

Effects of Crude Aqueous Extract of the Palmito on some Biochemical Parameters in Serum of Healthy Rats

Saba Z. AL-ABACHI
University of Mosul

Sameer M. AL-GORANY
Middle Technical University

Abstract: This study was designed to evaluate the protective effects of the cold crude aqueous extract of palmito and in different doses (100, 250, 500 mg / kg BW) on some biochemical parameters, including: hepatic enzymes, (alkaline phosphatase ALP, alanine aminotransferase ALT and aspartate aminotransferase AST), total protein, albumin, globulin, bilirubin (total, direct and indirect) and total lipid in the blood serum of healthy adult rats and compared with control group treated with distilled water orally, daily for two weeks. A (24) white adult male rats were used aging between (12-14 weeks) and weighting (215.3 ± 9.7 gm). They were divided randomly, each of which contain (6) animals. The results showed that the palmito aqueous extract led to the high significant of the liver enzymes activity, and in the concentration of total protein, albumin, globulin and in the concentration of total lipid, while led to a reducing in the concentration of total, direct and indirect bilirubin in the blood serum of healthy adult rats when compared with control group.

Keywords: Bilirubin, Hepatic enzyme, Liver, Palmito, Total protein

Introduction

Medicinal plants, also called medicinal herbs, are plants or parts of a plant, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesis hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Herbal medicine products are dietary supplements that people take to improve their health, but some of these products can cause health problems, while others do not have any effectiveness. And has taken a great place, especially in recent years, because people believe that herbal medicines have no side effects, as well as being cheap and available locally (Belay, et al., 2016 ; Thiagarajan and Venkatachalam, 2012).

Palmito: heart of palm is a vegetable harvested from the inner core and growing bud of certain palm trees . Harvesting of many uncultivated or wild single-stemmed palms results in palm tree death. When harvesting the cultivated young palm, the tree is cut down and the bark is removed, leaving layers of white fibers around the center core. During processing, the fibers are removed, leaving the center core or heart of palm (Masoomah et al., 2013).

The palmito are commonly medical used in supplements to improve prostate health, anti-cancer, balance hormone levels, and prevent hair loss in men (Masoomah et al., 2013). It's also associated with other benefits, including decreased inflammation and improved urinary function. Also, it is protect the stomach, kidney and liver (Baliga et al., 2011).

The aim of this research is to study the effect of the cold crude aqueous extract of palmito in different doses on some biochemical parameters, as (ALP, ALT and AST), total protein, albumin, globulin, bilirubin (total, direct and indirect) and total lipid in the blood serum of healthy adult rats.

- This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial 4.0 Unported License, permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

Materials and Methods

Preparation of cold crud aqueous palmito extract

The fresh date palm (type Al-Kastawi) was cut off which were collected from Baaquba city/ Dyala/ Iraq. The plant was positively identified by Biologist literature in Biological department /College of Science /University of Mosul / Iraq. the leaves and fibers were peeled from the base of the seed. After the cleaning and washing with tap water, the plant was cut into small parts. About (300 g) of the palmito was crush and mix with cold distilled water (1/3 w/v) with continuous stirring for one hour (Robyt and White, 1987). Then the mixture was subjected to the ultrasonic device and filtrated through glass woods, and separate the extract with a cooled centrifuge for 20 minutes at a speed of 33520 xg to obtain a clear extract, the volume of filtrate was measured, and then estimated the amount of protein by modified Lawry (Schacterle and Pollack, 1973). The volume of crude extract were concentrated by the lypholyzer device.

Experimental animals

In the study, (24) healthy male adult albino rats ageing between (12 and 14 weeks) and the main of weighting between (215.3 ± 7.9 g) were obtained from the animal house in the medicine faculty / Mosul University / Iraq. Animals were randomly distributed and placed in special plastic cages (10 x 20 x 40 cm) covered with stainless steel wire, (6) animals were placed in each cage. Rats were housed under standard laboratory condition, light and dark cycles of 12h, in a polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature (25±2°C), humidity (50±5%).

Experimental design

The experiment showed the effect of different concentrations of the crude aqueous extract of the plant on some of the biochemical variables in the healthy adult rats. In this experiment, 24 samples were distributed into four groups and in equal numbers, as follows:

Group I: Received (0.5ml/kg) body weight of distilled water orally for 2 weeks and used as the normal control. The animals in all groups were killed by anesthetic ether on the day 15.

