

**Effect of Baladi Rose Dark Red Petals
Fortification some of Buffalo Meat Products
by on Lipids Profile and Liver and kidney
Functions in Hyperlipidemic Rats.**

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**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

Introduction:

Buffalo meat is the healthiest meat among red meats known for human consumption (Abdolghafour and Saghir, 2014).

Buffalo meat is an excellent source of some nutrients. It contains 18-24% protein and 1-3% fat. It has low cholesterol (46 g/ 100 g). Buffalo meat also contains (omega 6 FA) and(omega 3 FA) which are important for human health (Uriyapongson, 2013).

Conjugated linoleic acid (CLA) has been reported to reduce serum cholesterol and so atherosclerosis and alter the low-density lipoprotein/high-density lipoprotein cholesterol, it was proved the existence of (CLA) in buffalo meat in study by (de Mendoza et al., 2005)

In recent years, several dietary and herbal formulations that have free radical scavenging potential have gained attention in treating such chronic diseases. In spite of the strong radical scavenging activity of synthetic antioxidants, they usually have side effects. Thus the interest in finding natural antioxidants, without undesirable side effects, has increased greatly (Abdel-Hameed et al., 2012).(Jin et al., 2016) study proved that there are few herbals like Chinese rose (Rosa

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

chinensis Jacq.) could be analogous or superior to green tea in antioxidant property values.

Roses are ornamental plants. Many species of roses had long been used in herbal and folk medicines to alleviate menstruation problems, treat blood circulation disorders and control cancer growth (Rezaie-Tavirani et al., 2013 and Choi et al., 2015). The results of (Kyung-Soon et al., 2015) proved that powder of Rosa (Rugosa Thunberg) reduces triglyceride concentrations in the blood, and could be used as an excellent natural antioxidant in the future.

In the food industry, organic cultured roses are known as edible flowers and phenolic compounds extracted from this plant have been used to make tea and functional beverages that exert beneficial effects on human health (Vinokur et al., 2006). Essential rose oil is rich in polyunsaturated fatty acids derived from α -linolenic acid, linoleic acid, ascorbic acid, and α -tocopherol that have a high antioxidant capacity and can regulate numerous functions (Choi et al., 2015). The results for (Ilyasoğlu., 2014) indicated that rosehip seed powder (RSP) may be utilized as a natural antioxidant in cooked meatballs.

Many studies had proven that reactive oxygen species (ROS) and free radicals play a vital role in maintaining human health. When the balance between the generating and scavenging of ROS and free radicals in vivo is destroyed, an oxidative stress would happen, which might

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

lead to extensive oxidative damage to cellular biomolecules, such as DNA, proteins, and lipids. Many chronic diseases such as hyperlipidemia, hyperpiesia, and cancer have proved to be associated with the existence of oxidative stress (Liu et al., 2010; Vamanu E and Nita S., 2013).

(Choi et al., 2015) study suggested that Rosa rugosa powder have the effect of lowering blood triglyceride levels be possible when it is used as a functional food.

So that, the aim of the current study is to study the effect of some Buffalo meat products fortified with different percentages of dried Baladi Rose dark red petals powder on hyperlipidemic rats.

Materials and methods:

- Fresh Buffalo meat (Round & Loin) obtained from Assiut slaughterhouse (animal 2 years old) and stored at -20 °C until used.

- Baladi Rose dark red Petals obtained from the Department of Horticulture in College of Agriculture at Assiut University. Petals were collected in April 2014 and dried at shade air-dried place and powdered using an electric mill, then stored in dark glass jars until use this powder without extraction.

- Preparation of meat products:

Four kilograms of meat were minced using a meat mincer. Four different were Prepared formulations

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

(meatballs 10%, meat ball 20%, luncheon 10% and luncheon 20%) as outlined in (Table 1 and 2) used without cooked.

Table (1): Formulations of Meatballs (g)*.

Ingredient	Meatballs(fortified with 10% Rose petals powder)	Meatballs (fortified with 20% Rose petals powder)
Meat	785	685
Bread	80	80
Onion	10	10
Garlic	5	5
Spice	10	10
Salt	10	10
Rose petals powder	100	200

*(Saba, 2010)

Table (2): Formulations of luncheon (g)*.

