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Full Length Research Paper

Therapeutic Value Of Camel Milk Supplemented With Echinacea Consumption By Albino Rat.

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This trial aims to clarify the role of the camel milk supplemented with three different ratio of the Echinacea and dates pulp on male albino rats fed on a high fat high cholesterol diet. The chemical content and GC-MS analysis for the Camel milk, dates pulp and Echinacea had been determined. Seventy male albino rats weight of 100±20 g. were divided randomly into seven groups, ten rats in each. They were fed on standard diets and the camel milk supplemented with dates pulp and the Echinacea (1,3and5 %) respectively, for 60 days. By the end of the experimental period, all rats were anesthetized and sacrificed to obtain blood tissues samples. A significant difference in AST, ALT, GCT, ALB , TP,TB,ALP, CK, LDH TC, TG, glucose, α-amylase, GSH, MDA ,IgM, IgG, IL-2, IL-1α and IgE levels were found between the high fat high cholesterol group and others groups. Finally, the destructive changes in the cellular tissues of the liver and kidney had been stopped in all treated groups compared with the high fat high cholesterol group. In conclusion, the camel milk was successfully supplemented with different ratio of the Echinacea and dates pulp which is a functional food, with stimulating potential antioxidant effect. Finally, it had been stimulate necessary bioactive components, which play a great a role in stimulating the immune system effect. The improvement in the liver and kidney functions and other metabolic indicators, it might be reflected in stopping greatly the destructive changes in the cellular tissues of the liver and kidney.

Keywords: aerial part of the Echinacea, date fruits, camel's milk, Antioxidant activity, Immune system.

INTRODUCTION

Functional foods have recently emerged as a novel sector of health-enhancing products¹. Camel's milk is an important nutritional source in several world regions either fresh or curdled and it has an important role in human nutrition in the hot regions and arid countries. Camel's milk contains all the essential nutrients found in bovine milk and it has high proportions of proteins such as lactoferrin, showed significant activity against Hepatitis C virus, amylase, α -lactalbumin and α -globulin. β -lactoglobulin is the major whey protein in cow's and buffalo's milks². Several studies proved that camel milk have therapeutic properties, such as anti-carcinogenic³, anti-hypertensive⁴, anti-diabetic⁵ and has been recommended to be consumed by children who are allergic to bovine milk⁶. Furthermore, Russia and Sudan camel's milk used for treatment for a series of diseases such as dropsy, jaundice, tuberculosis, and asthma⁷. Such nutritive value of camel milk proteins in dairy products, the liquid milk gives attention for more investigations for its possible use as a therapeutic value in many other protective healthy conditions. Clinical trials have shown that the daily consumption of a half liter of camel milk reduced the need for insulin medication by an average of 30% in patients with type-1 diabetes with better control of blood glucose and lipids profile⁵.

The date fruits (*Phoenix dactylifera* L) are a good source of moderate cost food and is an integral part of Arabian diet which is traditionally used in Ramadan to break the long fast day. Date fruits contain 70% carbohydrates (44-88%), fats (0.2-0.4%), proteins (2.3-5.6%), fibers (6.4-11.5%), minerals (Ca, Fe, Mg, P, Zn, Se & Mn) and many vitamins. Twenty-three different amino acids were found in dates proteins. Carbohydrates in dates are mostly of the simple type, in the form of fructose and glucose⁸ which are easily absorbed by the human body⁹ and generates higher source of energy for human organs and different activities. Aqueous extracts of dates have potential antioxidants (contain higher flavonoids, anthocyanins & carotenoids), antimutagenic activity¹⁰. Dates have the second highest antioxidant activity among 28 fruits commonly consumed in China¹¹. The highest antioxidant activity of date fruits have a potential effects in the prevention of chronic and degenerative diseases such as cancer¹², cardiovascular diseases and aging¹³.

Echinacea purpurea L, (purple coneflower) is an herbaceous perennial with a long, well-established tradition of medicinal use in an immune promoter, particularly for prevention and treatments of upper respiratory tract infections in Europe and North America¹⁴. part of *E. purpurea* had immunomodulatory properties in experimental animals and clinical trials¹⁵. Phenolic antioxidants inhibit the NF- κ B (nuclear factor κ B) and AP1, which participate in the production of proinflammatory cytokines interleukin 1 α (IL-1 α)¹⁶. A

mixture of camel milk and dates might proof to be beneficial for the treatment of various ailments¹⁷.

The present study postulated that incorporation of date pulp and camel's milk, may offer a potential source of many nutrients such as dietary fibers, antioxidants, vitamins and minerals, with different levels of the *E. purpurea* part (1, 3 and 5%) as functional food for feeding rats to study its effects on improvement the physiology, growth performance, metabolic disorder and stimulate immune system in albino rats.

MATERIALS AND METHODS

Materials

Chemicals

All chemicals used in this study were purchased from Sigma Chemical Co. USA with the highest grade and purity.

Raw materials used for animal diet

Camel milk samples

Thirty liter of camel milk samples were was purchased from a Camel farm Research Station- Desert Research Centre at Maryout, Egypt. Camel milk samples were collected from young camels in the morning before provender breakfast at 60 days of birth in white, clean polyethylene bottles previously washed with diluted nitric acid and de-ionized water and stored in 4°C till used.

Date fruits pulp

Dates fruits (*Phoenix dactylifera*) Cultivar named Al-Hayany commonly grown in Giza region were purchased from the local date market in Giza, Egypt. It was prepared by washing, cleaning, removing the peel and seeds, then quickly minced using an electric mixer (Brawn, Germany) and used directly after preparation.

Echinacea purpurea

Aerial part of the *Echinacea purpurea* was provided by El Nada farm at Elkelo-59 Cairo-Alexandria Desert Road, Giza Governorate, Egypt. 500gm of the Aerial part of *Echinacea purpurea* was collected and washed several times with deionized water, then dried at 45 °C and milled using a Moulinex mill machine and stored in 4°C till used.

Preparation of milk mixture

Camel's milk mixed with 10% of prepared date pulp and the mixture was homogenized with an electric blender then divided into two parts. The first part was used as a control without any additives and the second one was supplemented with different levels of crude *Echinacea* aerial part (1, 3 and 5%). The samples were pasteurized at 85 °C for 5 min. then cooled to 5 °C and stored in 4°C till used.

Analytical Methods for Raw materials

1. Chemical Analysis

Total solids (T.S), fat, total protein, lactose, ash, total acidity, specific gravity, pH values (using the Metter Toledo pH meter), crude fibers and total sugars were determined according to the methods described by the A.O.A.C¹⁸.

- Fe, K and Mg contents were determined after dry the ash, according to the method described by the A.O.A.C¹⁹, using an atomic absorption spectrometer (Perkin–Elmer, Model 3300, USA).

Camel's milk (30 liter) was purchased from a Camel farm Research Station- Desert Research Centre at Maryout, Egypt. -Dates fruits (*Phoenix dactylifera*) Cultivar named Al-Hayany commonly grown in Giza region were purchased from the local date market in Giza, Egypt aerial part of the *Echinacea purpurea* was provided by El Nada farm at Elkelo-59 Cairo-Alexandria Desert Road, Giza Governorate, Egypt. -All chemicals used in this study were purchased from Sigma Chemical Co. USA with the highest grade and purity.

2- Gas chromatography–mass spectrometry (GC-MS) analysis to fractionat active ingredient :

A. In camil milk.

Preparation of camel milk for (GC-MS) analysis

The camel milk samples were dried at 100°C till constant weight for GC analysis.

GC-MS Methods for analysis Camel milk

The Gas Chromatography GC was used a Agilent Technologies (7890A) interfaced with a mass-selective detector (MSD, Agilent 7000 Triple Quad) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) (J&W Scientific, Folsom, CA, USA) equipped with a capillary column (30 m × 0.25 mm i. d. and 0.25 µm film

thickness) and FID detector. Medium (Country) used for fractionations of the camel milk according to methods performed by Dionex²⁰.

