

Nutritional Effect of Mango Juices Inoculated with Probiotic Strain of *Lactobacillus plantarum* NRRL4496 as A Source of Probiotic on Hypercholesterolemic Rats

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ABSTRACT

The present study showed that probiotic enriched mango Juice can be used as a healthy food product due to its great amount content of health components like vitamins, antioxidant and polyphenols. Moreover, *Lactobacillus plantarum* NRRL4496 which were used as animal diet, Rats were classified into two groups, one received normal diet while the second fed with high cholesterol diet. Two groups were received with their diet mango juice or mango juice propagated with *L. plantarum* NRRL4496. The effect of those supplementations on the growth parameters, liver and plasma lipid profile and hematology were followed up after four weeks. Final body weight (FBW), gain in body weight (GBW). Daily food intake (DFI) and Food efficiency ratio (FER) were determined in rats received normal or high cholesterol diet. No significant differences were found in the (FBW) between rat groups. The highest increase in the (FBW) was for rats fed regular mango juice while, the control group was the lowest. On contrary. There was a significant decrease in the liver cholesterol concentration, plasma total cholesterol, and low density lipoproteins cholesterol (LDL-C) in the hypercholesterolemic rats group compared to the control. Insignificant decrease in the plasma triglycerides was recorded in normal rats group fed probiocated mango juice compared to the control. Also, No significant decrease in the HDL/LDL-C ratio in normal and hypercholesterolemic rat groups fed probiocated mango juice was detected compared to control.

Key words: *Lactobacillus plantarum* NRRL4496, hypercholesterolemic rats, and probiocated mango juice

INTRODUCTION

The focus of scientific investigation has moved from the primary role of food as a source of energy to the more important action as function food, biological active food, well-being and prevention of non-transmissible chronic diseases such as hypercholesterolemia (Granato *et al.*, 2010). Developing functional foods are rising day by day in order to improve human health. (Prado *et al.*, 2008). Several studies indicated that the risk of heart attacks and cardiovascular diseases in hypercholesterolemic individuals can be reduced by lowering their serum cholesterol. Because of dairy products are able to cause allergy and the requirements of cold storage, therefore new foods, Probiotic products and beverages have initiated to maintain the demand human therapeutic level (Ranadheera *et al.*, 2010)

The aim of this research is to investigate the ability of mango juice propagated with probiotic culture of *L. plantarum* NRRL4496 to lower blood cholesterol serum in hypercholesterolemic rats.

MATERIALS

Chemicals: All chemicals used in this study were of analytical reagents grade

Media: De Mann, Rogosa and Sharpe (MRS) broth was obtained from *Biolife (staly)*,

Cultures: Reference *Lactobacillus plantarum* NRRL 4496 was kindly supplied by Agriculture Research Service (ARS) culture collection (NRRL), USA pickled cabbage source and the recommended growth temperature 37°C.

Fruit Juice: Mango Juice of commercial brand was purchased from local store, Alexandria, Egypt

Experimental animals: Thirty male albino rats 8 weeks old weighted (134.6g ±6.4), were obtained from faculty of Medicine, Alexandria university.

Animal diet: skim milk, corn oil, and sugar were obtained from local store. Minerals mixture was obtained from local stores. Minerals mixture (Table 1) was obtained from Animal production department, Faculty of Agriculture Alexandria university. Vitamin mixture was obtained from the Medical professional for veterinary products and fodders additions Company, Ismailia Egypt.

Cholesterol (98%) was purchased from oxid (india).

METHODS

Preparation of media

MRS broth media was prepared according to the mammy caterers directions 55g of MRS broth, were dissolved in 1L distilled water, sterilized by autoclaving at 121 c° for 15 min, pH 6.4 ± 0.2.

Buffer preparation:

Ringer solution was obtained from El. Nasre chemical (Egypt), phosphate buffer saline (PBS), prepared from sodium chloride (130 mM) and sod phosphate (10mM), EDTA buffer was prepared from (g/L) NaCl(5.6), KH₂ PO₄ (0.8), KCl(0.1), EDTA (0.1). EDTA (3.7)Distilled water was used through this work.

