Alinteri J. of Agr. Sci. (2022) 37(1): 27-32
e-ISSN: 2587-2249
info@alinteridergisi.com

http://www.alinteridergisi.com/
DOI:10.47059/alinteri/V37I1/AJAS22006

RESEARCH ARTICLE

Efficacy of Senna Leaves Extract and Rosuvastatin on Blood Parameters of Inducing Hyperlipidemia Laboratory Rats

Rafal Ghadhban Al-Juburi[1] • Dr. Marwa Ibraim Abd Al Janabi[2*]

[1]*Department of Food Sciences, College of Agriculture, Tikrit University, Iraq.*

[2*]*Department of Food Sciences, College of Agriculture, Tikrit University, Iraq. E-mail: marwa984@tu.edu.iq*

ARTICLE INFO

Article History:
Received: 22.06.2022
Accepted: 30.07.2022
Available Online: 12.10.2022

Keywords:
Senna Leaves
Rosuvastatin
Blood Parameters
Hyperlipidemia Laboratory Rats

ABSTRACT

The current study was conducted at the Department of Food Sciences / College of Agriculture / Tikrit University. This study used 20 albino male rats obtained from College of Veterinary Medicine / Tikrit University: Their ages ranged between (3-4) weeks and weights ranged between (160-175 g); The period of experiment lasted for sixty days starting from (1-4- until 1-6-2021). They were randomly divided into 4 groups and each group consists of 5 rats. The first group considered as the control group. While the second group represented the fat-treated group, in which the rats feedon diet at a concentration of 500 mg.kg-1 weight of the animal. The third group has given the Rosuvastatin which was Effect of hyper pad concentration of 500 mg.kg-1 weight of the animal, and on day 30 it was treated with Rosuvastatin at a concentration of 20 mg.kg-1 weight of the animal for a period of 30 days until the end of the experiment. The fourth group represented the Sana makki, which was fed a high-fat diet at a concentration of 500 mg/kg of animal weight, and on day 30 it was treated with Sana makki at a concentration of 0.5-1mg.kg for a period of 30 days until the end of the experiment. Blood was drawn from all groups from the eye socket at time 0, i.e. before starting the experiment and its treatments, and then after the end of the specified period of the experiment, the rats were prevented from eating for approximately 24 hours, then blood was drawn from the eye socket after placing it in the inventory box to prevent it from moving, as it collected approximately (1-2) ml of blood for the purpose of conducting blood parameters, then the animals were killed, and 3 ml of each animal was taken and placed in tubes containing an anticoagulant substance to separate the serum and conduct biochemical tests. It was observed through the current study that the effect of lipids was clear by causing a significant decrease While Rosuvastatin caused a significant decrease in the level of white blood cells when compared with the control group and when compared with the time of the start of the experiment, while Rosuvastatin caused a significant increase in (total number of red blood cells, hemoglobin concentration, platelets and packed cells volume) within the same group when compared with the start time of the experiment. While the aqueous extract of Sana makki had a clear role by returning the levels of blood parameters to their normal status when compared with the fat-treated group. Regarding the biochemical parameters (cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) it was found that the use of fat in the ration caused significant increase (0.05) in all parameters except high-density lipoprotein HDL when compared with the control group on the one hand and when compared with the start time of the experiment on the other hand.

* Corresponding author:
marwa984@tu.edu.iq

When using Rosuvastatin, it was noted that it caused a significant decrease (0.05) in cholesterol level, while Rosuvastatin contributed to the return of (triglycerides, high-density lipoprotein (HDL)) to normal, and Rosuvastatin caused a significant increase in (low-density lipoprotein, LDL, Very high density when compared with the control group on the one hand and when compared with the start time of the experiment on the other hand. The positive effect of the aqueous extract of Sena was clear through its effect on all parameters, causing them to return to their normal values. We conclude through this study that Sena is considered as best natural compound to use for the purpose of controlling most of the biochemical parameters in the body and by preserving blood components from harmful effects, despite the role played by Rosuvastatin, but Sena is considered the best sometimes because it is free of chemical substances.