Group II: Received (0.5 ml/kg) body weight of cold crude aqueous extract of the palmito plant with a concentration of (100 mg / kg) orally by gavages tube daily for two weeks.

Group III: Received (0.5 ml/kg) body weight of cold crude aqueous extract of the palmito plant with a concentration of (250 mg / kg) orally by gavages tube daily for two weeks.

Group IIII: Received (0.5 ml/kg) body weight of cold crude aqueous extract of the palmito plant with a concentration of (500 mg / kg) orally by gavages tube daily for two weeks.

Blood collection

At the end of the experiment, anesthetize the animals for a few seconds and then draw the blood from all groups weekly from the Orbital sinus puncture using special capillary tubes, left stand in serum tubes for (30 minutes) to be coagulated. Serum samples were collected by centrifugation at (3000 rpm) for (20 minutes) at room temperature. The clear, non-haemolysed sera was separated and stored at (- 20 C°) for measurements of biochemical analyses of liver functions (Atta et al., 1983).

Estimation of serum biochemical parameters

-The ALT and AST activities were measured spectrophotometrically by using the method of Reitman and Frankle (Anderson and Cockayne, 1993).

-ALP was measured by the method devised by Bowers and McComb, which allows calculation of ALP activity basing on the molar absorptive of P-nitrophenol (Tietz, 1999).

- Total serum protein was measured by Biuret method using kit from BIOLABO (Bishop et al., 2005) and serum albumin was determined quantitatively by colorimetric method used bromocresol green (Doumas et al., 1971).
- The total proteins minus the albumin gives the globulin.
- The total and direct bilirubin concentrations were calculated by the colorimetric method used sulfanilic acid method (Walters and Gerarde,1970).
- The indirect bilirubin concentrations was estimated by the equation:

$$\text{Bilirubin indirect Conc. } \frac{\mu\text{mol/L}}{\mu\text{mol/L}} = \text{Total bilirubin Conc. } \frac{\mu\text{mol/L}}{\mu\text{mol/L}} - \text{Bilirubin direct Conc. } \frac{\mu\text{mol/L}}{\mu\text{mol/L}}$$

- The total lipid determined by heated the serum with concentrated sulphuric acid, then react the mixture with phosphovaniline reagent (Toro and Ackermann, 1975).

Statistical Analysis

The data presented in this investigation were expressed as the mean \pm Standard Deviation (mean \pm SD) of the mean. The significant difference among the means has been statistically analyzed by one-way analysis of variance Duncan, ($p < 0.05$) was considered as statistical significance using SPSS 19 Software (Snedecor and Cochran, 1986).

Results and Discussion

Amount of total protein and percentage in cold crud aqueous palmito extract

The amount of total protein in cold crud aqueous palmito extract was found and it was equal (8433.75 mg), then we found the percentage of the plant which estimate (2.318%), as shown in table (1).

Table 1. Amount of total protein and percentage in cold crud aqueous palmito extract

Extract	Protein concentration (mg/cm ³)	Total volume of extract (cm ³)	Total protein in extract (mg)	Protein percentage in plant (%)	Plant weigh (gm)	Practical weight of protein (mg)
Cold crud aqueous palmito extract	8.65	975	8433.75	2.318	300	6955

Effect of different doses (100, 250, 500 mg / kg) of crude aqueous extract of the palmito on some biochemical parameters in serum of healthy adult male rats:

Enzymes activity (ALP, ALT, AST):

The activity of alkaline phosphatase was evaluated in serum of healthy adult male rats and treated with cold crude aqueous extract of the plant. The treatment of (500 mg / kg) in the first week showed a significant increase ($p \leq 0.05$) compared with control group, while the two doses (100, 250 mg / kg) did not show significant differences when compared with the control group. When compared between the doses in the first week, the dose (250 mg / kg) was significantly reduced when compared to the two doses (100, 500 mg / kg) as shown in table (2). In the second week of the treatment, there were no significant differences in the treatment group with the cold crude aqueous extract of the palmito plant in the two doses (100, 250 mg / kg) compared with the control group. While the dose (500 mg / kg) showed a significant increase when compared with the control group. Statistical analysis showed that the two doses (100, 250 mg / kg) showed no significant differences between them, while the two groups showed a significant decrease in the activity of ALP enzyme when compared with the aqueous extract group of (500 mg / kg).

