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BIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF SOME TYPES OF PRICKLY PEAR IN AL-BAHA AREA ON HYPERLIPIDAEMIC RATS

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ABSTRACT

Hyperlipidaemic is the presence of high levels of lipids in the blood. It is not a disease but a metabolic derangement, which can be caused by various diseases, especially cardiovascular diseases. It is closely correlated with the terms "hyperlipidemia" (increased levels of lipids in the blood) and "hyperlipoproteinemia" (increased levels of lipoproteins in the blood). Therapeutic properties of prickly pear have very long been known in traditional medicine, however, the potential activities of the fruit, beyond nutritional benefits, have recently been explored. So, this study was done to evaluate the biological and immunological effects of some types of prickly pear in the Baha area on hyperlipidaemic rats. The study used (30) white Albino rats that were divided into 2 main groups; the first set of mice were infected with high levels of lipids and the second group was the negative control, contained non-infected mice. Then, the groups were divided into 5 sub-groups, including 4 groups fed with different concentrations of (5%, 10%, 15%) prickly pear and 1 control positive group infected with the disease and did not feed on the experimental diet and another negative control non-infected with this disease; means that all mice w were divided into five groups in each group of 6 mice. The results showed insignificant differences in HDL between all the groups of prickly pear and positive control, also no significant differences between both 5% and 10% prickly pear in triglyceride when compared with the negative control. Moreover, the results showed significant differences between all groups of prickly pear in serum IgM when compared with the control negative. The results indicated non-significant differences in total immunoglobulin between 10% prickly pear and 5% prickly pear groups when compared with the control negative. This study recommends using prickly pear for lowering LDL.

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Introduction

Prickly pear is a member of the *Opuntia* genus prickly pear tree is typically grown in sandy regions as the tree tolerates lack of water. Trees are grown for their fruits and also as windbreakers and fences to control erosion in deforested areas [1]. The chemical composition of prickly pear fresh is 1.56%, 5.81%, 0.10%, 87.70%, 4.28%, and 0.65% for protein, carbohydrates, crude fat, moisture, crude fiber, and ash, respectively [2]. **Butera et al., (2002)** reported that the oxidative stress induced by reactive oxygen species (ROS) has been concerned in over a hundred disease states, ranging from arthritis and connective tissue disorders to acquired immunodeficiency syndrome, infection, neurodegenerative disorders, aging, and carcinogenesis [3]. Antioxidants are helpful to scavenge these ROS and restore the physiological homeostasis The cactus forms a very good source of natural antioxidants.**Frati et al. (1990)** showed a statistically significant reduction of blood glucose and insulin levels when broiled prickly pear was administered to volunteers with non-insulin-dependent diabetes mellitus (NIDDM) also they showed that in NIDDM patients, a decrease in glycemia levels was noticed with crude and boiled blended preparations of prickly pear [4]. Dietary fibers such as pectin isolated from prickly pear fruits reduce the plasma LDL-c in animal models fed with high cholesterol diet. The natural antioxidant pigments found in prickly pear namely betalains protected LDL-c from oxidations. Hyperlipidaemic is the presence of high levels of lipids in the blood. It is not a disease but a metabolic derangement that can be caused by many diseases, especially cardiovascular diseases. It is closely correlated with the terms "hyperlipidemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (increased levels of lipoproteins in the

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blood) Therapeutic properties of prickly pear have very long been known in the traditional medicine, however, potential activities of the fruit, beyond nutritional benefits, have been explored only recently [5].

Aim of Study:

This work aimed to show the biological and immunological effects of some types of prickly pear in the Baha area on hyperlipidaemic rats

Materials and Methods:

Materials:

A- preparation of prickly pear: Washed and cleaned.

Drying was performed without exposure to heat or sunray in a clean place at room temperature with good airing for 7 days. Then it was stored in polyethylene bags far from moisture or heat, and kept until fresh crushing and admixtures with experiment diet.

B- Experimental animals: Thirty-six male Sprague Dawley albino rats, weighing 150±10g were used in the study.

Methods:

A- Biological experiment

Basal diet composition of tested rats:

The basal diet in the experiment consisted of casein (10%), com oil (10%), vitamin mixture (1%), salt mixture (4%), choline chloride (0.2%), cellulose (5%), methionine (0.3%), and the remained is corn starch (69.5%) according to [6] as seen in Table (1)

Table 1: Composition of basal diet

Ingredients	Amounts
Protein (casein)	10%*
Corn oil	10%
Mineral mixture	4%
Vitamin mixture	1 %
Cellulose	5%
Choline chloride	0.2 %
Methionine	0.3 %
Corn starch	Up to 100%

* 12.3g casein gives l0g protein.