B. Date fruits and *Echinacea purpurea* L

-Preparation of date fruits for GC-MS analysis

The 0.01gm of date treated with 40ml of ethanol/water 50% (V/V). The mixture was filtered through 0.43µm filter, and then the slurry was filtered with Wathman paper No. 41, and then centrifuged for 5 min. at 5000 RPM. The filtrate obtained from ethanol was evaporated to dryness at 40° C by a rotary evaporator (IKA® HB10 basic, China) and the extract was freeze-dried using a freeze drier system (Operon: FDB-5503, Korea). Finally, the dried extract was stored in 4°C until use²¹.

-Preparation of the *Echinacea purpurea* L. for GC analysis

Echinacea purpurea extract was prepared according to Punitha *et al*²². 1.0 gm of dried plant powder was taken and soaked in 20 ml of 70% ethanol for 48 hour. The slurry was filtered using Wathman No. 1 filter paper and centrifuged for 5 min at 5000 RPM. The filtrate was evaporated to dryness at 40°C by a rotary evaporator (IKA® HB10 basic, China) and the water extract was freeze-dried using a freeze drier system (Operon: FDB-5503, Korea). Finally, the dried extract was stored in 4°C until use.

Gas chromatography–mass spectrometry (GC-MS) Methods for analysis the *Echinacea purpurea* L and Date fruits.

Ethanol extracts of the *Echinacea* arial part or date pulp fruits were performed separately using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and samples were injected with a split ratio of 50:1; helium was used as carrier gas at 1.0 mL/ min. The column temperature was maintained at 100°C for 1 min after injection then increased at 10°C /min to 275°C which was sustained for 20 min. The time required for chromatography of one sample was 40 min.

Table 1. Formula and compositions of experimental diets

Groups *Ingredients %	Diets composition (%)					
	G1 Basal diet	G2 Camel milk	G3 Camel milk + date pulp	G4 Camel milk+ date pulp + Echinacea	G5 Camel milk+ date pulp + Echinacea	G6 Camel milk+ date pulp + Echinacea
Echinacea powder	-	-	-	1% (1g/100ml CM)	3 % (3g/100ml CM)	5 % (5g/100ml CM)
Camel milk (CM)	-	100 ml	100ml	100ml	100ml	100ml
Date pulp	-	-	10gm date	10gm date	10gm date	10gm date
Corn starch	20	20	20	20	20	20
Dex-corn starch	13	13	13	13	13	13
Fiber (cellulose)	5	5	5	5	5	5
Casein	15	15	15	15	15	15
Mineral mix	4	4	4	4	4	4
Vitamin mix	1.15	1.15	1.15	1.15	1.15	1.15
Corn Oil	10	10	10	10	10	10
Sucrose	10	10	10	10	10	10
L-Cystine	0.5	0.5	0.5	0.5	0.5	0.5
Tert-butyl hydroquinone	0.054	0.0154	0.054	0.054	0.054	0.0514
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25
Soybean oil	7	7	7	7	7	7
Fat	1	1	1	1	1	1
cholesterol	20	20	20	20	20	20

*AOAC, 1997(22),

Identifications Active Ingredients in raw materials by Thin-Layer Chromatography TLC Analysis

Identification and quantification of constituents: The relative percentage amount of each component was calculated by comparing its average peak area to the total areas (TLC) by apparatus software. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Experimental Design for Biochemical analysis

Experimental animals

Seventy adult male Albino rats weighting 100-120g, were purchased from the experimental animals house of National Organization for Drug Control and Research, Egypt.

Experimental Design

Seventy male albino rats were placed in pathogen-free cages with air and water *ad-libitum* and maintained at a temperature between (20–23°C) with a 12 hr. light/dark cycle (08:00–20:00h) and relative humidity of 50%. The animals fed on basal diet (experimental diets for acclimatization) for one week as an adaptation period and received 5 ml of mixture milk /rat /day by epigastric tube for 60 days from the start of the experimental period. The experimental protocols and procedures were approved with (Approval No. 21 at 1/3/2013) by the local Ethics Committee at the National Cancer Institute-Cairo-University –Egypt.

According to AOAC²³, the basal diet consisted of 15% casein, 10% corn oil, 4% salt mixture, 1.15 % vitamins mixture, 5% cellulose and 13% Dex-corn starch (starch 65%), 4% mineral mixtures as well as the dose and rout administration of camel milk only, 10% date pulp mixed with camel milk and date pulp mixed with camel milk and different levels of the *Echinacea* aerial part as cleared in Table 1.

Rats randomly divided into equal seven groups (n=10) according to the following scheme:

Group0: rats fed on basal diet only for 60 day. (Negative control).

Group1: rats fed on high fat diet (diet supplemented with

cholesterol and fats at a dose 1 and 20g/100 g diet, respectively) for 60 days (Positive control).

Group2: rats fed on high fat diet and received 5 ml camel milk only by gastric tube per rat per day for 60 days.

Group3: rats fed on high fat diet and received 5 ml of mixture milk (milk & date) by gastric tube per rat per day for 60 days.

Group4: rats fed on high fat diet and 5 ml of mixture milk (milk & date +1% *Echinacea*) by gastric tube per rat per day for 60 days.

Group5: rats fed on high fat diet and 5 ml of mixture milk (milk & date +3% *Echinacea*) by gastric tube per rat per day for 60 days.

Group6: rats fed on high fat diet and 5 ml of mixture milk (milk & date +5% *Echinacea*) by gastric tube per rat per day for 60 days.

The body weight and weight gain at time intervals at 0, 30 and 60 days were recorded.

Samples Collections

At the end of the experimental period (0.30,60) days, the animals were fasted for 12 hrs and the blood samples were taken from the retro-orbital plexus under light diethyl ether anesthesia²³. Blood was left to clot and centrifuged at 3000 rpm for 10 min. at 4°C and serum were obtained and frozen at -80 °C until used for analysis. Excised liver and kidney organs were also taken and kept in 10% formaldehyde to be used in histological examination.

Biochemical analyses

Metabolic parameters

The separated rat serum was used for analysis of the metabolic indicators profile as follows : total proteins (TP), total bilirubin (TB), albumin (ALB), glucose (Glu), urea ,creatinine , triacylglycerols (TAG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GCT), alkaline phosphatase (ALP), Creatine kinase (CK), L-lactate dehydrogenase (LDH), Total cholesterol (TC), and Triglycerides (TG) and Calcium (Ca). The indicators of the metabolic and Calcium profile were analyzed using the automatic analyser XT20i (Fischer Thermo Scientific, Finland) using standard commercial kits (Biovendor-Laboratorní medicína, CR).

Determination of spleen function tests

The α -amylase (AMY) was estimated in serum for spleen function using an analysis kits for each respective enzyme according to the manufacturer's instructions (Bio-Rad Scientific Inc., USA). Assays were read using an

automated blood chemistry analyzer (Photometer 5010, Germany).

Serum Malondialdehyde (MDA) determination

MDA level was measured by the double heating method described by Draper and Hadley²⁴. The optical density was measured at 532 nm. Serum Glutathione (GSH) determination was performed according to methods described by Goldberg and Spooner²⁵. MDA may serve as a good and sensitive marker of oxidative stress in the pathological process²⁶. Malondialdehyde is formed by peroxidation of unsaturated fatty acids containing three or more double bonds²⁷. Thus, malondialdehyde essentially serves as a marker for peroxidation of ω -6 arachidonate and the ω -3 lipids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA)²⁸.