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

Ingredient	Luncheon (fortified with 10% Rose petals powder)	Luncheon (fortified with 20% Rose petals powder)
Meat	755	655
Ingredient	Luncheon (fortified with 10% Rose petals powder)	Luncheon (fortified with 20% Rose petals powder)
flour	10	10
Onion powder	10	10
Garlic powder	5	5
Spice	10	10
Salt	10	10
egg	100	100
Rose petals powder	100	200

*(Saba, 2010)

Drying sample:

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

The sample was prepared in small glass dishes and dried in hot air oven at (55-600C) for 18 hour (AOAC 934.01.) cool dishes in discator then used electric mill and turn samples into a powder .

Determination of the Total Phenolic and Flavonoid Contents in Baladi Rose petals.

The total phenolic contents in aqueous extracts were measured according to the methods described by (Abdel-Hameed, 2009).The total phenolic content of plant extracts was determined using Folin-Ciocalteu's colorimetric method. The flavonoid content was determined by aluminium chloride method using rutin as a reference compound (Liu et al., 2010).

- Animal experiment:-

The study included (100) Albino Rats, their ws ranged between (100g – 120g).They were obtained from the animal house of the faculty of Medicine, Assiut University. The animals were housed as groups in wire cages under the normal laboratory conditions ,the rats were divided into (10) groups, each group consisted (10) rats and were fed on basal diets for two weeks as adaptation period. Then the rats were fed on high fat diet for three weeks then fed on treatment martials for four weeks. The animals were group-housed and kept in a regulated environment at $25 \pm 1^{\circ}\text{C}$ and $60 \pm 5\%$ relative humidity under (12 h) light/ (12 h) dark

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

conditions. The animals had free access to water and normal or high-fat diet.

Group (1): the negative control group (fed on basal diet)

Group (2): the positive control group (fed on fat rich diet)

Group (3): fed on fat rich diet in addition to 10 % powder of buffalo meat.

Group (4): fed on fat rich diet in addition to 20% powder of buffalo meat

Group (5): fed on fat rich diet in addition to 10 % powder of Baladi Rose Petals

Group (6): fed on fat rich diet in addition to 20 % powder of Baladi Rose Petals

Group (7): fed on fat rich diet in addition to 10 % powder of fortified luncheon with 10 % powder of Baladi Rose Petal

Group (8): fed on fat rich diet in addition to 10 % powder of fortified luncheon with 20 % powder of Baladi Rose Petals

Group (9): fed on fat rich diet in addition to 10 % powder of fortified meat balls with 10 % powder of Baladi Rose Petals.

Group (10): fed on fat rich diet in addition to 10 % powder of fortified meat balls with 20 % powder of Baladi Rose Petals.

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

Table (3): Ingredients of Basal Diet*:

Ingredients	Percentage
Corn starch	67.8 %
Casein	12.5 %
Ingredients	Percentage
Corn oil	10 %
mixture of vitamins	1 %
mixture of salt	3.5 %
Choline Chloride	0.2 %
Cellulose	5 %

*(AIN): American Institute of Nutrition

**High fat diet =basel diet +1%cholesterol+0.7cholic acid.(
Gollisch et al, 2009).**

- Preparation of Biochemical Samples:

After the experiments, the blood samples were collected. The collected blood was placed 30-40 min for clot formation, and then the serum was separated by centrifugation at 3000 rpm for 15 min.

- Measurement of Biochemical parameters:

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

The activity of serum aminotransferases (ALT and AST) was determined according to the kinetic method described by Schumann and Klauke (2003) using reagent kits purchased from AL-GOMHORIA COMPANY for diagnostic materials under trade mark from Human Gesellschaft für Biochemical und Diagnostic mbH, Germany.

Serum total cholesterol and triglycerides levels were determined according to Cole et al. (1997) respectively, using DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Serum HDL-cholesterol and LDL-cholesterol levels were determined according to Lopes-Virella et al. (1977) and Wieland and Seidel (1983) respectively, using DiaSys reagent kits purchased from AL-GOMHORIA COMPANY for diagnostic materials under trade mark from DiaSys Diagnostic System GmbH, Germany

Serum creatinine activity was determined according to the kinetic method described by Bartels and Bohmer (1973) respectively, using DiaSys reagent kits purchased from AL-GOMHORIA COMPANY for diagnostic materials under trade mark from DiaSys Diagnostic System GmbH, Germany.

Serum urea activity was determined according to the enzymatic colorimetric method described by Fawcett and Scout (1960) respectively, using DiaSys reagent kits purchased from AL-GOMHORIA COMPANY for

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

diagnostic materials under trade mark from DiaSys Diagnostic System GmbH, Germany

Serum uric acid level was determination according to the method of (HOWORTH and ZILVA 1968) respectively, using DiaSys reagent kits purchased from AL-GOMHORIA COMPANY for diagnostic materials under trade mark from DiaSys Diagnostic System GmbH, Germany

- Statistical Analysis

All data were expressed as mean \pm S.D The significance of differences between the means of the treated and un-treated groups had been compared by one-way analysis of variance (ANOVA), followed by Student's T test and P values less than 0.05 were considered significant (SAS, 1996).

