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Study the Effect of Different Laurel Plant (Laurus Nobilis) Extracts at Different Periods on Some Biochemical Markers in Experimental Rats Induced by Diabetes

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Abstract

This study included isolating and extracting the active compounds from the leaves of the bay leaves (Laurus Nobilis) plant and studying their effect on alloxan-induced diabetic rats.

Oils, flavonoids, and tannins were extracted in the extraction device (Saxolites) in successive stages using several solvents. The study used (72) white adult male rats, whose ages ranged between (12-14 weeks) and their weights (135.5±7.9 gm) were randomly divided into (6) rats per group, to perform three experiments.

The experiments showed the effect of the oily, flavonoid, and tannin extracts of the Laurus Nobilis plant at a dose (250 mg/kg of B.W.) on some biochemical changes associated with diabetes such as glucose, total cholesterol (TC), triglycerides(TG) and high-density lipoprotein cholesterol (HDL-C), as well as their effect on the activity of liver enzymes (alanine aminotransferase ALT, aspartate aminotransferase AST, and alkaline phosphatase ALP) in the serum of healthy, adult and newly introduced diabetic rats treated with the three extracts orally for 15 and 30 days, possess the natural products extracted from the plant and represented by oils, flavonoids and tannins have anti-alloxan activity and an important role in the treatment of diabetes mellitus. This current study aimed to evaluate the beneficial effect of compounds extracted from Laurus Nobilis, on some biochemical parameters related to diabetes mellitus such as blood glucose, lipid profile, liver function, and the effect on body weight in alloxan-induced diabetic rats.

Keywords: Alloxan, Diabetic, Laurus Nobilis, Liver enzymes, Triglyceride.

Introduction

Medicinal plants are materials or preparations obtained from various groups of plants or from the treated parts of the plant (World Health Organization, 2019), which contain substances with

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therapeutic properties and other benefits for human health (Aina *et al.*, 2020). These plants are characterized by the fact that they contain in one or more of their different parts one or more chemicals with certain concentrations, and they have the physiological ability to treat a specific disease or at least reduce symptoms of Infection with this disease (Waheed and Abboud, 2017). Various medicinal activities of herbal plants occur as a result of the presence of secondary metabolic compounds, including phenols, alkaloids, saponins, steroids, terpenoids and tannins. These compounds are widely distributed in plants and contain many properties such as antioxidant, antibacterial, and anti-inflammatory.

Laurel plant is one of the traditional herbs that is used to treat many diseases such as cardiovascular diseases and diabetes (Hasan *et al.*, 2020) and can heal wounds, protect nerves, antioxidant, anti-ulcer and anti-fungal (Al-Samarrai, 2018), and many health benefits have been scientifically achieved, such as its role in reducing blood glucose and low-density lipoprotein cholesterol (LDL-C), and increasing levels of high-density lipoprotein cholesterol (HDL-C) in diabetic patients. It has an activity that enhances insulin secretion and helps in the cure of diabetes. Laurel plant can enhance glucose metabolism in diabetic patients where its activity relates to the presence of active compounds such as flavonoids, alkaloids , and tannin.

Diabetes mellitus is a chronic disease related to defects in glucose and lipid metabolism (Al-Chalabi *et al.*, 2020), and it is one of the metabolic diseases characterized by high blood sugar levels (Widyawati *et al.*, 2019). The disease occurs when the body is unable to produce enough insulin hormone or is unable to use insulin effectively (Proboningsih *et al.*, 2020), as it causes an imbalance in insulin secretion, insulin action, or both. (Al- Chalabi *et al.*, 2020).

The first step to prevent complications of diabetes is to control blood sugar levels, as the treatment of diabetes consists of oral antidiabetics such as metformin insulin injection (Al-Chalabi *et al.*, 2020). Anti-diabetic drugs have been used to treat the disease and treatment has become more difficult, because traditional oral anti-diabetic drugs cause undesirable effects such as hypoglycemia (Ghadge and Kuvalekar, 2017). Therefore, it has become important to search for new and effective natural drugs against diabetes, especially in developing countries. There are many plant spices such as fenugreek, olive leaves, cloves, turmeric and bay leaves that have activity that enhances insulin secretion and helps in healing from diabetes (Al-Chalabi *et al.*, 2020).

Materials and Methods:

Preparation of laurel plant:

Leaves of plants were purchased from local markets in Mosul, the leaves were cleaned and dried at room temperature and grounded for (2 minutes) with the aid of using electrical grinder.