Estimation serum Immune globulins ELISA analysis

Some immunological parameters as IgG and IgM in blood serum were determined by chemiluminescence's methods using Immulete 2000 analyzer, with the application of ready kits of R&D Company and measurement of absorption at wavelength of 405 nm, by spectrophotometer. Serum IgE levels were measured using Genesis Diagnostics ELISA kit. It is a solid phase enzyme linked immunosorbent assay based on the sandwich technique. Organon Teknika Microwell system was used to estimate the concentration of IgE at 450 nm.

Estimation of serum cytokines for IL- 1 α and IL-2

Rat's serum interleukin IL-1 α and IL-2 had been measured by highly sensitive ELISA kits (DIAsource ImmunoAssays, Nivelles, Belgium)

Histopathological examination

Liver and kidney for all animals were excised specimens after sacrificing animals. One piece of liver and kidney tissues was fixed in 10% neutral formaldehyde. The slices tissues were embedded in paraffin for histological section. The slices tissues were embedded in a paraplast automatic tissue processor (Citadel 2000, Shandon Scientific, and Cheshire, UK), sectioned (4 μ m) and stained with hematoxylin and eosin (H&E) stain. The slides were mounted with neutral balsam.

Sensory evaluation

The Sensory evaluation of camel's milk, date pulp and

Table 2. Chemical composition of fresh *Echinacea* ariel part and date pulp

Samples Constituents %	Echinacea powder	Date pulp
Protein	18.81	3.5
Total sugars	*N.d	31.8
Crude Fiber	4.7	7.5
Ash	0.9	1.2
Iron (mg/100g)	1.5	2.9
Magnesium (mg/100g)	2.59	3.32
Potassium (mg/100g)	7.31	4.17
Moisture	7.5	56

*Nd: Not detected

Table3. Physio-chemical properties of fresh functional camel's milk blends

Treatments Properties	Control	T1	T2	T3	T4
Total solids (TS %)	12.97 ^e	15.32 ^d	17.61 ^c	18.94 ^b	20.21 ^a
Fat %	3.30 ^b	3.40 ^a	3.40 ^a	3.40 ^a	3.40 ^a
Protein %	3.72 ^c	4.42 ^b	4.44 ^b	4.46 ^a	4.49 ^a
Lactose %	4.92 ^a	4.92 ^a	4.92 ^a	4.92 ^a	4.92 ^a
Ash %	0.73 ^e	0.88 ^d	0.96 ^c	1.103 ^b	1.11 ^a
pH values	6.62 ^a	6.42 ^b	6.40 ^c	6.37 ^d	6.34 ^e
Total acidity	0.17 ^d	0.18 ^c	0.18 ^c	0.19 ^b	0.20 ^a
Specific gravity	1.0328 ^b	1.0342 ^a	1.0341 ^a	1.0341 ^a	1.034 ^a

Control: fresh camel milk without addition.

T1: camel milk + 10% dates pulp.

T2: camel milk +10% dates + 1% Echinacea

T3: camel milk + 10% dates + 3% Echinacea

T4: camel milk + 10% dates + 5% Echinacea

abcd : Means in the same column with different superscripts differ significantly ($p > 0.05$).

Echinacea mixtures were assessed by regular taste panel (appearance,5-body& texture,5-and flavor ,10) by the staff-members at Food Sci. Dept., Fac. of Agric., Ain Shams University according to Clark *et al.*,²⁹.

Statistical analysis

The data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA; 2007). Differences between groups were considered to be significant when $p \leq 0.05$. Hypothesis testing was done by one way analysis of variance (ANOVA). The Duncan's multiple was carried out by using the PROC ANOVA procedure of Statistical Analysis system range test at $p < 0.05$ according to the method reported by SAS³⁰.

RESULTS

Chemical composition of fresh *Echinacea* Aerial part and Al-Hayani date pulp

Table 2 showed that some chemical composition of fresh *Echinacea* aerial part and dates pulp. *Echinacea* aerial

part had higher protein content (18.81%) more than two fold date pulp content (3.50%). Total sugar in date pulp content had 31.8% while the *Echinacea* Aerial part had free sugar. The crude fiber contents of the *Echinacea* aerial part and dates pulp were 4.7and 7.5%. The ash content in the *Echinacea* aerial part and dates pulp were 0.9 and 1.2 %, respectively (Table, 2). Both iron and magnesium were elevated levels in date pulp compared with *Echinacea* while the potassium levels had been increased in the *Echinacea* compared with date pulp. Moreover, the moisture content of date pulp had higher than the *Echinacea* (Table 2).

Physio-chemical properties of fresh functional camel's milk blends

Table 3 presented some physio-chemical properties of fresh functional camel's milk supplemented with 10% date's pulp and different levels of the *Echinacea* powder (1, 3 and5%). Total solids, fat, protein and lactose contents ranged from 12.97, 3.30, 3.72 and 4.92 to 20.21, 3.40, 4.49 and 4.92, respectively. Camel's milk supplemented with 10% dates and 5% powder of the *Echinacea* (T4) had the highest acidity and ash contents,

Table 4. The sensory evaluation of fresh functional camel's milk blends

Treatments Properties	Control	T1	T2	T3	T4
Appearance (5)	5.0 ^a	4.8 ^a	4.0 ^b	3.8 ^c	3.3 ^d
Body & texture (5)	4.6 ^b	4.8 ^a	4.4 ^b	4.2 ^c	4.0 ^c
Flavor (10)	9.0 ^b	9.8 ^a	8.5 ^c	7.5 ^d	6.0 ^e
Overall (20)	18.6 ^b	19.4 ^a	16.9 ^c	15.5 ^d	13.3 ^e

See Table (3)

Table 5. Active components of camels milk using GC.Ms

No.	Retention time	Name	Peak area %	Compound nature	Pharmacological activity
1	3.069	Galactonic phenylhydrazide	3.02		Antioxidant
2	3.193	Cholesterol	2.68		
3	3.348	Gentamicin a	2.82	sesquiterpenes	Antifungal
4	3.607	Lactose	16.17		
5	3.963	β-(3-tert-Butyl-5-chloro-2-hydroxyphenyl)(phenyl)methanone	12.09	sesquiterpenes	
6	4.275	Actinobolin	1.37		
7	6.706	E-11-Hexadecenoic acid, ethyl ester	6.74		
8	7.187	5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone	5.04	sesquiterpenes	
9	7.267	6-Phosphogluconic acid	5.38	sesquiterpenes	
10	7.469	Methyl 3-hydroxydecanoate	1.44		
11	8.953	Methoxyacetic acid, 2-tetradecyl ester	6.15	sesquiterpenes	
12	9.286	Paromomycin I	11.73	sesquiterpenes	Antibacterial
13	9.726	2-Hydroxy-1-methylpropyl stearate	2.65		
14	10.582	α-Hydroxyisobutyric acid	1.45		
15	12.194	Gentamycin X2	0.88	sesquiterpenes	
16	12.365	L-Tryptophan	5.93		radical scavenger
17		Retinoic acid			has role in human metabolite
	14.666	3,7-dimethyl-9-(2,6,6-trimethyl cyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid	0.33		
18	14.733	δ-Tocopherol	0.30	polyphenole	radical scavenger
19	14.76	Retinyl propionate	10.03		
20	14.978	(S)-(-)-Citronellic acid	1.34		
21	15.576	Ascorbic acid, permethyl-	2.47		Antitumor

Compounds identified through mass spectra library matching for the peaks were listed at the table *Classification of compound obtained from references^{31&32}; **Pharmacological activity information obtained from references^{33&34}. The Camel milk contained a complex mixture consisting of a high proportion of sesquiterpenes polyphenol, whereas the fraction of monoterpenes was small.

while, the control samples (camel's milk without any additives) had the lowest content of acidity and ash. Significant differences were observed in total solids, total acidity and protein and ash contents in all treatments of camel's milk. Addition of dates to the camel milk caused a significant increase in total solids, protein and ash contents, but this addition had no effects on fat and the lactose contents. These could be due to the chemical composition of dates (Table, 2). Supplementation the camel milk /date with different levels of the *Echinacea* aerial part powder caused gradually significant an increase in protein and ash contents and acidity compared with (T1). These may be due to the high contents of the *Echinacea* aerial part powder in protein and ash contents. Increasing the levels of the *Echinacea*

aerial part powder in camel milk /date mixtures caused an increase in the specific gravity of the milk mixture. This may be due to the increase of total solids in all treated samples compared with control sample.