Please cite this paper as follows:

Al-Juburi, R.G. and Dr. Abd Al Janabi, M.I. (2022). Efficacy of Senna Leaves Extract and Rosuvastatin on Blood Parameters of Inducing Hyperlipidemia Laboratory Rats. *Alinteri Journal of Agriculture Sciences, 37*(1): 27-32. doi: 10.47059/alinteri/V37I1/AJAS22006

Introduction

Healthy nutrition and eating habits are among the most important factors that affect health, physical and mental state of people. The quality of food is necessary because it has great importance on the health and safety of the body from diseases and their causes. In addition, the quality of food helps the body performs its various vital functions. Bad eating habits and chronic and dangerous diseases are closely related (Al-Sadiq, 2011). Liver cells synthesize cholesterol with the help of 3-Hydr0xy-3-Methyl-Glutaryl-CoA reeducates enzyme. Several experiments were conducted in order to find out a way to inhibit the activity of this enzyme. In 1976, the statin that inhibits this enzyme was discovered, which is the main substance for cholesterol synthesis (Endo, 2015). Statins are one of the most widely used means in the treatment of hyperlipidemia, which in turn lead to the prevention of many diseases that may infect kidneys, liver, and heart (Ni Chroinin et al., 2013). In studies carried out by many researchers and scientists in the pharmaceutical and chemical disciplines, including Mahan, (2012) it was stated that cholesterol is a waxy, fatty substance that is found in the human body and in the bodies of animals. This substance is considered as an essential part of the body and plays a role in the production of hormones and the metabolism of fats. In another study conducted by Tietz (1986), it was found that the body can produce 1000-2000 (mg. day-1) of cholesterol, and the body can get rid of a percentage between 200-800 (mg. day-1) through feces excretion. The body can also dispose of cholesterol through the skin to a percentage ranging between 100-300 (mg. Day-1). High blood lipids are one of the main causes leading to atherosclerosis. The most significant type of high blood lipid, which is directly related to the development of the atherosclerosis, is cholesterol, where the majority of it (approximately 70%) is of an esterified form with fatty acids (Murray et al., 2003). The inhibition of cholesterol synthesis in the hepatic parenchymal cells, which is done by inhibiting the enzyme 3-Hydroxy 3-methyl glutaryl Co enzyme - A reductase, is not sufficient to prevent the accumulation of cholesterol inside the cell if its level in the blood continues to rise due to its high level in food. In addition, the increase in the level of cholesterol inside the cell and its accumulation in it will lead to a reduction in the activity of (LDL-C) receptors and thus a decrease in its metabolism (Mayne, 2005).

Statin drugs inhibit the activity of the enzyme HMG-CoA reductase, which is the required enzyme in production. This happens due to the similarity between statins and the enzyme in their composition and as a result statin replaces enzymes. Thus, statins reduce the production of mevalonate, which is the second molecule in the sequence that ends with the production of cholesterol. The search for cholesterol-lowering drugs initiated by the biochemist Erica Endo in 1971, where he discovered that a high proportion of cholesterol is produced in the liver, which is known as auto-synthetic cholesterol. This cholesterol is made by activating the HMG-CO (hydroxymethyl glutaryl coenzyme) enzyme. There are a number of microorganisms working on the formation of metabolic substances as a defensive factor to protect themselves from other organisms. However, it was found that these substances have the ability to inhibit the main enzyme used in the formation of cholesterol called HMG-CO which is Mevastatin. Mevastatin was isolated and produced from the fungus *Penicillium citrinum*. This statin was not sold because of its side effects, some of which led to a high rate of fatalities in laboratory dogs (Endo, 2015).

As stated above, the aim of the study was to determine the effectiveness of Rosuvastatin and Senna leaf extract to improve some biochemical parameters, especially the lipid profile in fed rats, which were experimentally induced by hyperlipidemia through the following.

Materials and Methods

Feeding Formulation of Laboratory Animals

The Formulation of the Main Feed

The base diet that is available in the 'animal house' at the university of Tikrit consists of the following ingredients. Table (1) shows below.