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Extraction of natural products from the laurel plant:

Oils were extracted from dry laurel plant with a weight of (60 g) in the Soxhlet apparatus thimble, for 3 days. After soaking the plant in the solvent (petroleum ether) for 3 days, then the process of evaporating the solvent was carried out using a rotary evaporator (Al-Samarrai, 2018). Followed by the extraction of flavonoids from the bagasse resulting from the extraction of oils using an ethanol solvent (Al-Chalabi *et al.,* 2020), and then the process of extracting the tannins from the bagasse resulting from the extraction of flavonoids using distilled water as a solvent (Tzanova *et al.*,2020).

Induction of Diabetes mellitus:

Diabetes mellitus (DM) was induced in overnight fasting rats by injecting a single dose of alloxan (alloxan monohydrate) (100 mg / Kg B.W), which was prepared by dissolving (1 mg) of alloxan in (1 ml) of normal saline solution. The animals were given (5%) of glucose solution for (24 hours) with drinking water to prevent drug-induced hypoglycaemic mortality (Male *et al*., 2018).

Experimental design:

After adopting the animals to the appropriate conditions, the animals were divided into (12) groups, with four groups for each experiment, as follows:

Group one: represented as the control group and rats have been given distilled water and a standard diet.

Group two: represented as diabetes group and rats were injected by Alloxan (100 mg / kg body weight).

Group three: rats have been given crude bay leave extract (oil, flavonoids and tannin) at dose 250 mg/kg body weight.

Group four: diabetic rats have been treated by crude bay leave extract at dose 250 mg / kg body weight (Male *et al*., 2018).

Collection of blood and biochemical analysis:

Blood samples were collected from animals in the following stages: before starting the experiment, 15 days after the experiment and at the end of the experiment by anesthetizing the animals for a few seconds and then with drawing blood from the eye socket pocket using special capillary tubes and leaving it for half an hour at room temperature and separating the serum using

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a centrifuge. The separated serum was placed in special tubes to be frozen and kept at a temperature of (- 20 °C) (Atta *et al*.,1983).

Biochemical analyses were carried out by measuring the concentration of blood glucose, TC, TG, HDL-C, LDL-C, VLDL-C as well as estimating the activity of ALT, AST, ALP using ready-made analysis kits from the company Biolabo French.

Statistical analysis:

The results were analyzed by adopting the statistical program SPSS 14 to find standard statistical methods to determine the mean and the standard deviation (SD), using one-way analysis of variance (ANOVA) and Dunkel's choice was also adopted to compare the results between more than one group (Steel and Torrie, 1980).

Results and discussion:

The results in Figure (1) showed that the treatment of rats with oily, flavonoid, and tannin extract for 15 days resulted in a significant decrease in glucose concentration in both groups treated with the plant extract (the group of healthy animals treated with the plant extract and the group treated with the plant extract after suffering from diabetes) with no occurrence of any significant differences in the control group and the alloxan group. It was found that the tannin extract reduces the concentration of glucose in the serum of rats when compared with the effect of the oily and flavonoid extract.