Sensory evaluation of fresh, functional camel milk blends

Four blends of camel's milk supplemented with 10% date's pulp and different levels of the *Echinacea* powder (1, 3 and 5%) were sensory evaluated and the results are shown in **Table 4**. The data indicated that, there were significant differences in appearance, body & texture and flavor scores between all treatments. Addition of the

Table 6. Fractionation and identification of phytochemical components of date pulp extract by GC-MS

Peak No.	Retention Time	Identified constituents	Peak area %	Compound nature*	**Pharmacological activity
1	3.815	L-Glucose	3.61		Antioxidant
2	4.050	Galacto-heptulose	2.66		Antioxidant
3	5.397	Phloroglucinol	2.52	Polyphenole	radical scavenger
4	5.021	1,3-Cyclohexanediol	3.66		
5	5.417	trans-Levogluconenone	5.71	Polyphenole	radical scavenger
6	5.891	3-hydroxy-4-(trimethylammonio) butanoate (L-Carnitine)	3.39	enantiomer	transport of fatty acids
7	6.069	l-Gulonic acid, γ -lactone	0.66	Polyphenole	
8	6.482	Octadecanedioic acid	3.19	Polyphenole	radical scavenger
9	6.890	β -D-Lactose	1.29		
10	7.231	Pyranone	0.71	Polyphenole	radical scavenger
11	7.812	d-Altronic acid	10.43		
12	8.665	n-Undecoic acid	4.41		
13	9.018	4-Guanidinobutyric acid	2.5		
14	9.290	D-2-Deoxyribose	2.24		
15	9.528	Gentamicin B	0.46	sesquiterpenes	antiinfective agent
16	9.686	D-Melezitose	2.25		
17	10.096	Isopropyl palmitate	11.74	Fatty Acyls	
18	10.840	11-Aminoundecanoic acid	3.18		Immounomodulatory
19	11.019	2-Deoxy-D-galactose	1.28		
20	11.416	cis-Vaccenic acid	7.89	Sesquiterpene	
21	11.883	Dihydro-3-oxo- β -ionol	1.80	Polyphenole	radical scavenger
22	12.663	Isochiapin B	1.12	Polyphenole	
23	12.809	d-Lyxo-d-manno-nononic-1,4-lactone	0.55	Polyphenole	Antioxidant
24	14.479	Butylated hydroxytoluene	1.66	sesquiterpenes	.
25	14.761	10-Octadecenoic acid, methyl ester	0.43	C18H34O2	
26	15.258	n-Pentadecanoic acid	7.61	carboxamide	antitumor
27	16.024	1-Heptatriacotanol	0.19	Polyphenole	Antioxidant
28	17.154	Ethyl 14-oxotetradecanoate	9.66		
29	17.414	Palmitic acid	2.67	Fatty Acyls	hypocholesterolemic
30	18.81	16-hydroxy-1,2-Dipalmitin	0.52	Polyphenole	Antioxidant

Compounds identified through mass spectra library matching for the peaks were listed at the table *Classification of compound obtained from references^{31&32}; **Pharmacological activity information obtained from references^{33&34}. The **date pulp** contained a complex mixture consisting of a high proportion of Polyphenole and sesquiterpenes, whereas the fraction of monoterpenes was small.

Echinacea aerial part powder to blends caused significant decrease in appearance and body & texture scores. While, when dates added to milk might be improve the body & texture of final products. This improve may be due to improve of the viscosity in camel milk with dates. Addition of the *Echinacea* powder to milk and dates decreased the appearance and body & texture scores. This is could be due to the presence of sediments in milk and dates blends due to increase ratio of the *Echinacea* aerial part powder. It is recommended that the consumers must be shaking the product before used it.

Fractionation and identification of effective constituents of camel milk

The effective constituents of camel milk fractions were separated by GC analysis and the produced peaks were

identified using GC/MS (**Table, 5**). More than 21 components were identified in camel milk. Antibiotics were the first major group identified in camel milk. The main antibiotic was Paromomycin I (11.73%) followed by germacrene (gentamicin a) (2.82 %) and gentamycin X2 (0.88%). Sugar identified in camel milk components was Lactose, which possesses a very strong sugar detected with high level (16.17 %), whereas, Cholesterol was found in a moderate amounts (2.68%). Retinyl propionate derivatives (Vit. A) and ascorbic acid (Vit.C) were the predominant vitamins, and their concentrations were 10.03% and 2.47%, respectively. Also, δ -Tocopherol (Vit. E) (0.30%) and Retinoic acid (Vit. A) (0.33%) found in small amounts. Four esters were represented in the table identifying camel milk effective constituents. Esters may play a role in the camel milk odor. The main esters compound identified were E-11-Hexadecenoic acid ethyl ester (6.74%) followed by

Table 7. Fractionation and identification of phyto-components of *Echinacea* ariel part extract by GC/MS

Peak No.	Retention Time	Identified constituents	Peak area %	*Compound nature	**Pharmacological activity
1	3.556	4-Methylcatechol	1.11		
2	8.967	Geranyl isovalerate	0.93		
3	9.904	Linoleic acid	0.16	Fatty acid	
4	11.462	Oleic Acid	0.18	Fatty acid	
5	12.318	α -Bisabolol	29.48		
6	12.15	Dodecanoic acid	0.31	Polyphenole	
7	13.004	Dodeca-2E,4E-dienoic acidisobutyl amide	3.78	aliphatic alkylamides	inhibited lipopolysaccharide
8	13.524	dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide	0.44	alkylamides	Exerted modulatory effects on cytokine expression
9	13.631	4-methyl-3-Methoxy-2,4,6-trimethylphenol	0.76	sesquiterpene	Antioxidant
10	14.693	δ -Tocopherol	1.06	sesquiterpene	Antioxidant
11	14.77	Retinyl propionate	1.16		
12	14.87	Carminic acid	3.92		
13	15.395	Phenol, 2,5-bis(1-methylethyl)-	0.86	Polyphenol	
14	15.549	Resveratrol (3,5,4'-trihydroxy- <i>trans</i> -stilbene)	2.5	stilbenoid	Antitumor
15	15.647	p-Nonylphenol	3.98	Polyphenole	
16	15.754	β -Methylionone	6.43		
17	15.915	4-tert-Octyl-o-Cresol	3.19		
18	16.275	γ -Ionone	0.81	Polyphenole	
19	16.355	Globulol	2.05		
20	16.456	9-cis-Retinal	1.98		
21	16.577	(-)-Isolongifolol, methyl ether	1.22		
22	16.93	Caryophyllene oxide	0.36	sesquiterpene	Anrioxidant
23	17.632	α -Monolaurin	23.87		
24	17.87	Xanthinin	0.46		
25	18.037	Octadecanedioic acid	0.98		
26	18.303	Biotin	8.99		Antioxidant
	18.495	Phenol, 3,5-di-tert-butyl-	0.43	Polyphenole	Anrioxidant
		Isopulegone,			Antioxidant

Compounds identified through mass spectra library matching for the peaks were listed at the table. *Classification of compound obtained from references^{31&32}; **Pharmacological activity information obtained from references^{33&34}. *Echinacea* aerial part contained a complex mixture consisting of a high proportion of sesquiterpenes, alkylamides and anandamide and polyphenole whereas the fraction of monoterpenes was small.

