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TO STUDY ON ABRESHAM (BOMBYX MORI): COCOON, FORMULATION ON HYPERLIPIDEMIC PATIENTS

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Abstract:

Background: *The silk worm Bombyx mori is a domesticated insect, which feeds exclusively on mulberry leaves to produce raw silk in the form of cocoon. Commonly it is known as Abresham and possesses the antioxidant activity and rich flavonoid contents. Since the ancient times it has been used to treat cardiac ailments due to the lipid lowering properties attributed to it which are well documented.*

Objective: *The objective to explore the potential of Bombyx mori cocoon formulation (khamira Abresham Hakim Arshad Wala) in the reduction of hyperlipidemia.*

Methodology: *Fifty hyperlipidemic male and female patients were taken and their lipid profile (cholesterol , triglycerides, HDL, LDL) liver function test (bilirubin toal, bilirubin direct, SGPT, SGOT, alkaline phosphate) and renal function test (blood urea,serum creatinine) were studied before, and after six and twelve weeks of treatment with Bombyx mori cocoon formulation. After 6^{th} and 12^{th} week, all the parameters were studied to evaluate the mean difference in their values.*

Results: *In lipid profile parameters there was decrease in cholesterol level (mg/dl) 197.36 ± 12.86 to 194.00 ± 17.60 after six and twelve weeks of treatment. There was decrease in triglycerides (mg/dl) 122.640 ± 23.4640 after the 6^{th} week and little increase to 123.94 ± 17.50 after the 12^{th} week of treatment. In the liver function test of total bilirubin (mg/dl) the value was decreased to 0.612 ± 0.1081and 0.56 ± 0.08 after 6^{th} and 12^{th} week. There was no change in the values of direct bilirubin before and after the treatment. The values of SGOT were also decreased to 26.90 ± 5.92 and 25.96±5.81mg/dl after the 6^{th} and 12^{th} week of treatment. In the renal function parameters the blood urea mean level was decreased to 34.740± 6.4581and 34.04± 6.43 after 6^{th} and 12^{th} week.*

Conclusion: *By the utilization of Bombyx mori cocoon formulation a decrease in cholesterol, triglycerides, LDL, total bilirubin, SGPT, SGOT, an increase in blood urea, while no change in direct bilirubin was observed.*

Key Words: *LDL, HDL, SGPT, SGOT, Cholesterol.*

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INTRODUCTION:

The fats medically are termed as lipids that are present in the blood, to perform many important functions in the human biological system at the normal ranges (150 mg/dl to 200 mg/dl), when the level of fats goes beyond normal values it can result in serious health related issues the. Hyperlipidemia term is used for increased level of fats or lipids in the blood (Bonow *et al.,* 2011). Normally there is smooth and unobstructed blood flow in the arteries from the inside, as the age increases an obstruction medically termed as plaque sticks with the inside lumen of the arteries. Fats are one of the major contributive factors in the formation of plaque, making arteries more narrow and stiff from the inside as the plaque increases in its volume. Finally, the plaque increases to a size decreasing the proper flow of the blood from the arteries. This results in a condition termed atherosclerosis in which the risk for heart disease rises and also there is rise for threats of developing conditions like atherosclerosis, hemorrhage and other problems associated with blood vessels (Bonow *et al.,* 2011).

The same person can also face other life threatening conditions like diabetes, hypertension, obesity and hyperlipidemia, it is important to assess, diagnose and treat the people at right time with right medication and therapies to prevent them from developing the hyperlipidemia and cardiovascular disease like life threatening conditions, and for that purpose ample number of qualified physicians and health specialists are required which is not the case with the countries like Pakistan. According to report there is also an increase in the average life of the people to sixty-six years of age, while nutritional and environmental standards for old age people are decreased to a very low level. The incomes are not up to the mark and the ideal health facilities are not easily available (Goff *et al.,* 2014).