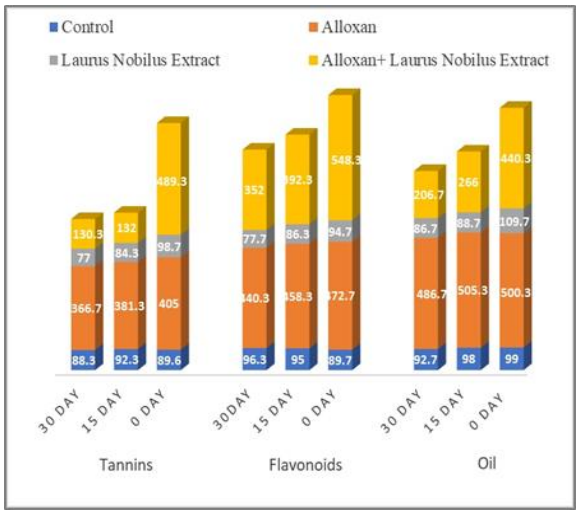

Fig. (1): The effect of the three extracts

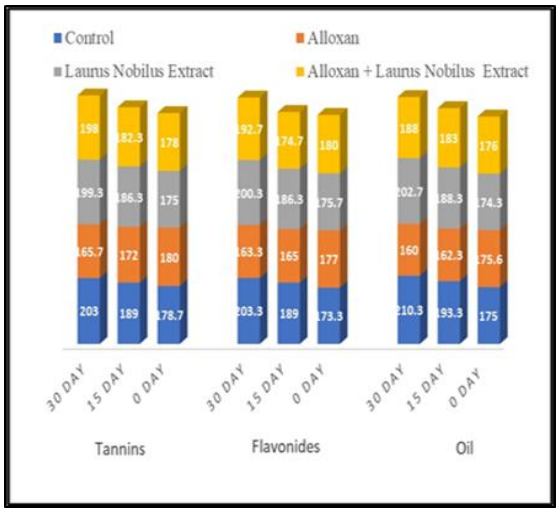

Fig. (2): The effect of the three extracts

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on glucose concentration on body weigh

These results are consistent with many researchers, where they showed that the reason for the effectiveness of the oil extract and its ability to reduce the concentration of glucose is attributed to the ability of the essential oils of the leaves of the laurel plant, represented by the presence of compounds such as (1,8-cineole, 1-(s)-α-pinene ,and limonene) present in the composition of the oil extract, which inhibit the activity of glucosidase and thus reduce glucose absorption (Heghes *et al.*, 2020). In the same way, other studies showed that treating rats with the flavonoid extract of the laurel plant leads to a significant decrease in glucose concentration, indicating that the reason is due to the presence of various phenolic compounds such as flavonoids in the extract formula, which can prevent the depletion of endogenous antioxidants through removing free radicals that tend to increase insulin signaling and the amount of proteins involved in glucose transport (Al-Chalabi *et al.*, 2020). The significant decrease in the concentration of glucose in the blood serum of rats treated with the tannin extract of the leaves of the plant is in agreement with the results of a study in which it was observed that the leaves of the plant reduce the level of sugar in the blood of diabetic patients as a result of what they contain of many compounds, including the tannins (Khan *et al.*, 2009).Tannin has anti-diabetic potential firstly due to its ability to lower glucose levels by delaying intestinal glucose absorption and insulin-like effect on insulin-sensitive tissues and secondly by delaying the onset of insulin-dependent diabetes by regulating the antioxidant environment of beta cells in the pancreas (Serrano *et al.*, 2009).

The results also showed in figure (2) that the treatment of diabetic group rats with oily, flavonoids and tannin extract for 30 days resulted in a significant increase in their body weights, as well as when the healthy non-diabetic group was dosed with oil, flavonoids and tannin extract after 15 days of treatment and continued moral elevation until the end of the experiment. The tannin extract increases the body weight of rats by a large percentage when compared with the effect of the oily and flavonoid extract.

The results in this study indicate a significant increase in the body weight of the rats treated with the oily, flavonoids and tannin extract, which may be because the oil extract enhances insulin secretion from the pancreas and thus prevents the processes of protein catabolism to its constituent fatty acids. The results of a previous study indicated that the reason for the increase as a result of treatment with the flavonoid extract may be attributed to the ability of the extract to prevent the catabolism of proteins, enhance insulin secretion in diabetic animals, and reduce the activity of the lipase enzyme that leads to lowering the concentration of cholesterol in the blood (Al-Chalabi

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et al.,2020) for laurel leaves. In addition to the ability of the tannin extract to enhance protein s