Methoxyacetic acid, 2-tetradecyl ester (6.15%); 2-Hydroxy-1-methylpropyl stearate (2.65%) and Methyl 3-hydroxydecanoate (1.44%). Two lactones namely (3-tert-Butyl-5-chloro-2-hydroxyphenyl) (phenyl) methanone and 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone were identified with concentration (12.09 and 5.04 %, respectively).

Lactones were known to be responsible for aroma aldehyde identified in volatile camel milk components was Galactonic phenylhydrazide which detected with a moderate amounts (3.02%). Organic acids may react with the alcohols leading to formation of aromatic esters. 6-Phosphogluconic acid and S-Citronellic acid were also detected (5.38 and 1.34%, respectively) (Table, 5). L-Tryptophan (Amino acid) was also detected (5.93%). Compounds identified through mass spectra library matching for the peaks were listed at the table(5). Classification of compound obtained from references^{31&32}; Pharmacological activity information obtained from references^{33&34}. According to the compounds

classifications and Pharmacological activity information of the camel milk contained a complex mixture consisting of a high proportion of sesquiterpenes, whereas the fraction of monoterpenes was small.

Fractionation and identification of effective constituents of date pulp fruits

The effective constituents of date pulp fruits fractions were separated by GC analysis and the produced peaks were identified using GC/MS (**Table, 6**). More than 30 components were identified in date pulp fruits. Six sugars were presented in moderate amounts namely, L-Glucose (3.61%), Galacto-heptulose (2.66%), β -D-Lactose(1.29%), D-2-Deoxyribose(2.24%), D-Melezitose (2.25%) and 2-Deoxy-D-galactose (1.28%). Furthermore, four different alcohols were detected and identified from the total separated volatile components. 1,3-

Table 8. Body weight gain of male albino rats feeding on basal diet and functional camel's milk blends

Feeding period *Groups	Initial body weight at zero time (g)	Pre-final body weight after days 30 (g)	Change at days 30 compared with zero time %	Final body weight after 60days (g)	Change at days 60 compared with zero time %
Group 0	119.40±0.93	125.9±0.79	18.80	135.53±1.42	30.3
Group 1	119.45±0.93	128.9±0.79	13.80	143.53±1.42	16.4
Group 2	120.31±1.16	148.10±0.18	16.00	161.92±2.19	31.0
Group3	119.75±0.67	143.21 ±0.37	16.40	158.35±1.52	31.3
Group4	120.84 ±0.47	140.2 ±0.84	18.50	157.90±2.25	28.2
Group5	119.61±0.93	139.9±0.53	15.30%	156.81 ±1.50	31.9%
Group6	120.83±0.55	137.5 ±0.43	15.50%	155.70 ±1.19	28.9%

*Groups :(n=10)

Table 9. Effect of feeding on functional camel's milk blends on serum biochemical aspects against high fat diets in Albino male rats

Groups Parameter	G0	G1	G2	G3	G4	G5	G6	P-Value
Serum Kidney Function								
Creatinine (mg/dl)	0.68±0.40	2.20±0.40	2.1 0 ±0.73	1.9 0 ±0.73	1.60 ±0.73	1.5 0 ±0.73	0.86 ±0.73	0.012
Urea (mg/dl)	25±0. 71	59.0±0.03	57.0±0.05	55±0.01	48±0.03	41±0.04	32±0.09	0.031
Serum glucose (µmol/l)	140.18±0.27	150.18±0.27	145.78±0.29	143.78±0.29	142.78±0.29	144.78±0.29	146.78±0.3	0.014
Lipid Profile								
Total cholesterol (mmol/l)	228±0.09	295±0.11	277±0.08	268±0.08	251±0.08	248.08±0.07	237±0.08	0.012
Triglycerides (TG) (mmol/l)	150±0.04	172±0.05	169±0.02	162±0.05	158±0. 13	153±0.12	151±0.04	0.033
Liver Function Serum								
Alanine aminotransferase (ALT) (IU/l)	30.65±0.12	55.11 ± 1.70	53.3 ± 1.90	52.11 ±1.90	50.1 ±10	49.94 ±0.90	45.8 ±1.70	0.023
Aspartate aminotransferase (AST) (IU/l)	40.96±0.19	66.82±12.30	47.89±17.34	46.03 ±0.8	45.6 ±0.3	44.91±0.4	44.25 ±0.8	0.041
Total bilirubin (T.B) (mg/dl)	0.16 ± 0.09	1.15 ± 0.09	0.16 ± 0.70	0.31 ± 0.07	0.8 ± 0.07	0.62 ±0.05	0.56 ±0.03	0.012
Albumin ALB(g/dl)	4.4 0±1.30	3.23± 0.42	4.2 0 ±0.80	4.2 0 ±0.80	4.19 ±0.80	4.29 ±0.80	4.30 ±0.80	0.042
Total protein (TP) (g/dl)	5.71±0.03	1.90 ±0.53	2.90±0.26	2.66 ±0.43	3.13 ±0.86	3.93 ±0.79	4.81 ±0.47	0.05
Heart Function Serum								
Alkaline phosphatase (ALP) (IU/l)	162.7 ±1.6	187.2 ± 1.4	175.2 ± 1.1	169.3 ± 0.6	167.2 ± 0.2	165.5 ± 1.9	164.7 ±1.6	0.035
Creatine kinase (CK) (IU/l)	1277.58±37.4	1290.25±58.3	1310.58±74.5	1353.32 ±62.3	1287.58±68.0	1265.58±94.1	1270.58±87.4	0.037
L-lactate dehydrogenase (LDH) (IU/l)	165.2 ± 0.8	166.1 ± 0.2	162.1 ± 1.8	164.7 ± 2.1	161.1 ± 1.9	164.2 ± 1.4	165.2 ± 0.8	0.011
γ-glutamyl transferase (GGT) (IU/l)	16.65 ±1.55	16.74 ±1.90	16.10±1.55	16. 40±1.56	16.34 ±1.34	16.51 ±1.47	16.65 ±1.55	0.015
Pancreatic function								
α-amylase (AMY) (IU/l)	1540.43±39.1	1562.09±54.8	1557.43±39.0	1552.43±39.0	1553.2 3±39.01	1549.43±39.0	1546.43±39.1	0.046
Mineral								
Calcium (mEq/L)	10.50 ±0.19	7.52±0.06	8.90±0.32	9. 32±0. 24	10.06±0.09	10.16±0.45	10.38±0.19	0.049

Values are expressed as are means ± SD (n =10). **G0**: Control basal diet only; **G1**: high fat basal diet (Positive control); **G2**:Camel milk + Fat basal diet; **G3**:Camel milk + Fat basal diet+ Dates; **G4**:Camel milk + Fat basal diet+ Dates +Echinacea (1%); **G5**: Camel milk + Fat basal diet+ Dates +Echinacea (3%); **G6**: Camel milk + Fat basal diet+ Dates +Echinacea (5%).

Cyclohexanediol and Phloroglucinol were the predominant alcohols and their concentrations were 3.66 and 2.52%, respectively. Dihydro-3-oxo-β-ionol(1.80%) and Heptatriacotanol (0.19%) were also identified (Table,

6).