Table 1. Main Food Contents, (NRC, 2002)

Material	Weight (Gram.KG^{-1})
Cellulose	50
Casein	158.5
Sunflower oil	100
Ready-made vitamin group	5
Minerals Group	50
Starch	536.5
Glucose	100

High-calorie Feed Preparation

The high-calorie diet for rats feed was prepared from the following ingredients showed in the Table (2) as percentages of the total calories.

Preparation of Rosuvastatin: The drug, Rosuvastatin, was used, commercially known as Cristor, which contains the active ingredient Atorvastatin (Lennernas, 2003). The concentration of the active substance in it was 20 mg kg^{-1} according to the data from the manufacturer (a Swedish company). The substance was well ground and powdered, and 0.04 mg of it was taken, dissolved in water and then dosed to animals and according to the requirements of the experiment.

Preparation of Senna Makki: The aqueous extract of Senna was prepared based on the method that was ccording to (Mbiantcha, 2011). A quantity of 150 g of ground leaves was taken and placed in 1500 ml of distilled water for four hours under room temperature. Then the scent was filtered by filter paper and the scent was used to feed the groups that was being studied with a daily average dose of between 0.5-1 mg. Kg, (Heikal, 1993).

Laboratory Animals

Preparation of laboratory animals: Laboratory animals, Albino white male rats, were brought from the 'animal house' at the Faculty of Veterinary Medicine at the University of Tikrit. The number of rats was 20 of ages ranged from 3 – 4 weeks and weights ranged from (160-175 g). Through follow-up in terms of health indicators and motor activity and the examination by the veterinarian, it was confirmed that the animals were free of diseases. The animals were placed under controlled environment in terms of ventilation and temperatures, ranged between 22 to 30 °C. The humidity was at 50%. The animals were provided with sterile water through the container attached to the cage and allocated for this purpose. The animals were placed inside the cages before the date of the actual trial for 10 days in order to adapt and get used to the place. Three rats, excluded from the previous number of rats, were isolated outside to conduct a preliminary test to ensure the ability of fat to raise cholesterol within a dose of 1 ml per day throughout taking blood samples from the eye area before and after feeding that lasted a week. A significant rise in the total amount of cholesterol was observed (McSweeney and Fox 2013).

Design of the Experiment: The rats were randomly divided into four groups in which for each group five rats. After the end of the conditioning period, which lasted for a week, the rats were treated daily for 60 days according to the group they belong. The initial weights of the rats were recorded and they were treated by oral dosing using the medical syringe and some with feeding as follows:

1. Control Group: The rats were treated by feeding them with standard feed.
2. Fat group: The rats were treated by dosing distilled water and by feeding a mixture of fat and feed with concentration of 400 (mg. kg^{-1}).
3. Rosuvastatin Group: It was fed by high-fat feed and in concentration 400 (mg. kg^{-1}). The animal weight was recorded through sixty days. On day thirty, the animals were treated with Rosuvastatin with concentration of 20 (mg. kg^{-1}).
4. Senna Group: It was fed on a high-fat feed and in a concentration of 400 (mg.kg^{-1}). On day 30, The animals were treated with a 10% of Senna concentration of 10% for thirty days to the end of the experiment.

Collection of Samples at the End of the Trial Period: Blood was drawn from all groups from the eye orb at the time (zero) which is before the beginning of the experiment and its treatments. Then after the end of the specified period for the experiment, the rats were halted from eating for about 12 hours. After that, the blood samples were withdrawn from the eye orb after placing it in the specific box to fix it from motion. Around 1 – 2 (ml) of blood was collected for the purpose of conducting blood tests. Then the animals were killed and 3 (ml) of blood from each animal was taken and placed in tubes containing Ethylene Diamine Tetra Acetate (EDTA), anticoagulant, for biochemical tests. This sample was left for about a quarter of an hour in a water bath at a degree of 37 ° C. Then, the serum was obtained through a Centrifuge with a speed of 3000 (rpm^{-1}). The serum kept at (-20) ° C in new and clean Plane Tubes until conducting the special biochemical tests (glucose, cholesterol, Triglycerides, high-density lipoproteins for cholesterol, low-density lipoproteins for cholesterol, very low-density lipoproteins for cholesterol).