Source: [6]

Table 2: Composition of salt mixture%:

Compound	**Amount**
$CaCO_3$	600 mg
K_2HPO_4	645 mg
$CaHPO_4.2H_2O$	150 mg
$MgSO_4.2H_2O$	204 mg
NaCl	334 mg
$Fe(C_6H_5O_7)26H_2O$	55 mg
KI	1.6 mg
$MnSO_4.4H_2O$	10 mg
$ZnCl_2$	0.5 mg
$CuSO_4.2H_2O$	0.06 mg

Source**: [7]**

Table 3: Composition of vitamin mixture:

Vitamin	Amount
Vitamin E	10 Iu
Vitamin K	0.50 Iu
Vitamin A	200 Iu
Thiamin	0.50 mg
Riboflavin	1.0 mg
Pyridoxine	0.40 mg
Niacin	4.00 mg
Vitamin C	20.0 mg
Pansonic acid	4.0 mg
Vitamin D	100 Iu
Choline chloride	200 mg
Folic acid	0.02 mg
Inositol	25 mg
Para- amino- benzoic acid	0.02 mg
Vitamin B12	2.00 mg
Biotin	0.02 mg
Corn starch	Up to 100 g

Preparation of hypercholesterolemic rats

Normal healthy adult albino rats were fed on a special diet for inducing hyperlipidemia for 3 weeks according to the method described by Rashwan, 1994 Normal rats fed a special diet for inducing hypercholesterolemic, the diet was prepared from fine ingredients per 100g according to [8].

Experimental Design and Animal Groups:

36 white male Sprague Dawley albino rats, weighing about 150 ± l0g were used in the study. Rats were housed in wire cages under the normal laboratory condition and fed on a basal diet for 1 week as the adaptation period. Water was given using glass tubes projecting through the wire cage from an inverted bottle supported to one side of the cage and diet was given in non-scattering feeding cups to avoid contamination or loss of food.

The rats were divided into 5 groups each with six rats. The groups of rats were as follows:

- Group (1): Control negative group, in which the normal rats fed on a basal diet (control"-").
- Group (2): hypercholesterolemic control positive group, in which alloxan injected rats fed on a basal diet (control"+")
- Group (3): hypercholesterolemic group fed on 5% Prickly pear.
- Group (4): hypercholesterolemic group fed on 10% Prickly pear.
- Group (5): hypercholesterolemic group fed on 15% Prickly pear.

Biological evaluation:

During the experimental period (28 days), the consumed feed was recorded every day, and body weight recorded weekly. The body weight gain (B.W. G. %), food efficiency ratio (F.E.R), and also organs weight were determined according to [9].

Blood sampling:

Blood samples were collected at the end of the experiment after 12-hour fasting. Using the retro-orbital method by means of microcapillary glass tubes, blood was collected into a dry clean centrifuge tube and left to clot in a water bath (37ºC) at room temperature for 30min. The blood was centrifuged at 3000 rpm for 10 minutes to separate the serum; a part of which was subjected to glucose determination and the reminder was carefully aspirated and transferred into clean quit fit plastic tubes and stored at -20ºC until analysis. The spleen, heart, kidney, and liver were removed and washed with saline solution, weighted and kept in 10%formalin according to [10].

- **Biological evaluation:**

Biochemical analysis:

1) Measurement of serum glucose: serum glucose was determined using chemical kits according to [11]

2) Determination of serum lipids:

2.1) Triglycerides:

Enzymatic calorimetric determination of triglycerides was performed as described by [12]

2.2) Total cholesterol

Determination of the principal use of the total cholesterol was done according to [13]

2.3) HDL-cholesterol:

Magnesium ions and phosphotungstic acid selectivity precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant was determined by the same method used for total cholesterol, according to [14].

2.4) V-LDL and LDL- cholesterol:

The determination of VLDL (very low-density lipoproteins) and LDL was done as described by (***Lee and Nieman, 1996)***

3) Determination of liver functions:

3.1) Determination of Alanine transferase (ALT):

ALT was determined as described by [15].

ALT catalyzes the transfer of the amino group from L-alanine to a-Ketoglutarate leading to the formation of L-Glutamate and pyruvate.