Esters were the first major group identified in the aroma of date pulp fruits. Three esters were represented in the table with high concentration. The main ester compound

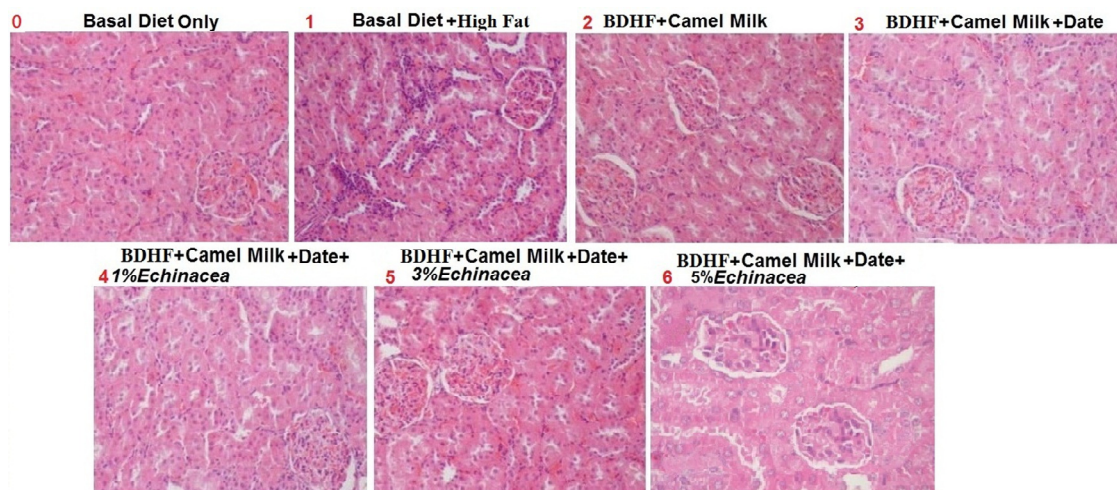


Figure 1. Light micrographs of hematoxylin-eosin (H&E) stained liver sections, represented that in Control group showed normal (G0) hepatic cell with well preserved cytoplasm, group1 (G1) hematoxylin and eosin sections of the liver revealed sever congestion sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change; in the group2(G2) were observed marked congestion sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change. Further, moderate sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change in group3(G3) as well as Mild congestion sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change in group 4 and 5 (G4&G5), while in the in group 6 (G6) animal fed on mixture of date fruit and camel milk with a high dose of Echinacea (5%) on the day sixty of the experiment showed normal parenchymal architecture with cords of hepatocytes nearly similar to normal live tissues(400×) compared with the positive control (G1)

identified was Isopropyl palmitate (11.74%) followed by Ethyl 14-oxotetradecanoate (9.66%) whereas, 10-Octadecenoic acid, methyl ester was found in a trace amount (0.43%).

L-Carnitine (3.39%) and Butylated hydroxytoluene (1.66%) were the major antioxidant compounds. Whereas, Gentamicin B (0.46 %) was the only antibiotic compound fractionated in date pulp fruits.

Five fatty acids were identified from date pulp fruits. n-Pentadecanoic acid, n-Undecoic acid and Octadecanedioic acid were the predominant fatty acids and their concentrations were 7.61 , 4.41and 3.19%, respectively. Moreover, Palmitic acid (2.67%) and 4-Guanidinobutyric acid (2.5%) were also identified. On the other hand, d-Altronic acid (10.43%) and cis-Vaccenic acid(7.89%) were the major organic acids identified in date pulp fruits. 11-Aminoundecanoic acid (3.18%) was the only lipoprotein compound fractionated in date pulp fruits.

Two lactones namely, l-Gulonic acid, γ -lactone and d-Lyx-d-Manno-nononic-1, 4-lactone were identified with trace concentration (0.66 and 0.55%, respectively). Furthermore, Levoglucosenone was the predominant ketone (5.71%) followed by Pyranone which found in a small amount (0.71%) (Table 6). Compounds identified through mass spectra library matching for the peaks were listed at the table(5). Classification of compound obtained from references^{31&32}; Pharmacological activity information obtained from references^{33&34}. According to the compounds classifications and Pharmacological activity information of the date pulp fruits contained a complex mixture consisting of a high proportion of polyphenols

,sesquiterpenes, whereas the fraction of monoterpenes was small.

Fractionation and identification of effective constituents of the *Echinacea purpurea*

The components of the *Echinacea* aerial part fractions were separated by GC analysis and the produced peaks were identified using GC/MS. More than 26 components were separated and identified in the *Echinacea* aerial part extract. The oxygenated compounds commonly named alcohols and esters are considered to be the most important flavor compounds. Alcohols were the major groups identified and α -Bisabolol was the major alcohol component of volatile fractions giving the highest peak area percentage 29.48%. Other seven alcohols namely p-Nonylphenol (3.98%), 4-tert-Octyl-o-Cresol (3.19 %), Resveratrol (2.50%), Globulol(2.05%), 4-Methylcatechol (1.11%), Phenol, 2,5-bis(1-methylethyl)(0.86%), and, 4-methyl-3-Methoxy-2,4,6-trimethylphenol (0.44%) were also identified in the *Echinacea* aerial part extract (Table, 7). As seen in the same table, two esters were identified of the *Echinacea* aerial part extract effective constituents namely, (-)-Isolongifolol, methyl ether (1.22%) and Geranyl isovalerate (0.93%).

Vitamins were the most dominant group identified in the *Echinacea* extract. The main vitamin was Biotin (Vit. H) which possess a very strong vitamin detected with high concentrations (8.99%) followed by 9-cis-Retinal and Retinyl propionate (Vit. A derivatives) and their concentrations were 1.98 % and 1.16%, respectively.

Table10. Effect of feeding on functional camel's milk blends on serum Malondialdehyde and Glutathione in healthy and high fat Albino male rats

*Groups Parameters	G0	G1	G2	G3	G4	G5	G6	P-Value
Malondialdehyde (MAD) (mg/dL)	3.2 ± 0.13	7.95 ± 0.43	6.76 ± 0.23	5.83 ± 0.18	4.87 ± 0.32	3.83 ± 0.18	3.40 ± 0.18	0.031
Glutathione GSH (mg/dL)	44.53 ± 1.35	32.53 ± 0.53	35.15 ± 0.17	37.32 ± 0.17	39.36 ± 0.24	40.28 ± 0.88	42.13 ± 0.53	0.038

*See Table (9)

Table11. Effect of feeding on functional camel's milk blends on serum Cytokine and Immunomodulatory concentrations in healthy and high fat Albino male rats

*Groups Parameters	G0	G1	G2	G3	G4	G5	G6	P-Value
**IL-2 (pg./ml)	0.214 ± 0.015	0.194 ± 0.015	0.200 ± 0.08	0.203 ± 0.06	0.207 ± 0.08	0.209.73 ± 0.01	0.206 ± 0.02	0.25
**IL-1α (pg/ml)	19.4 ± 1.84	24.4 ± 1.84	23.68 ± 3.94	21.74 ± 1.70	20.52 ± 1.51	19.91 ± 1.46	19.07 ± 1.46	0.10
***IgG (mg/ml)	19.4 ± 0.70	12.4 ± 0.50	17.34 ± 1.06	17.95 ± 0.85	18.53 ± 0.41	18.72 ± 0.76	18.91 ± 0.76	0.03
***IgM (μg/ml)	283 ± 8.24	200.1 ± 8.24	233.2 ± 7.64	245.4 ± 7.59	261.4 ± 8.86	270.5 ± 8.11	280.5 ± 9.11	0.04
***IgE (IU/ml)	151.4 ± 0.023	1045.15 ± 0.01	903.34 ± 0.03	712.42 ± 0.0	632.41 ± 0.04	464.0 ± 0.02	164.10 ± 0.03	0.002

*See Table (9)

**serum levels in cytokines (IL-1α and IL-2)

*** serum immunoglobulins (IgG, IgM and IgE)

Also, δ-Tocopherol (Vit. E) found in a small amount (1.06%).