The dose (500 mg / kg) of the cold crude aqueous extract of the palmito plant by oral resulted in a significant increase of the activity of ALP in the serum of adult male rats compared with control group during the first and two weeks as shown in table(2). The reason for the high activity of ALP enzyme is due to the presence of the

enzyme in the liver cells and when affected these cells by the overdose of the extract will lead to the collapse of some of these cells and then released the enzyme into the serum causing increased of the activity of this enzyme (Uskokovic et al., 2007).

The treatment with the cold crude aqueous extract of the palmito plant in doses (100, 250, 500 mg / kg) showed no significant differences when compared with control group at the first week, also when comparison of each group with the other in the activity of ALT in the serum of healthy adult male rats as shown in table (2).

In the second week, the treatment with the cold crude aqueous extract of the palmito plant showed an effect on serum ALT activity. Statistical analysis showed that the dose (250 mg / kg) showed a significant decrease when compared with control group, while the groups which are treated at doses (100, 500 mg / kg) showed no significant differences compared with control group. When comparing the three experimental groups, there were no significant differences between them as shown in table (2).

The treatment of the cold crude aqueous extract of the palmito plant at doses (100, 500 mg / kg) for male rats by oral did not show significant differences in ALT activity when compared with control group in the first and second weeks. This indicates that the activity of enzyme was not affected by these doses of extract. For the group of (250 mg / kg) dose, it was significantly decreased and improved well when compared with control group for both weeks.

Statistical analysis in the first week of treatment showed that the three trial groups (100, 250, 500 mg / kg) showed no significant differences compared to control group, when measuring the activity of the AST in the serum of healthy adult rats. When comparing groups between them, there was no significant difference between the two groups (100, 250 mg / kg) and between the two groups (250, 500 mg / kg) while the dose (500 mg / kg) showed a significant increase when compared with the dose (100 mg / kg).

In the second week, two doses of treatment (100, 250 mg / kg) showed a significant decrease when compared with control group, while dose group (500 mg / kg) has no significant differences when compared with control group. In addition, the two groups of doses (100, 250 mg / kg) showed no significant differences between them in the activity of serum AST, while the dose group (500 mg / kg) gave a significant increase compared to the previous two doses as shown in table (2).

The treatment with the cold crude aqueous extract of the three-dose of palmito plant showed no significant differences in AST activity during the first week when compared with control group, while in the second week of the treatment, the two groups of doses (100, 250 mg / kg) were significantly lower than the control group.

These results are identical to those found by (Abuelgassim, 2010), noting that the concentration of some biochemical measured (glucose) in serum of healthy rats after two and four weeks of treatment with date palm leaf extract did not give any significant changes compared with the non-treated control group. The date palm leaf extract was found to reduce the concentration of cholesterol significantly in the serum of healthy rats four weeks after the extract was given, while no significant difference was observed in the concentration of triglyceride at the same time in serum of healthy rats. Researchers have shown that the palm extract contains effective compounds that act as scavenger free radicals. And that the moral effect found after the four weeks indicates that the plant extract contains effective antioxidants that reduce oxidative stress (Saafi et al., 2010).

The cold crude aqueous extract of the palmito plant causes a significant decrease in the activities of AST and ALT. These decrement due to the antioxidant contained in the plant extract (Chaira et al., 2007). Or may be due to the presence of multiple phenolic compounds that play an important role in the synthesis of proteins and improve liver function, or may be due to the fact that the extract may have systematic effects of mitochondrial and cellular membrane permeability, resulting in increased membrane stability (Masoomah et al. , 2013).

Table 2. Effect of cold crude aqueous extract of the palmito plant at doses (100, 250, 500 mg / kg) on the biochemical variables (ALP, ALT, AST) in serum of healthy male adult rats

Groups	ALP U/L		ALT U/L		AST U/L	
	1 st week	2 nd week	1 st week	2 nd week	1 st week	2 nd week
Control	275.60±11.31 ab	287.67±9.01 a	67.17±9.39 a	66.90±4.58 b	232.80±10.82 ab	254.05±15.05 b
100 mg/kg	304.72±13.15 b	287.50±16.55 a	63.90±9.46 a	49.17±6.68 ab	191.67±12.66 a	203.82±8.14 a
250 mg/kg	267.45±10.05 a	318.73±9.48 a	45.57±2.76 a	39.43±3.22 a	228.57±8.07 ab	172.17±6.77 a
500 mg/kg	340.46±12.08 c	358.60±1.94 b	54.43±11.86 a	51.67±2.65 ab	246.07±27.29 b	246.56±11.80 b

Different letters vertically mean a significant at ($p \leq 0.05$).
The values above refer to the mean \pm standard deviation.