Lactate dehydrogenase catalyzes the simultaneous oxidation of NADH to NAD and the reduction of pyruvate. The resulting rate of absorbance reduction is directly related to ALT activity.

3.2) Determination of Aspartate Transferase (AST):

The determination of AST was done as described by ***Henry (1974)*** **[16].**

4- Determination of Some parameters for:

4.1) Determination of Creatinine

Creatinine was determined according to the kinetic method of [16], by the following reaction:

4.2) Determination of urea:

Urea was determined according to the enzymatic method described by ***Patton and Crouch (1977)*** [17].

5) Determination of immunity indices

6) Organs weight:

After taking retro-orbital blood samples, each rat was rapidly opened, the organs (liver, kidney, heart, spleen, lungs, brain were removed and washed in saline solution, weighed and kept in 10% V/V formalin solution as described by [10]; then it was compared with the control group.

7-Statistical Analysis:

Statistical analyses were calculated using a one-way classification. Analysis of variance (ANOVA), and least significant difference (LSD) according to [18].

Results and Discussion

This work aimed to show the biological and immunological effects of some types of prickly pear in Al-Baha area on hyperlipidaemic rats.

Table (4) demonstrates the effect of feeding different concentrations of prickly pear on serum total cholesterol, triglyceride, LDL, and HDL in both normal and hyperlipidaemic rats after 4 weeks of feeding.

- ***Serum total cholesterol*** in normal rats group was 162.54±12.23 mg/dl, while hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, 15% prickly pear) showed serum total cholesterol values (354.33±19.45, 288.25±14.36, 314.22±13.25, and 336.88±11.35) mg/dl, respectively. The statistical analysis showed non-significant differences between both 5% and 10% prickly pear when compared with the negative control.

- ***Serum triglyceride*** value in normal rat group was 86.32± 10.41 mg/dl, while in hyperlipidaemic rat groups fed on basal and supplemented diets with different levels of prickly pear was (292.52±12.31, 258.20±46.35, 270.45±21.02, and 285.25±13.10) mg/dl, for (positive control, 5%, 10%, 15% prickly pear), respectively. The results showed non-significant differences between both 5% and 10% prickly pear when compared with the negative control.

- ***Serum LDL*** in the normal rat group was 102.24±4.30 mg/dl. Hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, 15% prickly pear) showed serum LDL values of 189.15±8.15, 155.24±1.25, 163.28±6.10, and 178.4±7.10 mg/dl, respectively. The results showed non-significant differences between 5%, 10%, and 15% prickly pear and negative control.

- ***Serum HDL*** in the normal rat group was 72.65± 0.02 mg/dl. While hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, 15% prickly pear) showed serum HDL values of (33.80±0.05, 55.14±0.05, 49.99±0.02, and 34.83±0.02) mg/dl, respectively. The results proved insignificant differences between the prickly pear and positive control groups.

The obtained results are in line with those reported by [19] who assessed the effects of prickly pear chromium on 40 people with high cholesterol, or placebo for 2 months.

Table 4: Effect of 5%, 10%, and 15% prickly pear on the total cholesterol, triglyceride, LDL, and HDL of hyperlipidaemic rats

Groups / Parameters	Control (-ve)	Control(+ve)	5% Prickly pear	10% Prickly pear	15% Prickly pear
Total cholesterol (mg/dl)	162.54 ± 12.23[a]	354.33 ± 19.45[b]	288.25 ±14.36°	314.22 ± 13.25[b]	336.88±11.35[b]
Triglyceride (mg/dl)	86.32 ± 10.41[a]	292.52 ± 12.3 l[c]	258.20 ±46.35[b]	270.45 ± 21.02°	285.25±13.10[c]
LDL (mg/dl)	102.24± 4.30[a]	189.15 ± 8.15[c]	155.24 ± 1.25[b]	163.28 ±6.10°	178.4± 7.10[C]
HDL (mg/dl)	72.65± 0.02[a]	33.80 ±0.05°	55.14 ±0.05[b]	49.99 ± 0.02[b]	34.83± 0.02°

Total Cholesterol (Best ■ <200 mg/dL, Borderline high = 200-239 mg/dL, High = 240 mg/dL or higher)
Triglycerides (Best = <150 mg/dL, Borderline high = 150-199 mg/dL, High = 200-499 mg/dL, Very high = 500 mg/dl or higher)
LDL Cholesterol (' Best = <100 mg/dL, Good = 100-129 mg/dL, Borderline high = 130-159 mg/dL, High = 160-189 mg/dL, Very high = 190 mg/dL or higher)
HDL Cholesterol (Low = <40 mg/dL, Best = 60 mg/dL or higher)

Table (5) illustrates the effect of different concentrations of 5%, 10%, 15% prickly pear on the liver function of hyperlipidaemic rats.