α-Monolaurin was the only monoglyceride (Emulsifier) giving the highest peak area percentage 23.87%. Furthermore, four fatty acids were identified in the *Echinacea* extract and represented in trace amounts as Octadecanedioic acid (0.98%), Dodecanoic acid (0.31%), Oleic acid (0.18%) and Linoleic acid (0.16%). Furthermore, β-Methylionone was the predominant lactone (6.43%) followed by Isopulegone (3.78%) whereas, γ-ionone found in a trace amount (0.81%) (**Table 7**). Carmine acid (red pigment or dye/ organic acid) represented about 3.92%. Compounds identified through mass spectra library matching for the peaks were listed at the table (5). Classification of compound obtained from references^{31&32}; Pharmacological activity information obtained from references^{33&34}. According to the compounds classifications and Pharmacological activity information of the *Echinacea* aerial part contained a complex mixture consisting of a high proportion of sesquiterpenes, alkylamides and anandamide and polyphenole whereas the fraction of monoterpenes was small.

Influence of feeding on functional camel milk blends on growth rate in rats

Body weight was assessed at days 0, 30 and 60 days

and showed an increase in body weight in all rats groups compared with control group. There was no mortality rate in all groups all over the experimental period (**Table8**). A positive data in the body weights of the rats increased markedly at day 30 throughout the study and recorded a net body weight gain ranged from 13.8 to 15.5 % compared with the control group. The body weights of the rats increased slightly at day 60 throughout the study and recorded a net body weight gain ranged from 28.9 to 31.3 % compared with the zero time of body weight (30.3%).

Influence of feeding on functional camel milk blends on biochemical examination in rats

Table (9) summarized the changes in serum enzymes for different experimental groups. Feeding on the camel milk, the dates pulp and the *Echinacea* aerial part revealed that there no statistical difference in all parameters under estimation as blood serum glucose, TB, TP, ALT, AST, ALB CK, LDH, BUN, Creatinine, CHOL, TG, MDA, GSH, α-amylase (AMY) and Calcium (**Table 9**).

The serum levels of the myocardial markers and liver enzymes showed significant difference in the ALT, AST, CK and LDH serve as sensitive indices to assess the extent of cardiac food effect before and after food additive between groups under investigation compared with a positive control group (G1) (Table, 9). Feeding on high fat basal diet (G1) (positive control) had significant

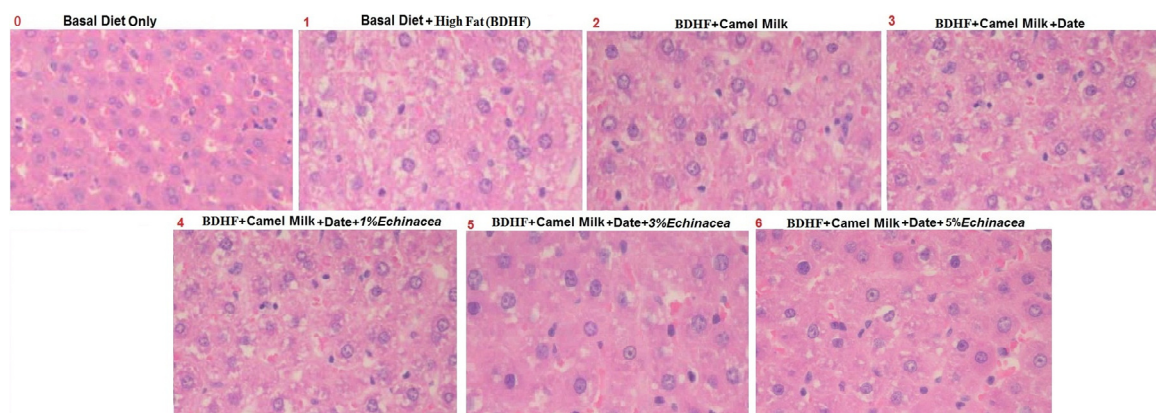


Figure 2. Light micrographs of hematoxylin-eosin (H&E) stained kidney cortex sections (400×) represented that (G0) control kidney showing, normal renal parenchyma, normal tubules and normal glomeruli. (G1) positive control of kidney showing renal inflammatory cells were severe infiltrated into the interstitial (G2) treated kidney with camel milk only showing normal renal parenchyma normal tubules and normal glomeruli slightly disappeared of inflammatory cells. (G3) treated kidney with mixture from camel milk and date fruit showing normal renal parenchyma normal tubules and normal glomeruli mild disappeared of inflammatory. (G4&G5) treated kidney with mixture from camel milk and date fruit and Echinacea (1,3)% respectively showing normal renal parenchyma normal tubules and normal glomeruli more disappeared of inflammatory. (G6) treated kidney with mixture from camel milk and date fruit and Echinacea (5%) respectively showing normal renal parenchyma normal tubules and normal glomeruli inflammatory cells infiltrated the interstitial had been disappeared

increases in the serum levels of glucose, triglycerides and cholesterol but their levels were reduced in the other groups which received the camel milk with the dates fruits and the Echinacea.

Stability of the bile acid levels, which is synthesized in the liver from cholesterol or derived from plasma lipids leading to stability in cholesterol levels post-treatment with the camel milk and/or the dates pulp and the Echinacea.

Table 9 also shows a marked increase in serum urea and creatinine levels in rats fed on high fat basal diet (G1) (positive control) compared with normal healthy control (G0) ones. Pre-and post-treatment with the camel milk and/or the dates pulp and the Echinacea induced a significant improvement of the urea and the creatinine levels compared to control (G1).

The present data in **Table 9** showed that be attention to TP and ALB contents in blood serum of the rats, which were found equals to the reference values this indicated that there are negative or positive effect of the additive food combination on the metabolism of proteins and this indicated that there no cell damage exhibited a good correlation with the other enzyme.

Serum alkaline phosphatase in the test groups is related to the functioning of hepatocytes and did not increase in its activity is due to no increase synthesis in the presence of biliary pressure. **Table 9** revealed that there were significant difference within group on serum amylase activity in pre and post food supplementation groups compared with the group received high fat basal diet (G1). A significant change was observed ($p > 0.05$) in calcium levels of all treated groups compared with positive control group (G1) (**Table 9**).

The present data in **Table 10** revealed that there were significant differences in concentration of the serum MDA

in group (2) comparing with group(1)($p < 0.05$) Whereas, there was a significant differences can be observed in groups (4,5,6) which were dieting on the different types of food supplementation especially at the high dose of the Echinacea the aerial part compared with a control group. Data of statistical analysis showed that significant differences on MDA levels between different types of the food supplementation ($p = 0.031$) (Table, 10). Moreover, the serum levels of GSH in Table (10) were affected significantly by different types of food supplementation compared with higher fat diet (positive control) group ($p = 0.038$). Also, there were significant changes for the effect of food supplementation ($p = 0.828$), and the period of treatment effect ($p = 0.062$).

The present study revealed that the effect of dietary the camel milk, the dates pulp and the Echinacea aerial part on Ig and cytokine production in the rat serum had been examined. The results of Table (11) showed that the dietary adding to Camel milk as date pulp and /or different levels (1, 3 and 5%) of the Echinacea the aerial part increased the concentration of the serum IgG, IgM and IL-2 levels. The Echinacea part levels (1,3 and 5%) increased the concentration of the serum IL-2, and degrade the levels of the serum interleukin-1 α and IgE and the mean of IgE levels in healthy controls and treated were found to be 151.95 IU/L and (1045;903;712.42; 632.41; 464.0; 164.10) IU/L, respectively compared with the other groups and the positive control group(G1) with significant differences when the levels were compared with group fed on High fat basal diet ($P < 0.001$). Likewise, all three dietary types were found to stimulate the IgG, IgM and IL-2 production. In contrast, the absence of the Echinacea aerial part (dietary herbs) had marked significantly decreased in cytokines secretion as the IgA, IgM and IL-

2 levels while the IL-1 α and IgE levels had been increased (Table, 11).

