlapati P, Khalid M. Valproic acid induced acute liver injury resulting in hepatic encephalopathy- a case report and literature review. J Community Hosp Intern Med Perspect. 2018;8(5):311-4.
24. Ibrahim. Evaluation of Hepatotoxicity of Valproic acid in albino mice , Histological and Histoistochemical studies. Life Sci J.2012;9(4) :153-159
25. Ghodke-Puranik Y, Thorn CF, Lamba JK, Leeder JS, Song W, Birnbaum AK, et al. Valproic acid pathway: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics. 2013;23(4):236-41.
26. Chang TK, Abbott FS. Oxidative stress as a mechanism of valproic acid-associated hepatotoxicity. Drug Metab Rev. 2006;38(4):627-39.
27. A M, Avanapu SR. Comparative Hepatoprotective activity of Selected Indian Medicinal Plants. Glob J Med Res. 2013;13:15-21.
28. Alchalabi S, Majeed D, Jasim A, Al-Azzawi K. Effect of ethanolic extract of Bay leaves on blood sugar level. Ann Trop Med Public Health. 2020;23.
29. Dias MI, Barros L, Duenas M, Alves RC, Oliveira MB, Santos-Buelga C, et al. Nutritional and antioxidant contributions of Laurus nobilis L. leaves: would be more suitable a wild or a cultivated sample? Food Chem. 2014;156:339-46.
30. Ham A-R, Shin J-H, Oh K-B, Lee S-J, Nam K-W, Koo U, et al. . Neuroprotective Effect of the n-Hexane Extracts of Laurus nobilis L. in Models of Parkinson's Disease. Biomol Ther. 2011;19: 118–25.
31. Vardapetyan H, Tiratsuyan S, Hovhannisyan A, Rukhkyan M, Hovhannisyan D. Phytochemical composition and biological activityof Laurus nobilis L. leaves collected from two regions of South Caucasus. J Exp Biol Agric Sci. 2013;1(45-51).
32. G G, Tiratsuyan S, Kazarian S, H V. Effect of Laurus nobilis extract on the functioning of liver against CCL4 induced toxicity. J Exp Biol Agric Sci. 2015;3:174-83.
33. Kaurinovic B, Popovic M, Vlaisavljevic S. In vitro and in vivo effects of Laurus nobilis L. leaf extracts. Molecules. 2010;15(5):3378-90.
34. Casamassima D, Chiosi F, Vizzarri F, Palazzo M, Costagliola C. The effect of Laurus nobilis on the blood and lenses antioxidant activity in rabbit under fat-enriched diet. Physiol Res. 2017;66(2):325-33.
35. Patrakar R, Mansuriya M, Patil PP, editors. Phytochemical and Pharmacological Review on Laurus Nobilis.2012.
36. Ravindran CA, Murugaiyah V, Kok-Khiang P, Xavior R. Hepatoprotective activity of leaf of methanol extract of Laurus nobilis.L against paracetamol induced hepatotoxicity in rats. Asian J Pharm Clin Res. 2013;6:153-7.
37. Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, et al. ROS signaling: the new wave? Trends Plant Sci. 2011;16(6):300-9.
38. Khan S, Shakoor K, Jan M, Khattak A, Shah S. Study of Histopathologic changes in the Liver of Albino Rats, Induced by Toxic doses of Valproic Acid. Gomal J Med Sci. 2005;3:15-8.