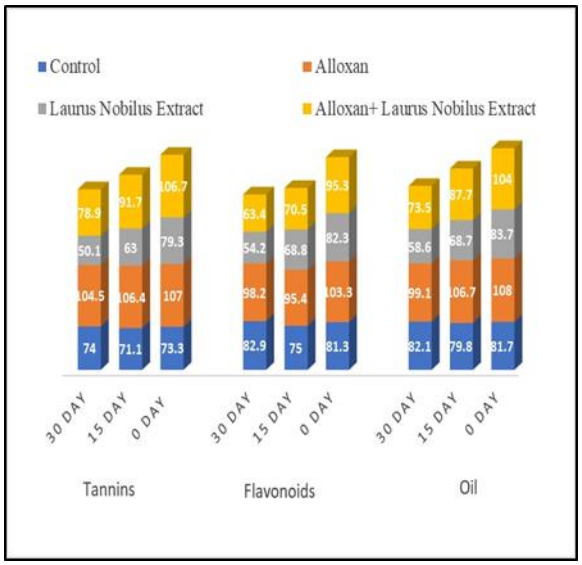

Fig. (3): The effect of the three extracts on TC concentration

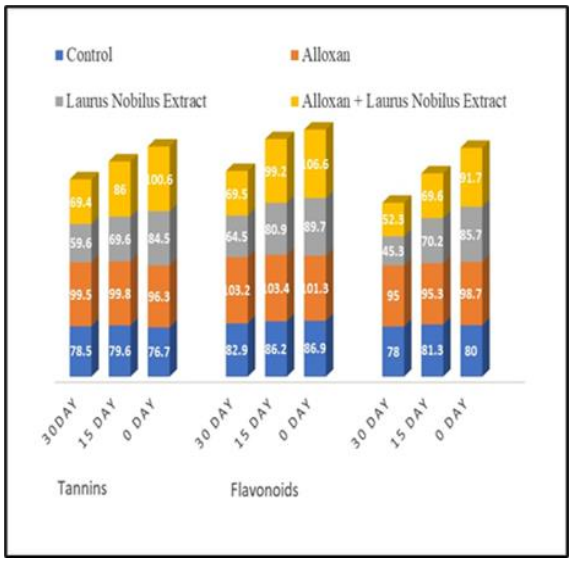

Fig. (4): The effect of the three extracts on TG concentration

The results in figure (3) show a significant decrease in cholesterol concentration after 15 days of treating the diabetic group with oily, flavonoid and tannin extract, and the decrease continued until the end of the experiment. The results showed a significant decrease in cholesterol concentration as a result of treating healthy animals with the oily and tannin extract for a period of 15 days, noting that there was no significant change in the concentration as a result of treatment with the two extracts within the time period (15-30) days. Also, we showed a significant decrease in the concentration of cholesterol in the blood of healthy animals as a result of treatment with the flavonoid extract for a period of 15 days, and the decrease continued until the end of the experiment. It has been shown that the tannin extract significantly reduces the concentration of cholesterol in the serum of rats when compared with the effect of the oily and flavonoid extract. The presence of a significant decrease in cholesterol concentration in serum of animals treated with flavonoid extract is consistent with the results of other study which, where they indicated that the reason for the decrease in cholesterol concentration may be attributed to the ability of the extract to improve insulin levels in diabetic animals and reduce the activity of the lipase enzyme that leads to a decrease in the concentration of cholesterol in the blood (Ganjali *et al.*, 2017).

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This has been shown by (Asmawti *et al.*, 2021) which indicated that tannin inhibits or reduces the absorption of cholesterol in the intestine through the formation of complexes with cholesterol and the deposition of cholesterol in the lumen of the intestine and its excretion with stool.

A significant decrease in the concentration of triglycerides in blood serum of diabetic male rats was found as a result of treatment with the oily and tannin extract for 15 days and the decrease continued until the end of the experiment, as well as a significant decrease in the concentration of triglycerides in blood of male diabetic rats. Result of treatment with flavonoid extract for 30 days as shown in figure (4). The results also showed a significant decrease in the concentration of triglycerides in serum of healthy group as a result of treatment with the oily and flavonoid extract for 15 days, and the decrease continued until the end of the experiment 15 days, noting that there was no significant change in concentration as a result of treatment with the extract within the time period (15-30) days.

These results are consistent with the results of other studies, where they observed that administration of lemon oil for eight weeks leads to a decrease in the concentration of total cholesterol, triglycerides and low-density lipoprotein (Lee *et al.*, 2018). The significant decrease in the concentration of triglycerides in the blood serum of animals treated with flavonoid extract may be attributed to the presence of Querecetin in the flavonoid extract, in addition to the ability of the extract to inhibit the secretion of triglycerides from the liver into the bloodstream (Rafienian-Kopaei *et al.*, 2013).

The results showed a significant increase in the level of high-density lipoprotein-cholesterol in the blood of diabetic male rats treated with oily, flavonoid and tannin extract for 15 days, and the increase continued until the end of the experiment, as well as in the group of healthy animals treated with flavonoid extract as a result of treatment for a period of 15 days with no significant change in the level of beneficial cholesterol in the serum of the healthy group of rats that were treated with oily and tannin extract as noted in figure (5).