Estimating the Level of Total Cholesterol in the Blood Serum: The level of total cholesterol in the blood serum was estimated using a test kit that is made available by the French international company Biolabo based on the enzymatic method by (Richmord, 1973). In this method, cholesterol is converted into Quinonimine pigment of pink color. The intensity of its absorption can be measured at a wavelength of 500 nm, using a Spectrophotometer according to the following equations:

The concentration of cholesterol was calculated according to the following equation:

$$\text{Cholesterol concentration (mg.100}^{-1}\text{mlblood)} = \frac{\text{Intensity of the test solution absorption}}{\text{Intensity of the standard solution absorption}} \times \text{concentration of standard solution (200mg.100}^{-1}\text{ml blood)}$$

Estimation of the Level of Triglycerides in the Blood Serum

The level of triglycerides in the blood serum was estimated using a ready-made test kit manufactured by the English company Randox and based on the method (Williams, 1987). The measurement was done at a wavelength of 500 nm, then the level of triple glycerides in the serum was calculated according to the following equation:

The concentration of triglycerides was calculated according to the following equation:

Intensity of the test solution absorption

Triglycerides concentration (mg. 100^{-1} ml blood) = ---------- × standard solution concentration (200 mg. 100-1 ml blood)

Intensity of the standard solution absorption

Estimation of the Level of High-density Lipoproteins HDL_C of Cholesterol in the Blood Serum

The concentration of high-density lipoproteins of cholesterol in the blood serum was estimated using a test kit from the English company Redox based on the enzymatic method used by Tietz (1986). The basis of the method is that LDL_C and VLDL_C precipitated with Phosphotungstic acid in the presence of magnesium ions at room temperature. The leachate was obtained after the centrifugal separation process that contains only HDL_C. It was estimated using the enzymatic solution of cholesterol.

The models were read by a spectrophotometer at a wavelength of (500) nm, and the following law was applied to calculate the concentration:

Intensity of absorption of test solution

Concentration HDL_ C (mg. 100-1 ml) = ---------- standard solution concentration × (55 mg. 100-1 ml blood)

The intensity of its absorption of the standard solution

Statistical Analysis

The experiment was carried out under Complete Randomized Design, (CRD) within the Ready-made Statistical Program (SAS), (2001). The results were compared to the use of the Duncan test to determine the significant differences at the probability level of 0.05.

Results and Discussion

Cholesterol Level

The results indicated that the concentration of cholesterol in the blood of rats of the group that treated with fat caused a significant difference (0.05) between cholesterol levels of 122.7 (mg/dL). This difference is due to the increased activity of the Cholesterol Acyl Transferase Enzyme responsible for that affects pancreatic beta cells under the influence of active oxygen varieties. This increases the level of cholesterol absorption by the intestines (Maechler, 2017). These findings are also in consistent with what Morishita et al., (2018) have stated. They pointed to the possibility of an increase in the average body weights of laboratory animals that were fed on a high-cholesterol feed as it led to the accumulation of fat in different areas of the body, causing a significant weight gain over time.

For the group treated with Rosuvastatin, a significant decrease in cholesterol level was observed in the group treated with Rosuvastatin 60 days in which 65.5 mg/dL were recorded. The results are in consistent with Nazari et al., 2016. They showed that statins have a very significant impact on the process of weight reduction and elimination of fat around the abdominal area if taken on a daily basis and within acceptable levels in addition to their ability to reduce the average sizes of fat cells in all adipose tissues. This happens due to its ability to improve metabolism and restore the valence of fatty acid distribution in addition to improving bowel movement and thus enhancing efficiency for digestion and the digestive system in general. Cengiz et al., (2015) have stated that Statins stimulate the secretion of various enzymes (amylolytic, proteolytic lipolytic) which help in the digestion of nutrients in the gut and in turn improve metabolic processes within the intestine. Also, they help the process of weight loss and normalize the high-density of lipoproteins HDL.