Media and buffer solution were autoclaved at 121C° for .15 min.

Preparation of reference (*Lactobacillus plantarum* NRRL 4496)

The pure confirmed culture (1ml) was transferred into a sterile 1.5ml eppendorf contain 200 ml of sterile glycerol and stored at- 180c°. Prior twice those fears of strain was sub-cultured (10% V/V) t in MRS broth, and incubated aerobically for 24hr at 37c°. purity of reference lactobacillus strain was examined repeatedly and at the beginning of each experiment.

Animal experiment:

Animals and diet:

The animals were housed in plastic cages on a 8hr light and 16hr dark at room temperature (20-25 c°). The animals were randomly divided into six groups each of fine animals, and fed on a normal diet and tap water for 1 week, then They were assigned to 2 dietary treatments (Table 3 and 4) groups were assigned to the normal diet and other 3 groups were assigned to the normal diet and other 3 groups were assigned to a high cholesterol diet (0.5% cholesterol) for 3 weeks to induce hyperchlosterolemia.

Blood samples of one of each group were withdrawn from eye vine to determine of serum total cholesterol.

Liver cholesterol

Liver cholesterol was determiedcolorimetry according to Rudel and Morris (1973).

Table 1: Rat groups and diet treatment used through the present experiment

Groups (30 rat each)	Experimental diet
group (15 rats)	Normal diet (1 week)
Sub group 1.a (5 rats)	Normal diet (3 week)
Sub group 1.b (5 rats)	Normal diet (4 week)
Sub group 1. c (5 rats)	Normal diet (ND) regular mango juice (4 week)
Group II (15 rats)	Normal diet (ND) probiocated mango juice high cholesterol diet (HCD) (3 weeks)
Sub group 2.a (5 rats)	HCD (4 weeks)
Sub group 2.b (5 rats)	HCD mango juice (4 weeks)
Sub group 2.c (5 rats)	HCD probacted mango juice (4 weeks)

ND= Normal diet.

HCD= High cholesterol diet.

Analysis of blood samples:

plasma analysis

cholesterol according tothe Meiattini*etal.*(1978)

Triglyceride (TG) were determined using enzymatic colorimetric according to Fossati*etal.*, (1982).

High density lipoproteins cholesterol(HDL.C), precipitating reagent (Bios tans, Barcelona, spain) according to the method described by Grove (1979).

Low density lipoproteins cholesterol (LDL) LDL-C was calculated by using the equation of Friedewald (1972) LDL.C= total cholesterol- HDL-(TG/ 5)

Total protein: Total protein in the plasma was determined using enzymatic color imetrickit (Biosystems, Barcelona, spain) according to Gomallet *al.*, 1949).

Creatinine: creatininwas determined using entymatic colorimetric kit (Biolabo, Maizy, france) according to fabiny and Ertingshausen(1971).

Total Bilirubin: Total bilirubin in the plasma was determined using enzymatic colorimetric kit (vitro scient, (vitro scient, Egypt) according to jendrassik-Grof (Dounaset *al.*, 1973).

Hematological parameters:

The following hematological parameters were measured in the blood samples .

(1) henogloblin (2) hematocrit (3) (RBC) and Red bloodcells.

(4) Total leuncocytes count (TLC) Mind ray BC-3200 Hematology analyzer, china.

Statistical analysis : The results are expressed as mean ±SD (standard deviation). The analysis of variance (one way ANONA) was carried out by STATASTICA 80 .Soft ware (stat soft, Inc, USA) and differences among the meams were determined for significance at P<0.05 using Fisher least significant Differences test (LSD 0.05).

RESULTS AND DISCUSSION

Generally, our data in table (2) revealed a non significant differences between control and treated rats. In the contrary Xie *et al.*, (2011) found a decrease in BWG amounted by13.3 %in rats group received high cholesterol diet plus Lactobacillus plantarum.

Table 2: Effect of regular and probiotic enriched mango juices on final body weight, gain in body weight, Daily food intake, and food efficiency of rats received normal or high cholesterol diet.