The results in this study indicate that the tannin extract increases the concentration of HDL-C in the blood serum of rats by a large percentage compared to the effect of the oily and flavonoid extract. These results are similar to the results of research that showed that eating bay leaf with food led to a significant decrease in the concentrations of cholesterol, triglycerides, glucose and a decrease in the activity of liver enzymes (Casamassima *et al.*, 2017). These results also agree with the results of a study that showed that the reason for the increase in the concentration of lipoprotein may be due to the presence of active substances in the leaves of the bay plant, which have a role in inhibiting the activity of free radicals because of their antioxidant effect inside the body, as it is considered one of the most important types of antioxidants as natural oxidation

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(Arora *et al.*, 2000). The results of this study showed a significant increase in the concentration of HDL-C as a result of treatment with the tannin extract of the leaves of the laurel plant. Similarly, it was noted (Asmawti *et al.*,2021) that a significant increase in the concentration of HDL-C occurred in the blood of rats. The result of treatment with tannin extract, indicating that the reason for the increase is due to the action of the tannin extract polymers to raise the concentration of HDL-C and reduce the concentration of VLDL-C.

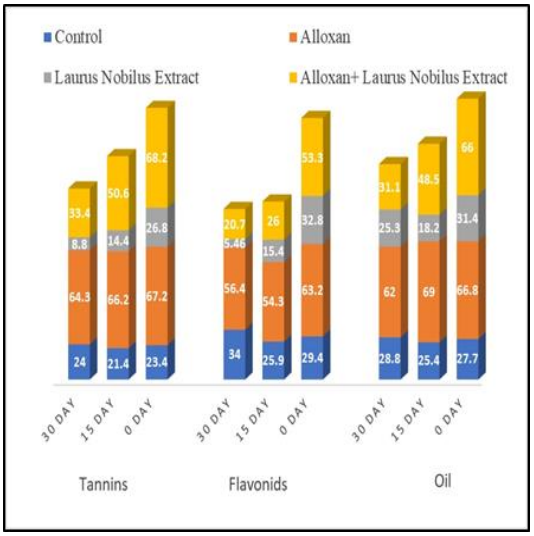

Fig. (5): The effect of the three extracts on HDL-C concentration

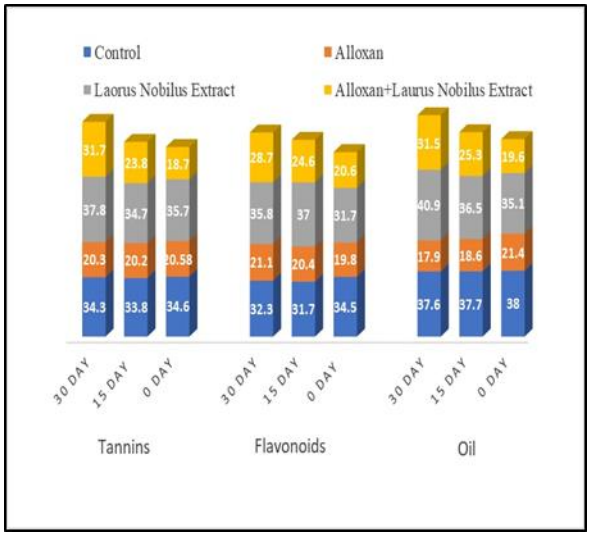

Fig. (6): The effect of the three extracts on LDL-C concentration

The results in figure (6) showed a significant decrease in the concentration of LDL-C in serum of healthy rats treated with oily and tannin extract for 15 days, with no significant change at the end of the experiment. As well as a significant decrease in the level of LDL-C in the serum of diabetic rats as a result of treatment with oily and tannin extract for 15 and 30 days, with no significant change in the serum of other groups. These results are consistent with a laboratory study on healthy volunteers. They took bay leaf powder in the form of capsules and found that it contributes to lowering the concentration of low-density lipoprotein cholesterol and raising the concentration of high-density lipoprotein (Chbili *et al.*, 2020).

When comparing the results in this study, it was found that the tannin extract reduced the LDL-C concentration in the blood serum of rats compared to the effect of the oily extract and the flavonoid extract.

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The results in this study indicate that a significant decrease occurred in the concentration of LDL-C as a result of treatment with tannin extract, which is in agreement with what was stated (Ravichandiran *et al.*, 2012), where it was shown that treating rats with tannin extract causes a decrease. Significantly in the concentration of total cholesterol and the concentration of cholesterol in low-density lipoprotein because of their effect on the catabolism of fatty acids in the kidney by controlling the breakdown of lipoprotein absorption and its metabolism.