Results:

People become more and more aware of the importance of taking care of their health by using healthy food that results in the need for new healthy products. One of the solutions is to enrich the favourite food with biologically active substances. By adding fiber-rich ingredients, the food value of the product increases. (Preedy et al., 2011).

-Total phenolic, flavonoids in Baladi Rose petals:

The total phenolic contents and biological activity were reported in some Rosa species (Yassa et al., 2009; Abdel-Hameed et al., 2012b; Baydar & Baydar, 2013).

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

**Table (4): Total amount of Total phenolic, flavonoid in Baladi
Rose petals (mg/100g) *:**

Sample	Total phenolic	Flavonoids
<i>Baladi Rose petals</i>	112.0	36.47

*on dry base.

Serum Total Cholesterol, Triglyceride, HDL-C & LDL-C (mg/dL):

Results recorded in Table (5) demonstrated the effect of feeding some Buffalo meat products fortified with different percentages of dried Baladi Rose petals for (4) weeks on serum Total Cholesterol, Triglyceride, HDL-c and LDL-C for rats induced to hyperlipidemia.

Results demonstrated that hyperlipidemic rats (positive control group) showed significant ($P<0.05$) higher values of serum Cholesterol, Triglyceride and LDL-C but significant ($P<0.05$) lowered HDL-C as compared to negative control group significant at ($P<0.05$).

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

Rats fed on two Buffalo meat with different concentrations(10%,20%) and rats fed on Baladi Rose petals with different concentrations(10%,20%) demonstrated a significant at P (0.05) increase values of HDL-C as compared to positive control group. On other hand, the mean values of serum TC, TG, and LDL-C were significantly decreased at (P<0.05) in the group fed on diets supplemented with Buffalo meat products fortified with different percentages of dried Baladi Rose petals, as compared to control positive group.

Table (5): Effect of feeding different meat products fortified with different percentages of dried Baladi Rose petals on lipid profile of hyperlipidemic rats (M±SD)*.

Biochemical Parameter Mg/dl		Cholesterol	HDL-c	LDL-c	Triglyceride
negative control group		100.14±3.02 ^a	32.2±1.2 ^b	49.4±2.5 ^a	92.7±3.1 ^a
positive control group		121.0±10.5 ^c	30.0±1.3 ^{ab}	62.0±2.5 ^f	144.2±11.4 ^f
buffalo meat Powder	10%	106.1±8.1 ^b	31.3±1.3 ^{ab}	53.5±3.5 ^{bcd}	125.1±2.8 ^e
	20%	105.0±5.3 ^b	31.2±1.3 ^a	52.0±2.9 ^{ab}	122±5.7 ^a
Baladi Rose Petals Powder	10 %	106.3±11.4 ^b	31.2±1.6 ^{ab}	52.7±1.6 ^{abc}	109.1±7.3 ^d
	20 %	103.1±4.8 ^a	31.2±1.8 ^a	50.66±2.03 ^{abc}	101±2.3 ^{bc}
10 % powder of fortified hamburger with	10 % powder of Baladi Rose Petal	107.4±4.6 ^b	31.2±1.4 ^{ab}	54.6±2.9 ^{cd}	101.9±2.9 ^{cd}
	20 % powder of Baladi Rose Petal	104.5±5.2 ^a	31.7±1.7 ^{ab}	52.06±4.7 ^{ab}	94.7±5.3 ^{ab}
powder of meat balls with	10 % powder of Baladi Rose Petals	107.7±10.2 ^b	31.1±1.7 ^{ab}	56.7±3.2 ^a	99.7±6.2 ^{abc}
	20 % powder of				

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

The results of lipid profile were recorded Levels close to each other for treated groups its showed significant improvement compared with positive group

Serum alanine aminotransferase (ALT) and Aspartate aminotransferases (AST) activities (IU/l):

Results in Table (6) illustrated the effect of different levels of buffalo meat, Baladi Rose Petals powder and buffalo meat products on serum concentration of AST and ALT as indicators of liver functions.