Total protein, albumin and globulin concentrations

The analysis showed that the group of dose (500 mg / kg) has a significant increase when compared with control group in the first week, while the two dose groups (100, 250 mg / kg) showed no significant difference. In the second week, shows that the two groups (250, 500 mg / kg) showed a significant increase compared to the control group, while the dose group (100 mg / kg) showed a non -significant difference compared to the control group as shown in table (3). When comparing the groups, the treatment group (100 mg / kg) showed a significant decrease when compared with the two groups of doses (250, 500 mg / kg) and the latter two groups showed no significant differences between them in the total protein concentration in healthy adult male rats.

Table 3. Effect of cold crude aqueous extract of the palmito plant at doses (100, 250, 500 mg / kg) on the biochemical variables (total protein, albumin, globulin) in serum of healthy male adult rats

Groups	Total protein gm/dl		Albumin gm/dl		Globulin gm/dl	
	1 st week	2 nd week	1 st week	2 nd week	1 st week	2 nd week
Control	5.26±0.32 a	5.76±0.09 a	2.84±0.26 a	3.31±0.03 a	2.42±0.12 a	2.45±0.24 a
100 mg/kg	6.03±0.29 ab	6.14±0.19 a	3.43±0.02 b	3.42±0.04 a	2.60±0.31 a	2.72±0.34 a
250 mg/kg	5.57±0.05 ab	6.90±0.26 b	3.52±0.19 b	3.79±0.05 b	2.05±0.33 a	2.11±0.18 a
500 mg/kg	7.01±0.34 b	7.05±0.69 b	3.40±0.04 b	4.13±0.19 c	3.61±0.06 b	3.92±0.38 b

Different letters vertically mean a significant at ($p \leq 0.05$).
The values above refer to the mean \pm standard deviation.

The albumin concentration in the first week for all doses showed significantly increased when compared with control group, while there are no significant differences compared between them. In the second week, the two groups (250, 500 mg / kg) showed a significant increase when compared with control group, while the dose (100

mg / kg) did not appear any significant changes when compared with the control group. When comparing the three dose groups among them, a dose of (500 mg / kg) was found to be significantly higher when compared with the dose (250 mg / kg) and the latter has significantly increased compare with the dose (100 mg / kg) in the concentration of serum albumin.

From table (3) and from the two weeks of the treatment with the cold crude aqueous extract of the palmito plant at dose (500 mg / kg) showed a significant increase when compared with control group in the concentration of globulin. The two dose groups (100, 250 mg / kg) did not show a significant difference when compared with the control group. In addition, the three dose groups did not show significant differences when compared among them at two weeks in the concentration of serum globulin of healthy adult male rats.

The increase in the cold crude aqueous extract of the palmito plant in total protein, albumin and globulin concentrations was due to the susceptibility of the extract to the stimulation the hepatic tissues, which led to increased protein synthesis in the liver and improved functional status of hepatic cells (Hussein et al., 2007).

Total bilirubin, direct, indirect and total lipid concentrations

From table (4) and from the first week in the measurement of total bilirubin concentration in serum adult rats, the dose group (500 mg / kg) showed a significant decrease when compared with control group, while the two doses (100, 250 mg / kg) did not show significant differences compared to the control group, and we note from the same table that the groups of three doses did not show significant differences when comparing among them during the first week of the treatment. In the second week, the treatment with the three-doses with aqueous extract showed better improvement than in the previous first week, with the statistical analysis that the three doses of the extract showed a significant decrease when compared with the control group. When compared with the doses, the dose group (100 mg / kg) showed a significant increase when compared with the two groups of doses (250, 500 mg / kg), while note the results showed that the last two doses did not show any significant differences between them in the total bilirubin concentration. These results were identical to those found by the researcher (Al-Azzawi, 2015) that the extract is the regeneration and repair of liver cells, which leads to the expansion and opening of bile ducts within the liver and the lack of flow of bilirubin into the blood.