The rapid increase is noticed in the impact and occurrence of hyperlipidemia around the globe and the numbers are rising consistently. Due to hyperlipidemia, in the European Union two million people are dying every year. The major reason for the death of the people was the heart disease developed due to the increase fat levels, sixteen percent of the deaths belonged to men and fifteen percent belonged to women. Seven million people in Romania are assessed to have a cardiovascular disease, one third of them are estimated to have the ischemic heart disease. The statistical analysis of the demographic data reflects that there is serious need to assess the risk factor associated to each individual for the purpose to attain the right therapeutic measures (Ginghina *et al.,* 2011).

Pakistan is located on that geographical region which is suitable for the growth of much herbal vegetation The herbal treatment was oriented by the Greek philosophers and came to the region during the period of Indus valley civilization. The herbal treatments were then flourished with the passage of time and many Muslim scientists also contributed in it. Currently, it is mostly practiced in tribal and rural areas where people do not have sufficient medical facilities. These herbs are cultivated in moderate northern regions and forests of subtropical regions of Pakistan (Bodekar and Kronenberg, 2002). The Unanipathy (Greek medication) was founded by a philosopher Galen. Later on, many Muslim Hakeem (scholars) like Avicenna, Alberuni, Alkhwarazmi, Alkindi, Alhythym and many more contributed their efforts in the field of Hikmat. The herbal practice was started in subcontinent by the time of Indus valley but some ancient Hindu scripts showed us the use of herbal plants for the cure, named as Ayurveda. Hikmat came here in 10th century by the Arab Muslim invaders (Goyal *et al.,* 2010).

Bombyx mori cocoon (Abresham) is also recognized as silk cocoon refers to Bombycid, Lepidoptera family. The common name for this aromatic plant is Raw Silk Worm-Moth and in Pakistan it is named as Abresham (Ahmad *et al.,* 2010). It consists of yellow, oval and rounded capsule like structure. Cocoon is nearly 2cm to 5cm long composed of fibroin (silk) threads paved together with a sheet of silk paste (sericin) secreted by silk worm as transitory from caterpillar to the chrysalis or pupa. These worms take food from the leaves of Morus Alba (Shaitut) which are nearly an inch long and half an inch wide. The powdered form of cocoon is utilized in medicines (Wang *et al.,* 2012).

The legendary book of medicine, written by Avicenna, was Al fi al Tib. In his book he highlighted the hepatic, cardiac and neurological functionalities, and the effect of herbal drugs on them. Abresham (*Bombyx mori* cocoon), is one out of 64 vital herbs mentioned in the book (Khan *et al.,* 2006). Khamira is the herbal paste formed by the mixing of powdered drugs in honey along with minerals and musk. Qiwam is the base of Khamira consisting of honey, sugar and water. It was first prepared as a royal product for the kings to gain eternity. Many stones like yaqoot, merjan sang e marmar are added when the decoction is fermented, some musk petals are also added (Malik *et al.,* 2009).

Cardiac medicines are prepared in such a manner to facilitate the effortlessly absorption and quick responsive. The Khamiras are usually named by the name of main herbal ingredient like Khamira Gaozban, Abresham, Sandal, Marwareed and so on. There are various other benefits in the ailment of stomach, palpitation, flu, cough, neurological and respiratory dysfunction, heart problems and hypertension (Yousuf *et al.*, 2005).

There are two types of Khamira Abresham available in the markets are Khamira Abresham Sada and Khamira Abresham Hakeem Arshadwala. Khamira Abresham Hakeem Arshadwala has some unique properties. It is beneficial to many body organs in the same time without any side effect. Some other benefits include melancholia ailment and stimulant tonic of brain, heart and liver. The major constituent is Abresham (*Bombyx mori* Cocoon) in all above Khamiras (Tan and Whiteman, 2002).