We also observed a significant decrease in the concentration of LDL-C in the blood serum of diabetic rats as a result of treatment with flavonoid extract for 15 days. Also, a significant decrease in LDL-C concentration occurred when healthy rats were treated with flavonoid extract for 15 and 30 days. These results are consistent with what was stated (Titilayo *et al.*, 2018). Where they showed that the reason for the decrease in the concentration of cholesterol, triglycerides and low-density lipoprotein cholesterol may be attributed to the role of the plant leaves in reducing liver enzymes that form fatty acids, or it may be due to the inhibition of the enzyme acetyl CoA synthetase, which is the basis in fatty acid synthesis.

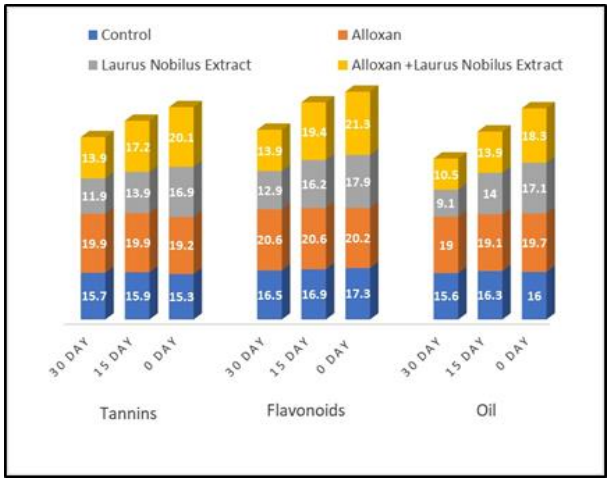

Fig. (7): The effect of the three extracts on VLDL-C concentration

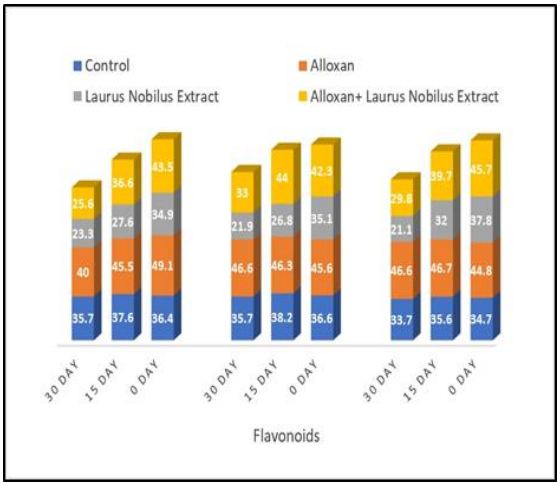

Fig. (8): The effect of the three extracts on ALT activity

The results in figure (7) indicate a significant decrease in the concentration of VLDL-C in the serum of healthy and diabetic rats as a result of treatment with the oily and flavonoid extract for 15 and 30 days, with no significant change in the concentration of VLDL-C in serum of the diabetic group. And treatment with the oil extract. It was noted that the treatment of diabetic animals of the alloxan group with the flavonoid extract for a period of 30 days led to a significant

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decrease in the level of VLDL-C. We also note that the treatment of diabetic animals of the alloxan group with tannin extract for a period of two and four weeks caused a significant decrease in the level of VLDL-C. Also, the treatment of healthy animals with tannin extract for two weeks led to a significant decrease in the concentration of VLDL-C.

When comparing the results in this study, it was found that the flavonoid extract significantly reduced the concentration of VLDL-C in the serum of rats, compared to the effect of the oily extract and the tannin extract.

It may be attributed to the role played by the extract as an antioxidant and its contribution to raising the concentration of cholesterol. The results were close to what was found (Rafienian-Kapaei *et al.,* 2013), which may be due to the role of flavonoids and their derivatives present in the laurel plant, which work to regulate and manage the percentage of fats present in the blood.

The results in figure (8) show that the treatment of diabetic group animals with oily extract for 30 days led to a significant decrease in the activity of ALT enzyme. While we note that treating animals with diabetes mellitus with flavonoid and tannin extract for a period of 15 days led to a significant decrease in the activity of the enzyme and the continuation of the decrease until the end of the experiment. In the same way, treatment of healthy animals with oily, flavonoid and tannin extract for a period of 15 days led to a significant decrease in the enzyme activity from the treatment, and the decrease continued until the end of the experiment and when comparing the results in this study, it was found that the flavonoid extract reduces the concentration of ALT in the serum of rats compared to the effect of the oily extract and the tannin extract.