The results of the direct bilirubin concentration in serum adult male rats showed that there was a similarity in the results of the statistical analysis between the first and second weeks of this characteristic. Table (4) shows that each dose of the three group doses showed a significant decrease when compared with the control group during the first and second weeks of treatment. When comparing between the three doses, the dose group (500 mg / kg) showed a significant decrease when compared with both doses (100 , 250 mg / kg) for both the first and second weeks of treatment, but there are no significant differences between the two doses in the above when

In table (4) and in the first week of treatment with the cold crude aqueous extract of the palmito plant, it was found that the three dose groups showed a significant decrease in indirect bilirubin concentration when compared with the control group. When comparing dose groups among them we showed that the dose (500 mg / kg) did not show a significant difference when compared with the two doses (100, 250 mg / kg), while the dose group (250 mg / kg) was significantly lower when compared with the dose group (100 mg / kg). In the second week, we note from table (4) that the three dose groups showed a significant decrease when compared with the control group, especially the dose group (500 mg / kg). Compares among the doses, the dose group (500 mg / kg) decreased significantly with the dose group (250 mg / kg) and the latter decreased significantly compared to the dose group (100 mg / kg) in indirect bilirubin concentration in the serum. . The reason for the low concentration of direct bilirubin in both weeks is to increase the effectiveness of the hepatic enzyme glucuronyl transferase, which converts the direct bilirubin into indirect bilirubin. As a result of the increased the activity of this enzyme the reduced of the direct concentration of bilirubin in the serum (Travis and Anupam, 2014).

In the first week of treatment, we observed in table (4) that the two doses (100, 250 mg / kg) showed a significant decrease in total lipid concentration in compares with control group, while the dose (500 mg / kg) did not show a significant difference. The latter dose showed a significant increase when compared with the two groups of doses (100, 250 mg / kg), while the two doses did not show significant differences when comparing them.

In the second week of treatment, we note from the same table that the two dose groups (250, 500 mg / kg) showed a significant decrease when compared with the control group, while the dose group (100 mg / kg) was no significant when compared with the control group. When comparing the three doses, the dose group (500 mg

/ kg) was significantly lower than the dose group (250 mg / kg) and the latter also decreased from the dose group (100 mg / kg) in the total lipid concentration in adult male rats treated with cold crude aqueous extract of the palmito plant. This indicates that increasing the dose of cold crude aqueous extracts of palmito lead to a decline in total lipid decomposition process. These results are consistent with what he found (Abuelgassim, 2010). This reduction was due to the presence of compounds acting as antioxidants in date palm leaf extracts that reduce oxidative stress. The decrease may be explained by the fact that these antibodies are associated with bile acids, which inhibit the hydroxymethyl glutaryl coenzyme A enzyme, which reduces the level of cholesterol (Saafi et al. , 2010).

Table 4. Effect of cold crude aqueous extract of the palmito plant at doses (100, 250, 500 mg / kg) on the biochemical variables (total, direct, indirect bilirubin and total lipid) in serum of healthy male adult rats

Groups	Total bilirubin μmol/L		Direct bilirubin μmol/L		Indirect bilirubin μmol/L		Total lipid mg/dl	
	1 st week	2 nd week	1 st week	2 nd week	1 st week	2 nd week	1 st week	2 nd week
Control	5.19±0.51 b	7.39±0.43 c	2.03±0.36 c	4.38±0.29 c	3.16±0.38 c	4.01±0.47 c	398.5±16.03 b	568.83±35.79 c
100 mg/kg	4.18±0.38 ab	5.34±0.52 b	1.77±0.16 b	2.71±0.21 b	2.41±0.19 b	2.63±0.33 b	264.5±32.08 a	568.80±18.77 c
250 mg/kg	3.17±0.33 ab	4.73±0.27 a	1.67±0.22 b	2.85±0.18 b	1.50±0.27 a	1.88±0.11 ab	308±10.42 a	474.63±8.26 b
500 mg/kg	2.91±0.34 a	2.96±0.36 a	1.20±0.19 a	1.30±0.16 a	1.71±0.39 ab	1.66±0.14 a	418.4±12.15 b	385.85±20.38 a

Different letters vertically mean a significant at ($p \leq 0.05$).
The values above refer to the mean \pm standard deviation.

Conclusions

The treatment of adult male rats with the cold crude aqueous extract of the palmito plant in different doses (100, 250, 500 mg / kg) of body weight showed significant increases in the activities of hepatic enzymes, especially in the second week of the treatment from the concentration of total protein, albumin and globulin and to a significant decrease in total, direct and indirect bilirubin concentrations and in total lipid.