The obtained data showed that animals fed on the high fat diet recorded significant increase in serum ALT and AST activities compared with negative control group. On contrast, rats fed on high fat diet and treated with buffalo meat powder or Baladi Rose Petals powder alone in concentration 20% showed significant decreases in serum ALT and AST; as compared to positive control group. It should be noted that, both of ALT and AST still higher in different treated groups with fortified buffalo meat products with concentration 10% than that of treated groups with fortified buffalo meat products with concentration 20%. Even significant differences were observed between them.

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

Table (6): Effect of feeding different meat products fortified with different percentages of dried Baladi Rose petals on serum ALT and AST activity (M±SD)*

Groups		Parameter IU/L	ALT	AST
negative control group			23.6±2.1 ^a	29.1±3.1 ^a
positive control group			54.1±3.6 ^f	61.4±3.9 ^g
buffalo meat Powder	10%		44.9±3.3 ^{cd}	49.3±5cd ^e
	20%		37.1±3.4 ^b	45±4.1 ^c
Baladi Rose Petals Powder	10%		41.8±8.9 ^c	49±2.4 ^{cd}
	20%		35.3±4.1 ^b	39.9±4.1 ^b
10 % powder of fortified luncheon with	10 % powder of Baladi RosePetal		44.0±4.2 ^{cd}	49.1±2.4 ^{cde}
	20 % powder of Baladi RosePetal		37.1±3.8 ^b	39.3±2.8 ^b
10 % powder of fortified meat balls with	10 % powder of Baladi RosePetal		45.5±2.3 ^{cd}	44.1±3 ^{cd}
	20 % powder of Baladi Rose Petal		33.5±3.8 ^b	33.7±3.7 ^b

* All data are expressed as mean ± standard deviation (M±SD).

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

* The dissimilar superscript letters within the same column are significantly different at $p < 0.05$

Serum Creatinine, Urea & Uric Acid (mg/dl):

Results illustrated in Table (7) demonstrated the Effect of feeding different meat products fortified with different percentages of powder Baladi Rose Petals for (4) weeks at on serum Creatinine, Urea and Uric Acid of hyperlipidemic rats.

The obtained results revealed that animals induced to high fat diet recorded significant increase in serum Creatinine, Urea and Uric Acid as compared to negative control group. On contrast, all hyperlipidemic groups treated with powder Baladi Rose petals alone or combination with meat products (luncheon and meat balls) tended to have serum Creatinine, Urea and Uric Acid much lower than positive control group. It's also noticed that hyperlipidemic group treated with buffalo meat powder alone tended to have serum Creatinine, Urea and Uric Acid much lower than positive control group and higher from another groups. Even, significant differences were observed between treated groups and not treated groups. No significant differences has been detected for serum Creatinine between control negative group (healthy group) and treated groups with 10 % powder of fortified meat balls with 10 and 20 % powder of Baladi Rose Petals.

Discussion:

Hyperlipemia can cause some largest endocrine disease in the world, involving metabolic disorders of carbohydrate, fat and protein. Therefore, it is necessary to search for new drugs that can be used to amend this metabolic disorder without any side effect. Oxidative stress is currently suggested as a mechanism underlying hyperlipidemia, which is one of the major risk factors for coronary artery diseases (Liu et al., 2010 and Kyung-Soon et al., 2015)

The term hyperlipidemia is applied when the plasma cholesterol or triglycerides levels are increased over normal level. The degree of cholesterol or triglyceride elevation is a reflection of altered lipoprotein levels, which are often classified as lipoprotein phenotypes. Hyperlipidemia is caused by a variety of disorders, which identifies specific lipoprotein disorders that are associated with a variety of clinical features, the most prominent are atherosclerosis, pancreatitis, and xanthoma formation. These disorders are usually identified by measuring the plasma cholesterol and triglyceride level after 12-to14-hours fasting (Kelly et al, 2000 and Hari Kumar et al., 2013).

In Liu et al., (2010) study, TC, TG and LDL-C levels significantly increased in the hyperlipidemic animals fed on high-fat diet for 4 weeks. The liver is capable of removing cholesterol from the blood circulation as well as

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

manufacturing cholesterol and secreting cholesterol into the blood circulation. And liver damages are generally induced in the condition of hyperlipidemia as dramatic increase of serum ALT and AST levels. Many reports attributed the biological properties of roses to its high contents of phenolic compounds (Ng et al., 2004; Cai et al., 2005; Yassa et al., 2009; Amir et al., 2011; Abdel-Hameed et al., 2012b; Baydar and Baydar ., 2013 ;Abdel-Hameed et al., 2013).