Parameters	Rat groups					
	Group A			Group B		
	Sub Group A.1	Sup GroupA.2	Sup GroupA.3	Sup GroupB.1	Sup GroupB.2	Sup GroupB.3
Initial weight (g)	135.2 ± 4.15 ^a	134.0 ± 8.72 ^a	134.0 ± 7.31 ^a	135.6 ± 7.80 ^a	134.4 ± 6.66 ^a	134.2 ± 6.14 ^a
Final weight (g)	181.2 ± 18.1 ^a	196.3 ± 17.8 ^a	185.8 ± 17.1 ^a	182.6 ± 15.8 ^a	194.0 ± 22.6 ^a	186.5 ± 17.1 ^a
Gain in body weight (g)	46.0 ± 14.4 ^a	60.3 ± 10.9 ^a	51.5 ± 9.90 ^a	47.0 ± 9.80 ^a	59.0 ± 15.2 ^a	50.3 ± 12.8 ^a
Daily food intake (g)	14.74 ± 0.64 ^a	15.45 ± 0.98 ^a	14.81 ± 0.86 ^b	14.75 ± 0.65 ^a	15.47 ± 0.92 ^a	15.17 ± 1.07 ^a
FER*	3.12	3.90	3.48	3.20	3.90	3.31

- FER= Food Efficiency ratio

Subgroup A.1 gN= normal diet group

Sub group A.2 N+ M= normal diet + mango juice group

Sub group A.3 N+PM = normal diet + probiocated mango juice group

Sub group B.1 HC= high cholesterol diet group

Sub group B.2 HC+M= high cholesterol diet + mango juice group

Sub group B.3 HC+PM= high cholesterol diet + probiocatedmango juice group

-Values with different superscript letters in the same column are significantly different

Table 3: Effect of regular and probiocated mango juices on weights of rats liver and kidney received normal or high cholesterol diet .

Sub groups	Liver weight (g)	Kidney weight (g)
A.1 (control)	5.7 ± 0.8 ^a	1.7 ± 0.3 ^{ab}
A.2	5.7 ± 0.5 ^a	1.5 ± 0.1 ^b
A.3	5.7 ± 0.9 ^a	1.5 ± 0.4 ^{ab}
B.1	5.9 ± 0.8 ^a	1.9 ± 1.0 ^{ab}
B.2	6.9 ± 1.1 ^a	2.4 ± 0.7 ^a
B.3	6.0 ± 1.1 ^b	1.2 ± 0.3 ^b

Subgroup A.1 gN= normal diet group

Sub group A.2 N+ M= normal diet + mango juice group

Sub group A.3 N+PM = normal diet + probiocated mango juice group

Sub group B.1 HC= high cholesterol diet group

Sub group B.2 HC+M= high cholesterol diet + mango juice group

Sub group B.3 HC+PM= high cholesterol diet + probiocatedmango juice group

-Values with different superscript letters in the same column are significantly different at p<0.05)

Mean while, table (2) showed non significant differences in DFI between the two groups. This agree with the findings of Nguyen et al., (2007). our results disagree with those of wang *et al.* (2012) who found that liver and Kidney weight did not differ significantly among rats fed hyper cholesterol diet and rats received high cholesterol diet skim milk inoculated with 10⁹ Cfu/g lactobacillus strains isolated from koumiss products.

Generally, weight of liver and kidney hypercholesteremic rats fed probiocated mango juice were less than these fed regular mango juice. This means that probiocated mango juice is more effective than mango juice in this aspect

In general, results showed that feeding high cholesterol diet increased significantly the liver lipid content, compared to rats received the normal diet. liver lipids (%) in this research., for all groups did not exceed 10%, which has no effect on the liver function. These results are in agreement with Reddy and Rao, (2005), who reported that any change in

the liver function occurs when lipid content of the liver exceeds 10% by weight.

Table (6) showed non significant differences in both total protein % and plasma total bilirubin mg/dl), between experimental rat groups of both normal and hypercholesterolemic rats.