It was also noted that there is a significant difference in the level of cholesterol within the group treated with Senna at zero time (beginning of the experiment) and within the group treated with Fat. There is also a significant difference between the level of cholesterol in the group treated with Senna after the end of the experiment when compared with the Control Group, as this group was treated with the fat-containing feed for thirty days and then used the aqueous extract of senna. This extract led to reduce the level of cholesterol where it was recorded 97.4 (mg / dL) when compared with the group treated with fat that was 122.7 (mg/dL). Also, the level of cholesterol returned to normal when compared with the control group. These results we obtained were consistent with (Ajaiyeoba et al., 2018). This showed that Senna extract contains antioxidant compounds that inhibit the manufacture of cholesterol in the cells manufactured in the liver.

Table 2. Effect of treatment with fat, Rosuvastatin and aqueous extract of Senna on cholesterol level

Groups / Standards	Time	Control Group	Fat treatment group	Fat treatment group + Rosuvastatin	Fat Treatment Group + Senna
Cholesterol Mg $^{DSL-1}$	0	3.6 ± 84.4 a A	4.6 ± 83.8 b A	3.7 ± 87.3 a A	5.1 ± 88.1 b A
Cholesterol Mg $^{DSL-1}$	In 60 days	4.2 ± 89.3 a B	5.3 ± 122.7 a A	2.3 ± 65.5 b C	4.6 ± 97.4 a B

The values are expressed by the standard error rate ±

Animals in each group = 5 rats

The different lowercase letters within the same column indicate a significant difference at the level of (0.05)

The different uppercase letters within the same row indicate a significant difference at the level of (0.05)

Triglyceride Level in the Study Groups

The results showed that triglycerides significantly increased between the zero and 60-day times when treating animals with fat-containing feed (table 3). The treatment from day 30 to day 60 was 102.77 (mg/dL) due to several reasons including that fat treatment leads to a decrease in the activity of the enzyme Lipoprotein lipase, which is responsible for the fragmentation of triglycerides (Mohieddin et al., 1990). Also, the other reason may be attributed to the inhibition of the enzyme Triglyceride lipase, which is responsible for the fragmentation of triglycerides and thus causing an increase in fat metabolism and higher levels in the blood that were 102.77 (mg/dL) (Janabi, 2008). The results were in consistent with the studies carried out by Lawler et al., 2017; Taylor et al., 2016). They have shown that the Rosuvastatin drug has a high potential to lower total cholesterol, triglycerides and lipoproteins of low density and very low density by reducing the enzyme HMG-CoA which plays an important role in the manufacture of cholesterol in the liver that was recorded 72.44 (mg / dL). This is due to the similarity at the molecular level between Rosuvastatin and the enzyme in which Rosuvastatin replaces it. Thus, statins work to reduce the production of mevalonate which is the second molecule in the sequence that ends in the production of cholesterol. In the group treated with Senna, it was noted that the use of senna extract after the end of the trial (30 days of treatment) contributed to the normalization of triglyceride level when compared with the control group and with zero time from the start of the trial (74.88 mg/dL). The results were in consistent with (Amal et al., 2016). They showed the ability of the active substances in senna, antioxidants and flavonoids and their content of cynarine and chlorogenic which is composed of the mixture of Caffeic acid and Quinic acid necessary to restore triglycerides to a normal level when compared to the control group.

Table 3. Effect of treatment with Rosuvastatin and Senna extract on the level of triglycerides in rats induced hyperlipidemia

Groups / Standards	Fat Treatment Group + Senna	Fat Treatment Group + Rosuvastatin	Fat treatment group	Control Group	Time
Triglycerides Mg $^{DSL-1}$	4.3 ± 74.88 a A	2.5 ± 72.44 a A	4.5 ± 72.13 b A	± 73.76 3.5 a A	0
Triglycerides Mg $^{DSL-1}$	3.2 ± 80.42 a B	3.2 ± 77.37 a B	± 102.77 5.8 a A	± 77.56 4.2 a B	In 60 days

The values are expressed by the standard error rate ±
Number of animals in each group = 5 rats
The different lowercase letters within the single column indicate a significant difference at the level of (0.05)
The different uppercase letters within the same row indicate a significant difference at the level of (0.05)