Plant-derived foods contain a broad spectrum of secondary plant metabolites such as polyphenols that inhibit human low density lipoprotein oxidation, thus are made responsible for the beneficial effects on human health (Ginova et al., 2013)

As seen in the current study results, the serum levels of TC, TG, and LDL-C were significantly increased in rats fed with high-fat diet compared with other groups. The levels of TC, TG and LDL-C of those animals fed with fortified products

were significantly decreased compared with hyperlipidemic animals. Although there was no statistically significant difference in the serum HDL-C levels between normal and hyperlipidemia rats and animals fed with fortified products, an increasing tendency was exhibited in the groups administrated with fortified products. In summary, the administration of antioxidant compounds in meat products fortified with Baladi Rose powder could decrease the serum

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

levels of TC, TG and LDL-C these results were in agreement with (Liu et al., 2010;Kyung-Soon et al., 2015; Murathan et al., 2016) ,despite differences in Rose species used in their studies , It is noted in this study the convergence of the decline in TC levels in the buffalo meat and Baladi Rose powder groups and this may be due its contain greater concentration of conjugated linoleic acid (CLA)(1.83 mg/g fatty acid)(de Mendoza et al., 2005) ,CLA has been reported to reduce atherosclerosis and total serum cholesterol, and to alter the low-density lipoprotein/high-density lipoprotein cholesterol ratio in rabbits (Lee et al .,1994).

In the present study, the damage of liver induced by high-fat diet was also investigated, which included hepatic enzymes and results showed that animals treated with the high fat diet recorded significant increase in serum ALT and AST activities compared with animal control group. While the rats treated with high fat diet in combination with rats fed on meat products fortified with Baladi Rose powder , individually or in combination showed significant decreases in serum ALT and AST; respectively when both were compared to those treated with high fat diet only These results are consistent with (Liu et al., 2010).

The results suggested that Anti-oxidant compounds content(total poly phenols , flavonoids) in petals powder of Baladi Rose was probably a key factor that resulted a

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

decrease of lipid profile and hepatic enzymes which agree with studies of (Taneva et al., 2016; İlyasoğlu H .,2014).

There therefore a fundamental requirement to decrease the levels of lipids. The evolution of hyperlipidemia is associated with oxidative stress as total serum cholesterol is really an indicator of the amount of the free radical damage in the body. Higher the free radical level, higher the body needs to produce more cholesterol internally from liver to act as an antioxidant and free radical scavenger (Salama and Ibrahim 2015). Dry Baladi Rose could be used as preventive and therapeutic effective natural agents for diseases in which free radicals are involved after more in vivo and in vitro studies.

Experimental studies suggest that high lipid levels also may promote progression of the renal disease (Kao et al., 2010).

Abnormal blood cholesterol may be the first and only sign for a few years of live. High cholesterol levels are seen in renal failure also. (Wadhaw, 2003).

Though initial events involved in lipid-mediated renal damage are unclear, oxidative stress is thought to be especially important. Hyperlipidemia causes significantly higher rates of monocyte reactive oxygen species (ROS) generation, which is strongly associated with impairment of endothelium-dependent relaxation and elevated plasma levels of Ox-LDL. Arteries from hypercholesterolemic

**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

animals produced significantly higher rates of oxygen radical than control arteries (Ruan et al., 2014).

It is what has been seen in our results, whereas hyperlipideimic group had a sever disorder kidney functions at variance that it was noticeable improvement in renal function with decrease lipids in groups serum which had treated with fortified buffalo meat products by Baladi Rose powder, this is due to the products content of antioxidant factors like(total poly phenols, flavonoids and conjugated linoleic acid (CLA)).

Conclusion:

The main objective of the present study is to shed more light on the specific benefits of buffalo meat products fortification with Baladi Rose petals and give access to the best mixing ratio to get the highest impact on control blood lipid level and to become having the ability of therapeutic options for treatment of hyperlipidemia.

Higher dose of Baladi Rose petals powder is safe to liver and kidney function in hyperlipidmic rats and acts as effective hypolipidemic and hypocholesterolemic agent, in rats fed on high cholesterol diet.

The observations from the results of the study showed that the use of buufalo meat products fortified with Baladi Rose petals inhibition cholesterol and cholesterol fractions that can be beneficial in reducing the risk of cardiovascular diseases.

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**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

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**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

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**THE EFFECT OF SOME HERBAL BEVERAGES ON
THE LEVEL OF ADIPONECTIN HORMONE,
GLUCOSE AND LIPID PROFILE OF OBESE RATS**

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