The results show that the treatment of diabetic group animals with the oil extract for a period of 30 days showed a significant and clear decrease in the activity of AST enzyme as shown in figure (9). We also note that the treatment of diabetic animals of the alloxan group with flavonoid and tannin extract for 15 and 30 days led to a significant decrease in enzyme activity. The results also showed that the treatment of healthy animals with oily and tannin extract for 15 days led to a significant decrease in enzyme activity, and the decline continued until the end of the experiment.

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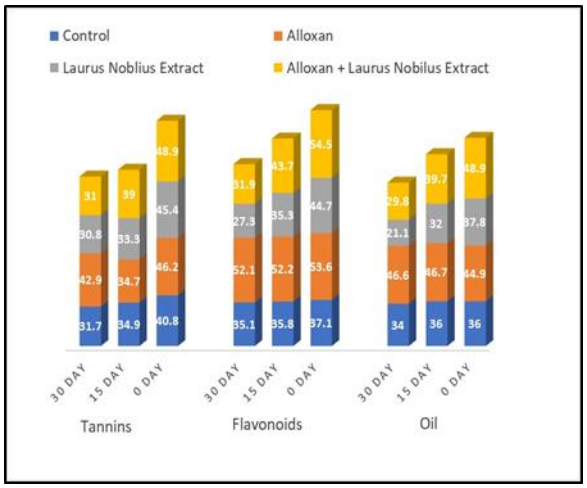

Fig. (9): The effect of the three extracts on the AST activity

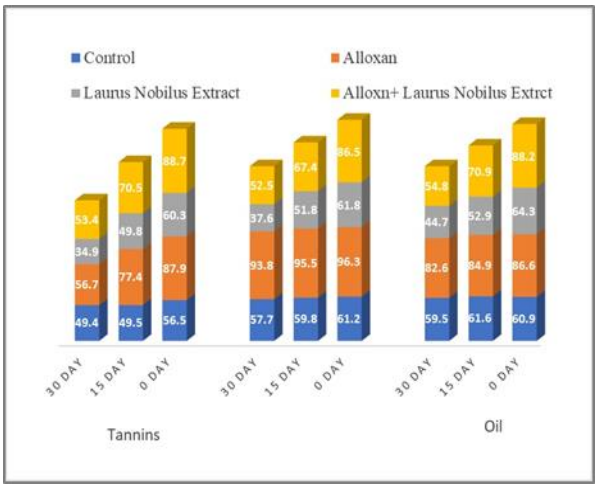

Fig. (10): The effect of the three extracts on the ALP activity

Also, the treatment of healthy animals with flavonoid extract for 30 days led to a significant decrease in the activity of AST enzyme. When comparing the results in this study, it was found that the tannin extract reduced the concentration of AST in the serum of rats compared to the effect of the oily and flavonoid extract.

The results in Figure (10) show that the treatment of diabetic group animals for 15 days with the oily and tannin extracts showed a significant decrease in the activity of ALP enzyme and the decline continued until the end of the experiment. The treatment of healthy animals for 15 days with oily extract, flavonoid and tannin extract showed a significant decrease in enzyme activity and the continuation of the decrease until the end of the experiment. It was found that the tannin extract reduced the concentration of ALP in the blood serum of rats compared to the effect of the oil and flavonoid extract.

The treatment of diabetic animals with the oil extract led to a clear decrease in the activity of the enzyme, as the biologically active components in the plant extract affected regulating and restoring the activity of the three enzymes (Mohammed *et al.*,2021). The reason for the significant decrease in the activity of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase enzymes in the blood serum of rats treated with flavonoid extract may be attributed to the presence of antioxidant compounds in the extract that prevent the oxidation of fats associated with diabetes and the consequent necrosis and fibrosis liver cells and thus the regulation of liver functions, as these compounds prevent programmed cell death as well as

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prevent liver damage by raising and improving the activity of antioxidant enzymes, which leads to a decrease in the secretion of enzymes represented by aminotransferase alanine, aspartate aminotransferase and alkaline phosphatase enzymes (Alam *et al.*, 2014) Likewise, also, others noted that administration of laurel leaf extract regulated the activity of liver enzymes (Gasparyan *et al.*, 2015).

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