References

- Abuelgassim, O. Abuelgassim, (2010). Effect of flax seeds and date palm leaves extracts on serum concentrations of glucose and lipids in alloxan diabetic rats. *Pakistan Journal of Biological Sciences* 13(23): 1141-1145.
- Al-Azzawi, H. H., (2015). Effect of smoking cigarettes on the activity of enzymes AST, ALT, ALP and the levels of electrolytes in the plasma of smokers. Master Thesis, College of Education for Pure Sciences, Diyala University, Iraq.
- Anderson, S., Cockayne, S., (1993). *Clinical chemistry: Concepts and Applications*. Philadelphia; W.B. Saunders Company. pp.248-264.
- Atta, A.H.; Shalaby, M.A.; Shokry, I.M. and Ahmed, A.A., (1983). Interaction between oral hypoglycemic and antibiotics on blood glucose level of normal fasted and alloxan-diabetic rats. *Vet. Med. J.*, 31(1): 11-18.
- Baliga, M.S.; Baliga, B.R.; Kandathil, S.M.; Bhat, H.P. and Vayalil, P.K., (2011). A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera L.*) *J. food Res Int*; 44: 1812-1822.

- Belay, B.; Belachew, B.; and Habitamu, D., (2016). Review on Application and Management of Medicinal Plants for the Livelihood of the Local Community. *Journal of Resources Development and Management*. Vol.22, pp.33-39.
- Bishop, M., Fody, E., Schoeff, L., (2005). *Clinical chemistry: Principle, Procedures, Correlations*. 5th ed., Baltimore: Lippincott Williams. Pp: 250-253. 476-486.
- Chaira, N.A.; Ferchichi, A. M. and Sghairoun, M., (2007). Chemical composition of the flesh and the pit of date palm fruit and radical scavenging activity of their extracts. *Pak. J. Biol. Sci.*, 10: 2202-2207.
- Doumas, B.T. ; Watson, W.A. And Biggs, H.G., (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*. pp: 392-402.
- Hussein, J. S.; Oraby, F. S. and El-Shafey, N., (2007). Antihepatotoxic effect of garlic and onion oils on ethanol-induced liver injury in rats. *J. Applied Sciences Research*, 3 (11): 1527-1533.
- Masoomah, D.; Hojatallah, K.J.; Nader, T. and Negar, A., (2013). Effects of heart of palm (palmito) extract on reproductive system of adult male rats. *Asian pacific J. of Reproduction* 2(4): 272-276.
- Robyt, F.J. and White, J.B., (1987). *Biochemical Techniques. Theory and Practice*. Brookes/Cloe Publishing Company, Monterey, California, pp. 115-118.
- Saafi, E.B.; Louedi, M.; Elfeki, A.; Zakhama, M.F.; Najjar, M. H. and Achour L., (2010). Protective effect of date palm fruit extract (*Phoenix dactylifera* L.) on dimethoate induced-oxidative stress in rat liver. *Exp. Toxicol pathol.* 14:15-22.
- Schacterle, G.R. and Pollack J.K., (1973). A simplified method for the quantitative assay of small amount of protein in biological materials. *Anal. Biochem.*, 51: 654-655.
- Snedecor G., Cochran, G., (1986). *Statistical Methods*. 4th Edn. Iowa State University Press, Ames, Iowa, USA.
- Thiyagarajan, M., and Venkatachalam, P.,) 2012). Large-scale in vitro propagation of *Stevia rebaudiana* (Bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb. *Industrial Crops Products*, 37(1): 111-117.
- Tietz, N.W., (1999). *Text book of clinical chemistry*, 3rd Ed. Burtis C.A., Ashwoodm E.R. , Saunders W.B.; pp.819-861.
- Toro, G. and Ackermann, P.G., (1975). *Practical Clinical Chemistry*. Little Brown, Boston, U.S.A., p: 354.
- Travis D. Hull and Anupam Agarwal, (2014). Bilirubin: A Potential Biomarker and Therapeutic Target for Diabetic Nephropathy, *Diabetes Volume* 63: 2613–2616.
- Uskokovic-Markovic, S. , Milenkovic, M. , Topic, A. , Kotur-Stevuljevic , J. , Stefanovic, A. , Antic-Stankovic, J., (2007). Protective effectives of tungstophoric acid and sodium tungstate on chemically induced liver necrosis in wistar rats. *J Pharm Pharmaceut Sci*, 10 (3): 340-349.
- Walters, M. and Gerarde, H., (1970). An ultramicro method for the determination of conjugated and total bilirubin in serum or plasma. *Micro chem. J.* 15(2):231-243.

Author Information

Saba Z. Al-Abachi

Mosul University, Iraq

Contact E-mail: Saba_alabachi@yahoo.com

Sameer M. Al-Gorany

Middle Technical University

Iraq