- ***Aspartate amino transaminase (AST)*** for the control (-) was (19.22±0.15) IU/L. While hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% prickly pear) showed serum AST values of (87.25±3.24, 46.22±5.25, 59.22±1.14, and 71.22±2.22) 1U/L, respectively. The results proved a significant difference in the AST values between the groups of prickly pear and the negative control.

- ***Alanine amino transaminase (ALT),*** in the normal rat group, was (31.77±6.18) IU/L. While hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% prickly pear) showed serum LDL values of (101.46±7.11, 63.45±3.25, 77.77±3.30, and 92.86±5.46) IU/L, respectively. The statistical analysis proved significant differences between all groups of prickly pear when compared with the negative control.

- ***Alkaline phosphate (ALP)*** in the normal rat group was (96.45± 4.45) IU/L. While hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% prickly pear) showed serum ALP values of (220.5±11.40, 168.29±9.74, 181.20±5.7, and 198.36±2.67) IU/L, respectively. The results showed significant differences between 5%, 10%, and 15% prickly pear when compared with the negative control.

- ***Total bilirubin,*** it could be concluded that TBIN in the normal rat group was (0.55±0.05) mg/dl. The hyperlipidaemic rat groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% prickly pear) showed serum TBIN values of (2.98±0.03, 2.35±0.03, 2.77± 0.03, and 2.98± 0.03) mg/dl, respectively.

- ***Albumin,*** it could be observed that albumin in the normal rat group was (4.85±0.23) g/dl. While hyperlipidaemic rats groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% prickly pear) showed serum Alb values of (2.10±0.45, 2.81±0.10, 2.69±0.11, and 2.44±0.3) g/dl, respectively. Significant differences were found between 5%, 10%, and 15% prickly pear when compared with the negative control.

Table 5: Effect of feeding different concentrations of Prickly pear on liver functions of hyperlipidaemic rats

Groups / Parameters	Control (-ve)	Control(+ve)	5% Prickly pear	10% Prickly pear	15% Prickly pear
SGOT (IU/L)	19.22± 0.15[a]	87.25± 3.24[d]	***46.22*± 5.25[b]	59.22± 1.14[c]	71.22± £.22[d]
SGGT (IU/L)	31.77± 6.18[a]	101.46± 7.11[d]	63.45±.3.25[b]	• 77.77± 3.30[c]	92.86± 5.46[d]
ALP (IU/L)	96.45± 4.45[a]	220.5± 11.40[d]	168.29± 9.74[b]	1S1.20± 5.7[C]	198.36± 2.67[c]
TBIN (mg/dl)	0.55± 0.05[a]	2.98± 0.03°	2.35±0.03[b]	2.59± 0.03[c]	2.77±0.03[c]
Alb (g/dl)	4.85± 0.23[a]	2.10± 0.45°	2.81±0.10[b]	2.69± 0.1 l[b]	2.44± 0.30°

Data are as Mean ± SE IU/L International Unit per Liter. Alb (Albumin) - Normal Range 3.4 - 5.4 g/dl; TBIN (Total Bilinibin) - Normal Range 03-1.9 mg/dl; ALP (Alkaline Phosphatase) - Normal Range 44-147 IU/L; SGPT (Serum Gamma Glutamyl Transpeptidase) - Normal Range 1-50 IU/L; SGOT (Serum Glutamic-Oxaloacetic Transaminase) - Normal Range 10-34 IU/L.

Table (6) illustrates the effect of feeding different concentrations of prickly pear on Kidney Function in both normal and hyperlipidaemic rats after 4 weeks of feeding.