High Density Lipoproteins Level

The results in Table (4) indicated that the level of high-density lipoproteins decreased significantly when rats were treated with cholesterol which recorded 41.22 (mg / dL). The reason for the decrease in HDL-chol is attributed either to the liver having some diseases, or as a result of high levels of cholesterol, triglycerides and LDL-chol because the function of HDL–chol is the reverse transfer of cholesterol from tissues to the liver (Schlant et al., 2016). If the concentrations of cholesterol and triglycerides in tissues and blood vessels increase, this constitutes an impediment to the efficiency of HDL-chol in transporting cholesterol. In addition, the oxidation of LDL and the destruction of internal cholesterol in the body leads to a decrease in the level of HDL, which is the basis for the process of transporting cholesterol from the cells of the body to the liver and thus reducing its level found in the blood vessels (Guyton and Hall, 2006). For the group treated with Rosuvastatin, a significant increase of 50.33 (mg/dL) in the level of high-density lipoproteins at a time of 60 days compared to zero time. These results are in consistent the results of (Cengiz et al., 2015). Statin compounds stimulate the secretion of various enzymes (amylolytic, proteolytic lipolytic) which help in the digestion of nutrients in the gastrointestinal tract which in turn improves metabolic processes within the intestine. Thus, it helps the process of reduction weight and normalize high-density lipoproteins HDL. It was also noted that there is a significant difference in the level of high-density lipoproteins in the group treated with Senna and in the group treated with Fat, while there is no significant difference between the level of high-density lipoproteins in the group treated with Senna after the end of the experiment when compared with the control group 51.22 -50.53 (mg/dL). As this group was treated with the fat-containing feed for thirty days and then used the aqueous extract of the Senna in which led this extract to reduce the level of proteins. The results we obtained were in consistent with (Ajaiyeeoba et al., 2018) which showed that the extract of Senna contains antioxidant compounds and works to inhibit the manufacture of high-density lipoproteins in the cells that produce it.

Table 4. Fat treatment and effect of Rosuvastatin aqueous extract of Senna on the level of high-density lipoproteins

Groups / Standards	Fat Treatment Group + Senna	Fat treatment group +Rosuvastatin	Fat treatment group	Control Group	Time
High Density Protein Fat HDL mg.dL-1	2.9 ± 50.53 a A	1.9 ± 49.65 a A	2.1 ± 50.56 a A	3.5 ± 52.13 a A	0
High Density Protein Fat HDL mg.dL-1	3.1 ± 51.22 a A	2.9 ± 50.33 a A	1.7 ± 41.22 b B	2.7 ± 55.21 a A	In 60 days

The values are expressed by the standard error rate ±
Number of animals in each group = 5 rats
The different lowercase letters within the same column indicate a significant difference at the level of (0.05)
The different uppercase letters within the same row indicate a significant difference at the level of (0.05)

References

Ajaiyeoba EO, Ashidi JS, Okpako LC, Houghton PJ, Wright CW. (2018). Antiplasmodial compounds from Cassia siamea stem bark extract. Phytother Res., 22(2): 254-5.

Ajaiyeoba EO, Ashidi JS, Okpako LC, Houghton PJ, Wright CW. (2018). Antiplasmodial compounds from Cassia siamea stem bark extract. Phytother Res., 22(2): 254-5.

Al-Janaby, K.A. (2008). The effect of aqueous extract of grassed on oxidative stress induced by hydrogen peroxide in male rat. Msc. Thesis. Tikrit University, IRAQ. 65 p.

Amal A Aloud, Chinnadurai Veeramani, Mohammed A Alsaif, Ahmed S El Newehy, Khalid Al-Numair. (2016). Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. Redox Rep., Nov; 22(6): 290-300.

Cengiz, O.; Koksal, B.H.; Tatl, O.; Sevim, O.; Ahsan, U.; Uner, A.G.; Uluta, P.A.; Beyaz, D.; Buyukyoruk, S.; Yakan, A. & Onol, A. G. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poult. Sci., 94: 2395-2403.