Khamira Marwareed (KM) and Khamira Abresham Hakim Arshadwala (KAHAW) are the two major Khamiras of Abresham. They have the anti-oxidant properties for the production of free radical. To reduce the weakening of cognitive functionalities, these Khamiras can be helpful to cure the patient. They are both semi solid paste prepared with *Bombyx mori* cocoon with the honey (Khan *et al.*, 2006). The nature of *Bombyx mori* silk produces dryness and heat in the body. It is a neurological stimulant tonic that enhances the speed of brain in understanding, learning, memorizing and quick response. It decreases the pathological phlegm from the blood and loosen the arteries due to smoking or hypertension (Mahmood *et al.*, 2015).

MATERIALS & METHODS:

Bombyx mori cocoon formulation:
The formulation containing *Bombyx mori* cocoon (khamira Abresham Hakim Arshadwala) was purchased from local market of Lahore Township. The formulation was semisolid, yellow colored, sweet in taste with aromatic odor.

Preparation method:
Bombyx Mori was prepared by the method of Kabir, 2003.

Study site:
Present research study was conducted at department of Food and Nutrition Minhaj University Lahore and duration of the study was three months.

Inclusion and exclusion criteria:
Male/female patients suffering from primary or secondary hyperlipidemia age ranging from 45 years to 70 years were included in the research study. The selected individuals were already diagnosed as hyperlipidemic patients from ward and OPD of the Tehsil Head Quarter Hospital Haveli Lakha District Okara Punjab.

Diabetic, alcohol addictive, cigarette smokers were excluded from the study. As advised by Ethics Committee, to remain on safe side due to adverse effects or low herb compliance we also excluded patients suffering from peptic ulcer, and thyroid disease. Patients with any kidney or liver disease or patients already on medicines due to any disease were excluded

Treatment and dose:
Patients were treated with the dose of 5gm (1/2) teaspoon twice a day (Kabir, 2003) with water for 12 weeks and the lab values of required parameters (lipid profile, renal function test and liver function test) were taken before the administration of formulation and then repeatedly on the 6th and the 12th week.

Sample size:
Fifty patients according to the inclusion criteria were selected for the research purpose.

Sample collection and serum separation:
By following the WHO guidelines after collecting blood samples by needle and syringe method the samples were applied to be centrifuged at 2500 RPM for 15 minutes with the desired serum separation to perform the lab tests according to the research required parameters (WHO, 2010).

Biochemical parameters:
Following biochemical Parameters were estimated.

Lipid profile
Liver function test (LFT,s)
Renal function test (RFT,s)
All the tests performed by using photometric kit method

Statistical Analysis:
The results related to all the parameters studied were recorded and analyzed statistically with 5 % level of significance by using SPSS software version 2016.

RESULTS & DISCUSSION:

Table1. ***Effect of Bombyx mori cocoon formulation on hyperlipidemic patients after being treated for twelve weeks.***

Parameter (n=50)	Test name	Week 0 (mean± std.dev)	6th week (mean± std.dev)	12th week (mean± std.dev)	P-Value (P<0.05)
Lipid profile	Cholesterol	198.900 ± 13.1789	197.360 ± 12.8615	194.000 ± 17.6010	0.002
	Triglycerides	125.480 ± 16.8949	122.640 ± 23.4640	123.940 ± 17.5027	0.001
	H.D.L	40.040 ± 2.9344	41.040 ± 3.0637	42.740 ± 3.8109	0.001
	L.D.L	130.820 ± 11.4369	130.000 ± 11.7213	129.520 ± 12.5165	0.003
Liver function test	Bilirubin total	0.630 ± 0.1249	0.612 ± 0.1081	0.568 ±0.0868	0.000
	Bilirubin direct	0.124 ± 0.0431	0.122 ± 0.0418	0.124±0.0410	0.003
	ALT (SGPT)	38.920 ± 5.4989	37.720 ± 5.3071	37.060±5.2931	0.000
	AST (SGOT)	27.600 ± 6.1246	26.900 ± 5.9221	25.960±5.8168	0.001
	Alkaline phosphate	202.380 ± 18.8213	200.400 ± 19.1108	201.440±22.4565	0.000
Renal function test	Blood urea	35.220 ± 6.6341	34.740± 6.4581	34.040± 6.4332	0.03
	Serum creatinine	0.904±0.1551	0.908 ± 0.1550	0.932±0.1584	0.000