For the creatinine plasma concentration, in the normal rat groups, there is a decrease in the rat group fed regular or probiocated mango juices compared to their control. The group fed regular mango juice showed the higher increase this increase could be explained by increasing in kidney weight of this group.

No significant differences was recorded in plasma total bilirubin among the experimental rat groups. In accordance of over results Hoyos *et al.* (2000) found no significant differences in creatinine, Total protein and total bilirubin between rats received normal diet and those received high cholesterol diet. Tsai *et al.*, (2004) reported that plasma total protein and total bilirubin of rat received *L.acidophilus* LA₅ were not significantly different from those of the control.

Table 4: Effect of regular and probiocated mango juices on total lipids percentage and cholesterol concentration in liver of rats received normal or high cholesterol diet.

Sub Groups	% Total lipids	Cholesterol (mg/g)
N (control) sub A.1	5.76±0.7 ^b	7.6±2.8 ^{bc}
N + M sub A.2	6.86±0.4 ^b	7.1±0.5 ^c
N + PM sub A.3	6.82±1.0 ^b	7.5±0.7 ^{bc}
HC (control) sub B.1	8.79±1.5 ^a	18.0±4.9 ^a
HC + M sub B.2	9.41±1.3 ^a	17.5±3.7 ^a
HC + PM sub B.3	9.61±0.5 ^a	12.3±4.4 ^b

- Groups as previously defined in Table (1)

HC+M= high cholesterol diet + mango juice group Sub group A.1

HC+PM= high cholesterol diet + probiocated mango juice group sub group A.2

HC= high cholesterol diet group sub group A.3

N+M= normal diet+ mango juice group sub group B.1

N+PM= normal diet + probiocated mango juice group sub group B.2

N= normal diet group B.3

-Values with different superscript letters in the same column are significantly different (at $P < 0.05$).

Table 5: Effect of regular and probiocated mango juices on plasma lipid profile of rats received normal or high cholesterol diet

Sup Group	Total cholesterol (mg/ dl)	IIDL-C (mg / dl)	LDL-C (mg /dl)	HDL/LDL	TG (mg/ dl)
Sub group A.1 N (control)	70.8±7.9 ^e	22.4±4.3 ^a	37.1±7.4 ^e	0.63±0.2 ^{abc}	56.6±3.8 ^{ab}
Sub group A.2 N + M	66.0±2.9 ^e	22.4±2.1 ^a	32.4±4.2 ^e	0.70±0.13 ^{ab}	56.0±7.8 ^{ab}
Sub group A.3 N+PM	66.5±10.3 ^e	26.1±9.3 ^a	31.0±6.5 ^e	0.89±0.48 ^a	47.0±6.2 ^b
Sub group B.1 A.HC (control)	98.6±12.9 ^a	22.4±8.1 ^a	63.7±5.9 ^a	0.35±1.3 ^c	62.8±12 ^a
HC + M	94.3±3.3 ^{ab}	21.4±4.4 ^a	61.0±5.1 ^a	0.36±0.1 ^c	59.0±8.9 ^a
HC +PM	82.7±6.8 ^b	22.4±2.8 ^a	48.6±7.7 ^b	0.47±0.12 ^{bc}	59.3±4.3 ^a

Sub group A1 N= normal diet group

Sub group A2 N+M =normal diet + mango juice group,

Sub group A3 N+PM= normal diet + probiocated mango juice group

Sub group B1 HC= high cholesterol diet group

Sub group B2 HC+M= high cholesterol diet+ mango juice group

Sub group B3 HC+PM= high cholesterol diet + probiocated mango juice group

These results disagreed with the results obtained during the present study.