- *Creatinine and BUN* levels were (0.70±0.02 and 10 12±0.55) mg/dl for the normal rat group. While hyperlipidaemic rats groups fed on basal and supplemented diets with different levels of prickly pear showed that creatinine levels were (2.79±0.11, 2.02±0.04, 2.39±0.01, and 2.56±0.02) mg/dl for (positive control, 5%, 10%, and 15% Prickly pear), respectively after 4 weeks of feeding on basal and supplemented diets with different concentrations of prickly pear. The same Table indicated that the creatinine level showed a gradual decrease with the increase in the concentration of Prickly pear. The results proved significant differences between all groups of prickly pear when compared with the negative control. The same trend was also observed for BUN; hyperlipidaemic rat groups fed on basal and supplemented diets with different levels of prickly pear showed that BUN levels were (16.85±0.45, 11.55±0.15, 12.86±0.01, and 15.20±0.12) mg/dl for (positive control, 5%, 10%, and 15% prickly pear), respectively. The results showed significant differences between all of RGS when compared with the negative control. The present results are going in the same line with those of [20]. They noticed the effect of proanthocyanidin on rats with renal ischemia-reperfusion. This was due to the increase of thiobarbituric acid (TBA) in the kidney and alteration of the activity of antioxidant enzymes (dismutase, catalase, and glutathione peroxides).

Table 6: Effect of feeding different concentrations of prickly pear on the kidney functions of hyperlipidaemic rats.

Groups / Parameters	Control (-ve)	Control(+ve)	5% Prickly pear	10% Prickly pear	15% Prickly pear
Creatinine (mg/dl)	0.70± 0.02[a]	2.79± 0.1 l[d]	2.02± 0.04[b]	2.39± 0.0 l[c]	2.56± 0.02[d]
BUN (mg/dl)	I0.12±0.55[a]	16.85± 0.45[d]	11.55± 0.15[b]	12.86± 0.40°	15.20± 0.12[d]

Data presented as Mean ± SE Normal Range of Creatinine is 0.6 - 1.2 mg/dl BUN (Blood Urea Nitrogen) - Normal Range 7-20 mg/dl

Table (7) presents the effect of feeding different concentrations of prickly pear on immunity indices, serum IgG, serum IgM, and the total immunoglobulin in normal and hyperlipidaemic rats after 4 weeks of feeding.

- *Serum IgG* in the normal rat group was (2850.5± 350.10) U/ml. While hyperlipidaemic rats groups fed on basal and supplemented diets at different levels (positive control, 5%, 10%, and 15% Prickly pear) showed serum IgG values of (2400.5±160.40, 3550.2±60.35, 2880.650±40.30, and 2550.45±30.22) U/ml, respectively. Significant differences between groups of prickly pear were found when compared with the negative control.

Serum IgM value in the normal rat group was (380.92±15.12) U/ml. While in hyperlipidaemic rats groups fed a diet with different levels of prickly pear it was (330.25±25.3, 465.30±65.15, 425.50±55.20, and 370.25±20.40) U/ml, for (positive control, 5%, 10%, and 15% prickly pear) U/ml, respectively. The results showed significant differences between all groups of prickly pear when compared with the control negative.

- *Total Immunoglobulin* value in the normal rat group was (3650.25±200.5) U/ml. While in hyperlipidaemic rat groups fed on basal and supplemented diets with different levels of prickly pear it was (3550.35±25.3, 4200.80±30.11, 3950.30±40.25, and 3595.45±22.4) U/ml, for (positive control, 5%, 10%, and 15% prickly pear) U/ml, respectively. The results showed non-significant differences between 10% prickly pear and 5% prickly pear when compared with the control negative. This result is in agreement with [21].

Table 7: Effect of feeding different concentrations of prickly pear on immunity indices of hyperlipidaemic rats.

Groups / Immunity Indices	Control (-ve)	Control(+ve)	5% Prickly pear	10% Prickly pear	15% Prickly pear
IgG (U/ml)	2850.5 ± 350.10[b]	2400.5 ± 160.40[c]	3550.2±60.35[a]	2880.650±40.30[b]	2550.45^0.22°
IgM (U/ml)	380.92± 5.12[C]	330.25 ± 25.3[b]	465.30±65.15[a,]	425.50±55.20[b]	370.25±20.40[c]
Total Immunoglobulin (U/ml)	3650.25 ±200.5°	3550.35 ± 55.8[C]	4200.80±30.11[a]	3950.30±40.25[b]	3595.45±22.4°

Recommendations

1. It is suggested to use different levels of prickly pear for hyperlipidaemic patients.
2. Different levels of prickly pear, especially 15%, may be used for the remedy of liver disorders.
3. Different levels of prickly pear may be suggested for lowering LDL.
4. Future studies may be suggested to evaluate the efficacy and advantage of using prickly pear as extracts versus dried powder.

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