Note. HDL (high density lipoproteins), LDL (Low density lipoproteins), ALT (Alanine aminotransferase), SGPT (Serum glutamic pyruvic transaminase), AST (aspartate aminotransferase), SGOT (Serum glutamic oxaloacetic transaminase

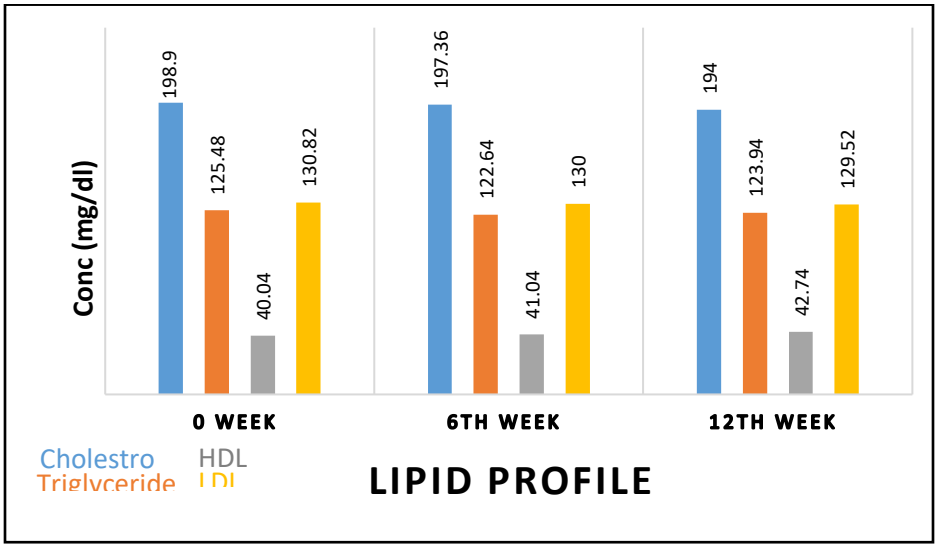

Figure 1: Graphical representation of Lipid Profile

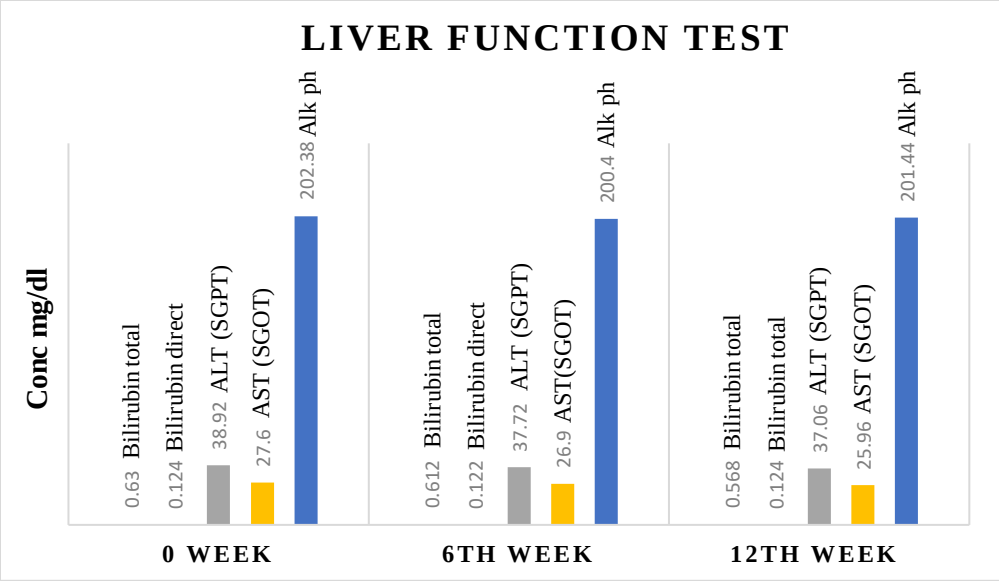

Figure 2: Graphical Representation of Liver Function Test

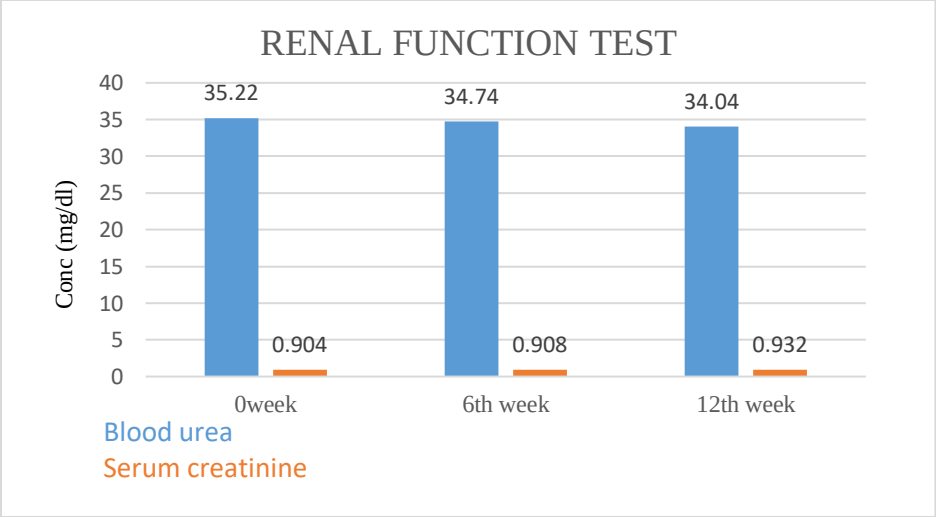

Figure 3: Graphical Representation of Renal Function Test

The current research work was designed with the objective to evaluate the importance of *Bombyx mori* cocoon formulation to lower the hyperlipidemia. So, the blood samples of patients were collected and their lipid, liver functioning test and renal function tests were performed and the results are compiled below

Lipid profile:

Cholesterol:

Cholesterol is a type of fat (lipid) found naturally in the human body which is obtained by the food intake and it is required by the human system to perform various biological functions but only at proper levels it can be beneficial if it gets above the border line (200 mg/dl).

During twelve weeks of treatment with 5gm of the formulation the results were compiled as before and after 12th week of provision of the formulation. The results related to the cholesterol levels of patients at different time intervals are mentioned in Table 4.1. It describes that the mean value of cholesterol before initiation of treatment was 198.90 ± 13.17 mg/dl, after the 6th week it decreased to 197.36 ± 12.86 mg/dl and after the 12th week the value was further decreased up to 194.00 ± 17.60 mg/dl.

A decrease in cholesterol level was found by Ali *et al* (2011) who studied the antihyperlipidemic property of *Bombyx mori* cocoon. These findings support the results of present study as a reduction in hyperlipidemia was observed.

Triglycerides:
Triglycerides are the main form of fat in the body mostly it is stored in the hips and belly regions of the body. Normal ranges of triglycerides are below 150 mg/dl. By today's modern life style where there is lesser physical activity and input of triglycerides through bulky or fatty food.