Table 6: Effect of regular and probiocated mango juice on biochemical parameters of rats received normal or high cholesterol diet

Sub group	Total protein (mg/dl)	Creatinine (mg/dl)	Total bilirubin (mg/dl)
Sub group A1 N(control)	4.8±0.6 ^a	0.75±0.17 ^b	0.71 ± 0.20 ^a
Sub group A2 N+M	4.5 ± 0.3 ^a	0.65 ± 0.29 ^{ab}	0.73 ± 0.28 ^a
Sub group A3 N+PM	4.4 ± 0.3 ^a	0.69 ± 0.04 ^{ab}	0.72 ± 0.11 ^a
Sub group B1 HC (control)	4.7 ± 0.4 ^a	0.68 ± 0.10 ^b	0.74 ± 0.07 ^a
Sub group B2 HC + M	4.5 ± 0.5 ^a	0.91 ± 0.05 ^a	0.72 ± 0.09 ^a
Sub group B3 HC + PM	4.6 ± 0.2 ^a	0.82 ± 0.10 ^{ab}	0.75 ± 0.15 ^a

Sub group A1 N= normal diet group

Sub group A2 N+M =normal diet + mango juice group,

Sub group A3 N+PM= normal diet + probiocated mango juice group

Sub group B1 HC= high cholesterol diet group

Sub group B2 HC+M= high cholesterol diet+ mango juice group

Sub group B3 HC+PM= high cholesterol diet + probiocated mango juice group

CONCLUSION

In any case the administration of mango juice enriched with probiotic of *Lactobacillus plantarum* NRRL 4496 in a dose of 10^8 CFU / ml/ day resulted in significant reduction in plasma total cholesterol and LDL in hypercholesterolemic rats as well as in the HDL/LDL ratio. Moreover, the administration of

the juice resulted in about 31.7% reduction in the cholesterol concentration compared to high cholesterol diet control group

In normal rats, the probiocated juice resulted in no significant reduction in the plasma triglycerides and improved the HDL/LDL ratio. As known

probiocated mango juice is a healthy drink or health life style from the researcher's opinion.

It is concluded that super bio-juices like a probiocated mango juice could play a key role in reducing risks of cardiovascular disease.

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الملخص العربى

التأثيرات التغذوية لعصير المانجو الملقح بالسلالة *Lactobacillus plantarum* NRRL4496m الداعمة للحويوية كمصدر لخفض الكوليسترول فى فئران التجارب

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جامعة الإسكندرية

لقد أوضحت الدراسة ان عصير المانجو المدعم حيويًا بالسلالة/ت NRRL 4496 يمكن ان يستخدم كغذاء صحى، نظرا لاحتوائه على كمية كبيرة من المكونات الصحية مثل الفيتامينات ومضادات الأكسدة ومركبات الفينولات العديدة لاحتوائها على هذه السلالة التى اختبرت استخدامها على فئران التجارب. تم تقدير وزن الجسم النهائى لفئران التجارب ومعدل الزيادة فى النمو ومعدل الغذاء المتناول يوميا ونسبة الاستفادة من الغذاء لكل من المجموعة الاولى المغذاه على وجبة عادية(منخفضة الكوليسترول) او المجموعة الثانية المغذاه على وجبة مرتفعة من الكوليسترول. ووضحت النتائج انه لا توجد فروق معنوية فى كل من نسبة الاستفادة من الغذاء من المجموعتين كما لوحظ زيادة فى وزن الجسم للمجموعتين بينما كانت الزيادة غير معنوية للمجموعة المغذاه على النسبة العادية فى الكوليسترول (المجموعة الضابطة) كما اوضحت النتائج انه بالنسبة للأوزان النهائية للجسم كان الترتيب على الوجه التالى:
أولاً: الفئران المغذاه على عصير المانجو فقط.

ثانياً: يليها تلك المجموعة المغذاه على عصير المانجو المدعم ببيولوجيا.

ثالثاً: كان اقلهم المجموعة الضابطة وقد اوضحت النتائج نفس اتجاه التأثير فى المجموعة المغذاه على المستوى المرتفع من الكوليسترول اما بالنسبة لمعدل الاستهلاك اليومى للغذاء فقد اظهرت النتائج نفس الاتجاه من التأثير حيث كانت المجموعة المغذاه على عصير المانجو هى الاعلى فى معدل الاستهلاك اليومى للغذاء وخلاصة القول ان المجموعتين سواء تلك المجموعة المغذاه على عصير المانجو فقط او المغذاه على عصير المانجو المدعم حيويًا كانت دائما اعلى فى كل القيم عن المجموعة الضابطة.