Endo A. (2015). "The discovery and development of HMG-CoA reductase inhibitors" (PDF). J. Lipid Res., 33 (11): 1569–82. PMID.

Guyton, A.C. and Hall, J.E. (2017) Textbook of Medical Physiology. Koraea: Bupmoon Education.

Heikal, M.E. A,A,R,O. (1993) Medicinal and aromatic plants-their chemistry –their production – their benefits.Knowledge Foundation in Alexandria – second edition.

Lawler, P. R.; MPH, M.D.; Akinkuolie, A.O.; MPH, M.; PhD, C.Y. A.; Kraus, W.E.; MSC, D.C.; MPH.P.R.; Chasman, D.I. & Mora, S. (2017). "Residual Risk among Individuals with Low-Density Lipoprotein Cholesterol" J. of the Amer. Heart Association, 6 (7), eoo5549.

Lennerna, H. (2003). Clinical pharmacokinetics of atorvaststin. Clinical pharmacokinetics, 42(13), 1141-1160.

Maechler, P., Wollheim, C., Bentzen, C., and Niesors, E. (2017): Importance of exogenous cholesterol in diabetic rats: Effect of treatment with insulin or with an Acyl-CoA: Cholesterol acyl tranceferase inhibitor. Ann. Nutr. Metab.; 37: 99-209.

Mahan, L.K., Escott-Stump, S., Raymond, J.L., Krause, M.V. (2012). Venous thromboembolism: annualized united states models for total, hospital –acquired and preventable costsutilising long – term attack rates. Thrombosis and haemostasis, 108 (08), 291-302.

Mayne, P.D. (2005). Clinical Chemistry in Diagnosis and Treatment, 6th ed, oxford Univresity Press, Inc., New York. PP. 225-241.

Mbiantcha, A. Kamanyi, R. Teponno, A. Tapondjou, P. Watcho, T. Nguelefack, J. (2011). Analgesic and Anti-Inflammatory Properties of Extracts from the Bulbils of Dioscorea bulbifera L. var sativa (Dioscoreaceae) in Mice and Rats. Evid Based Complement Alternat Med., 912935. doi: 10.1155/2011/912935.

Mc Sweeney, G. and Fox, F. Niter, M.D., Barrett, H.L., Fox Croft, K., T remellen, A., Willkinson., Lingwood, B., …& Callaway, L.K. (2013). Spring: an RCT study of probioticis in the prevention of gestaional diabetes mellitus in over weight and obese women. BMC pregnancy and child birth, 13 (1), 1-7.

Morishita, T.; Kegushi, Y.; Yajima, M.; Sakurai, T. and Yura, T. (2018). Multiple nutritional requirements of Lactobacilli: genetic lesion affecting amino acid biosynthetic pathways. J. Bact., 148: 64 – 71.

Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W. (2003). Harper's Illustrated Biochemistry. 26¬th ed. Mc Graw-Hill companies. pp: 111-120. 136-162, 205-229, 580-597.

Nazarii Kobyliak, Caterina Conte, Giovanni Cammarota, Andreana P. Haley, Igor Styriak, Ludovit Gaspar, Jozef Fusek, Luis Rodrigo and Peter Kruzliak.(2016). Probiotics in prevention and treatment of obesity: a critical view. Nutrition & Metabolism. vol, 1-13.

Ní Chroínín D; Francis N; Wong P; Kim YD; Nham S; D'Amours S, (2013). Older trauma patients are at high risk of delirium, especially those with underlying dementia or baseline frailty', Trauma Surgery and Acute Care Open, vol. 15; 279(2): 130-135.

NRC (National Research Councli Recommended) (2002). Dietary Allowance –and now: areview. Journal of agromedicine, (2), 85-93.

Richmond, W. (1973) Preparation and Properties of a Cholesterol Oxidase from Nocardia sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. Clinical Chemistry, 19, (12), 1350-1356.

Tietz, W. (1986). Text Book of Clinical Chemistry. JAMA, 256(1), 106-107.

Williams, L. (1978) in Disturbances in Lipid and Lipoprotein Metabolism. J. Lipid Res. pp. 229-249.