After the study of twelve weeks with the dose of 5gm of the formulation the results related to the triglycerides level are compiled as before and after in the Table 4.2 The results describe that before the start of the treatment the mean value of triglycerides was 125.48 ± 16.89 mg/dl and after the 6th month it decreased to122.64 ± 23.46 mg/dl while after the12th month it increased a bit to 123.94 ± 17.50 mg/dl

A decrease in the value of triglycerides was found by Shaila *et al* (1998) who studied the effect of three different drugs against experimentally induced hyperlipidemia and atherosclerosis. The results of the present work are advocated by the work of Shaila *et al* (1998).

HDL (High density lipoproteins):
HDL stands for high density lipoproteins which are considered as the good cholesterol because these do not contribute in producing the cardiovascular disease. The only type of cholesterol that needs to be above, versus below, at certain levels. These lipoproteins in fact transport the cholesterol towards the liver to be expelled out of the body. The common blood tests measure HDL in the parameter of mg/dl and the normal readings should be above 55mg/dl for males and 45mg/dl for females.

The results of the HDL level obtained before and after the administration of 5gm of the formulation are described in Table 4.3 The results revealed that before the start of the treatment, the mean value of HDL was 40.04 ± 2.93 mg/dl and after the 6th month it increased to 41.04 ± 3.06 mg/dl while after the 12th week the value was further increased up to 42.74±3.81mg/dl

This increase in HDL level is supported by the findings of Ghule *et al.*, (2006) who studied the antihyperlipidemic effect of the fruit extract of *Lagenaria Siceraria*. A similar increase in HDL level was reported.

LDL (Low density lipoproteins):
Low density lipoproteins are termed as "bad cholesterol" because they pose a risk for cardiovascular disease. LDL is not considered good for health so it is significant to control LDL level in body in order to prevent risk of cardiovascular diseases. Higher LDL levels can increase the risk of heart disease, the normal ranges for LDL are below 130mg/dl.

The results related to LDL level before and after the treatment of 5gm of the formulation are mentioned in Table 4.4. Which shows that before the treatment the mean value LDL was 130.82 ± 11.43 mg/dl and after the treatment of 6 weeks the mean value was decreased to 130.00 ± 11.72 mg/dl while after the 12 weeks the value further decreased to 129.52 ± 12.51mg/dl.

This decrease in LDL level was also found by Khan *et al* (2006) who worked on to observe the effect of Khamira Abresham Hakim Arshadwala to reduce the intensity of neurodegeneration.

Liver function test:
Bilirubin total:
A secretion produced by the liver which normally is formed as a result of the metabolic processes involved in the destruction of the hem. The part of the hemoglobin that is comprised of the RBCs. By using the complex enzymatic process, the liver manages to remove the excess of the bile from the human body.
The values of total bile obtained before and after the treatment of 5gm formulation are expressed in table 4.5 the mean value of total bile before the treatment was 0.63 ± 0.12 mg/dl and after the 6th week the value was decreased to 0.61 ± 0.10 mg/dl and after the 12th week the value decreased further to 0.56 ±0.08 mg/dl.

Bilirubin direct:
The component of the bile that has the property of being solved in the water is termed as direct bilirubin. The parameter of direct bile gives an insight to the level of bile that exists in conjugated form.

The bilirubin direct values obtained before and after the treatment are described in Table 4.6 the mean value before the treatment was 0.12 ± 0.04mg/dl and after 6th week without any deviation the value was 0.12 ± 0.04 and after the 12th week again without any change the value was 0.12 ± 0.04mg/dl.

SGPT (Serum glutamic pyruvic transaminase):
Liver is the organ where mainly the metabolic factory undergoes many enzymatic reactions. like other

biochemical reactions the proteins are broken down into further fragments the SGPT is an enzyme linked with metabolic fragmentation of the proteins. The level of this enzyme gives an estimation of the protein break down in the liver normally it is found in greater levels. Under normal circumstances this enzyme stays within the boundaries of liver but while in any of the conditions attributed to the liver disease the enzyme is released into the main blood circulation.

The values of SGPT level obtained before and after the treatment are described in Table 4.7 the mean value of SGPT before the treatment was 38.92 ± 5.49mg/dl and after the 6th week the value was decreased to 37.72 ± 5.30 mg/dl and after the 12th week the value was decreased further up to 37.06±5.29 mg/dl.

A decrease in SGPT level was found by Shan-hong *et al* (2004) who studied the effect of the male tussah Miller in protecting against liver and its enzymes. The results of SGPT of present work are in alliance with their findings.

SGOT (Serum glutamic oxaloacetic transaminase):
SGOT as many others is one of the metabolic enzyme of the liver playing part in many metabolic reactions. The elevated levels above the normal ranges of SGOT give an indication of the liver disease.

After the twelve weeks of treatment the values obtained before after the study are expressed in Table 4.8 the mean value before the treatment was 27.60 ± 6.12mg/dl and after 6 weeks of treatment the value was decreased to 26.90 ± 5.92mg/dl while after the 12th week the value was decreased further up to 25.96 ± 5.81mg/dl.

Ali *et al* (2006) studied the effect of *Bombyx mori* cocoons against hyperlipidemia and atherosclerosis by using the crude extract, and reported a decreased SGOT level. Their results are in accordance with current work.

Alkaline phosphate:
Alkaline phosphatase is secreted by the liver where it plays a role in the metabolic steps related to the proteins. It is found in blood stream in different forms, depending on where it originates. Mainly it is secreted by the liver.

The study results obtained of mean value of alkaline phosphate before and after the treatment are described in Table 4.9 the mean value before the treatment was 202.38 ±18.82 mg/dl and after the 6th week of treatment the value was decreased to 200.40 ±19.11mg/dl while after the 12th week of treatment the mean value was increased a bit to 201.44±22.45 mg/dl.

A decrease in the level of alkaline phosphate was found by seidavi *et al* (2011) who worked on to investigate the biodiversity of different local silkworm germplasm based on different enzymes aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, iron, sodium potassium and phosphate. The results of alkaline phosphate during present work are in range with their results.

Renal function test:
Blood urea:
Blood urea is the parameter to determine the function of kidneys. The kidneys mainly are concerned with the removal of toxic materials or metabolic wastes which are mostly the nitrogenous in nature like urea and uric acid etc. the high levels of these compounds may indicate an impaired renal function.

The study results of blood urea level before and after the treatment are described in Table 4.10 the mean value of blood urea level before the treatment was 35.22±6.63 mg/dl, after the 6th week the value was decreased to 34.74±6.45 mg/dl while after the 12th week it was decreased more to 34.04±6.43mg/dl.

Serum creatinine:
Creatinine is produced as a result of catabolic activities happening primarily in the muscles and under normal conditions is produced at a consistent rate breakdown product of creatinine phosphate in muscles and is usually produced at a consistent rate. The level of Serum creatinine in the blood stream gives an idea about the normal kidney function.

The values of serum creatinine obtained before and after the treatment of formulation are described in Table 4.11 the mean value of serum creatinine before the treatment was 0.90 ± 0.15 mg/dl after the 6th week without any deviation the value was 0.90 ± 0.15mg/dl while after the 12th week the value was increased to 0.93±0.15 mg/dl.

CONCLUSION:

It can be concluded from present study that *Bombyx mori* cocoons has a lipid lowering ability. The reduced levels of Cholesterol and increase in the HDL level since the HDL high density lipoproteins are considered as “good cholesterol” and LDL as “bad cholesterol” indicates that it is beneficial and safe to use against the condition of hyper lipidemia. So, the ancient claim associated with the formulations of *Bombyx mori* cocoons is true and the usage of

Bombyx mori cocoon should be encouraged since the herbal products impose lesser adverse effects and are more safe for human consumption.

Recommendation:
It is recommended that further work is required to explore and reveal the potential benefits of this herb including their extracts, dried powders with more sophisticated efficacy.

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