Influence of feeding on functional camel's milk blends on his topathological examination in rats

Figure 4 reprehensive that in G1, the hematoxylin and the eosin sections of the liver revealed sever congestion sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change. G2 observed marked congestion sinusoids and vacuolation of cytoplasm of hepatocytes/fatty change. Further, moderate sinusoids, and vacuolation of cytoplasm of hepatocytes/fatty change in group (G3) as well as, mild congestion sinusoids and vacuolation of the cytoplasm of hepatocytes/fatty change in groups 4 and 5 while in G6 on the 60ty day of the experimental period [Figure 6] respective no morphological changes in the liver tissues in rats fed on camel milk mixed with date fruit pulp at a higher dose of the *Echinacea* (5%) compared with the High fat high cholesterol group (G1).

Figure (5) reprehensive that in group1, hematoxylin and eosin sections of the liver revealed sever showed histological analyses as renal inflammatory cells were infiltrated into the interstitium in control and camel milk only fed rats (Fig.5). This tubulointerstitial pathology disappeared after treatment with mixture of camel milk, date pulp and three different levels of the *Echinacea*. Moreover, the kidney of albino rats received camel milk +date pulp and 5% of the *Echinacea* in group (G6) showed no obvious morphological changes compared with vehicle normal states, inflammatory cells infiltrated the interstitial had been disappeared.

DISCUSSION

The present results proved that rats fed on camel's milk mixed with date's pulp and different levels of the *Echinacea* arial part (1,3and5 %) gained more weight than other groups. These results are in agreement with the results obtained by Maass *et al.*,³⁵ who found that adding part of the *Echinacea* extract affected the growth performance equally in all groups of pigs. The scores of flavor of camel's milk sample supplemented with 10% dates pulp (T1) was higher than control sample. This means that, addition of date's pulp in camel milk improved the sensory attributes of the final product. Farahat *et al.*,³⁶ recommended that date pulp could be used up to 15% for making functional dates ice cream with high quality attributes. Control and (T1) samples (10% dates) had the highest flavors score than all treatments which supplemented with different ratios of the *Echinacea*., Supplementation camel's milk with different levels of part of the *Echinacea* reduced the flavor score of product compared with control sample.

Generally, camel's milk supplemented with 10% dates pulp (T1) showed the highest total score comparing with control. T2, T3 and T4 samples. Whereas, camel's milk supplemented with 10% dates pulp and different levels of the *Echinacea* aerial part (up to 3%) were acceptable by the regular panel test. Increasing the level of the *Echinacea* part to 5% decreased the scores of flavor and appearance of camel's milk /date product. This decrease may be due to the flavor and color properties of the *Echinacea* aerial part that contains carmine acid (red pigment or dye (3.92) and more than 50% phenolic compounds (Table 7).

Data of Table 3 (camel milk with 10% dates pulp with 3% *Echinacea* powder) are in agreement with the data recommended by Mansouri *et al.*⁸ they found that the manufacture of functional camel date milk with Algerian dates had acceptable sensory properties

The present study proved that the al-Hayani date pulp fruits and *Echinacea* arterial part were found to be rich sources of phenolic and bioactive compounds which have highest antioxidant activity. These results have been similar to documented by El-Farsi *et al.*³⁷ and FAO³⁸. Ghiaba *et al.*³⁹ reported that the dates have potent anthocyanins, carotenoids and phenolic compounds (protocaechuic, p-hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, hydroxy benzoic, mainly cinnamic acids) and flavonoids (flavones, flavonols and and flavanones). Also, they proved that the date's fruit have the unique distinction of being the only food to contain flavonoid sulfates which have antioxidant properties. Pharmacological activity information of the al-Hayani date pulp fruits, *Echinacea* arterial part camel milk contained a complex mixture consisting of a high proportion of polyphenole sesquiterpenes, whereas the fraction of monoterpenes was small. A spectrum of natural sesqui-terpenoids from herb oil has displayed anti-oxidant,⁴⁰ anti-inflammatory and anti-carcinogenic properties⁴⁰

Camel's milk is an excellent source of well-balanced nutrients and also exhibits a range of biological activities. Also, it has low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium) and high vitamin C, B2, A and E. It has also no allergic properties and it can be consumed by lactase deficient persons and those with weak immune systems. A series of metabolic and autoimmune diseases are successfully being treated with camel's milk³⁸. Also, camel milk contains fat with a relatively large amount of polyunsaturated fatty acids and linoleic acids, which are essential for human nutrition⁴¹.

The camel's milk in the present study was found to have low concentration of phenolic compounds. Addition of the Al Hayani dates pulp and the *Echinacea* aerial part (main sources of the phenolic and the flavonoid compounds) to camel's milk increased the total of bioactive compounds (the phenolic, the flavonoid and the δ -Tocopherol) and reached to maximum values led to

significant increase in serum glutathione levels in groups of rats diet fed on the dates pulp, the *Echinacea* aerial part and camel milk compared with group fed on a high fat diet (G1).

The results in Table (9) showed that serum cholesterol and triacylglycerol levels also were slightly reduced by use of camel's milk in combination with *Echinacea* powder in this study which is in agreement with results of Sakine *et al.*⁴² they found that there were non-significant differences between the experimental rats as regards serum amylase activity in pre and post food supplementation, this may support against pancreatitis and other exocrine function as well as intra-abdominal disease. The prepared formula includes the camel milk, the date pulp fruits and the *Echinacea* aerial part have good antioxidant properties.

The present data revealed that the TP and ALB contents in blood serum of the rats were reached to the reference values in G6 that received camel's milk and date pulp with 5% *Echinacea* aerial part powder than other groups which were received camel's milk (G2); the camel milk and the dates pulp group (G3); the camel milk and the dates pulp with the *Echinacea* 1 and 3% (G4 and G5, respectively). This indicated that there was no cell damage. The present data had been similar to the data reported by Wolfensohn and Lloyd⁴³. So, the food additives camel's milk and dates pulp with *Echinacea* 1 and 3% and 5% (G4, G5 and G6) respectively, have higher hepato-protective effect as stated by Suresh Kumar *et al.*,⁴⁴.

Extracts of the dates provided to the women after childbirth stimulate their immune system⁴⁵. A significant decrease in the levels of triacylglycerol was observed, possibly due to the high dietary fibers content in dates. Although an increase in the serum glucose level concentrations on postprandial samples was observed, the fasting serum glucose levels remained unaltered. The decreased serum triglyceride levels and the oxidative stress during the month of date consumption were suggested to be the reason for the significantly unaffected serum cholesterol levels⁴⁶.

The elevation in the serum ALP, ALT and AST levels and reduced GSH and histological had been evident the group of rats that fed on high fat diet only which induced oxidative stress effect had been reached to the normal values scavenging the free radicals on vital cells in groups fed on the mixture of the camel milk and the date pulp with the *Echinacea* (5%). Moreover the serum alkaline phosphatase in the tested groups was related to the functioning of hepatocytes and increase in its activity. These data are in agreement with study performed by Burtes and Atwood⁴⁷ they proved that the Alkaline phosphatase (ALP) is a membrane bound glycoprotein enzyme with higher concentration in sinusoids and endothelium, This enzyme reaches to the liver mainly from the bone and it is excreted into the bile. Therefore its elevation in the serum occurs in the hepatobiliary

diseases, as a results of that the present results indicated that the serum of ALT, AST enzymes and the alkaline phosphatase in the tested groups was related to the functioning of the hepatocytes.

So, there were marked stability of the alkaline phosphatase with non elevation of the serum bilirubin levels suggested the stability of the biliary function as well as the stability of the bile acids levels, which is synthesized in the liver from the cholesterol or derived from the plasma lipids leading to stability in the cholesterol levels as shown in groups fed on the dates pulp, the *Echinacea* aerial part and camel milk compared with the group fed on a high fat diet (G1) Table (9).

Table (9) showed that the total protein levels were significantly decreased in G2 rats if compared with the positive control group. These findings are in accordance with the data of Siddiqui *et al.*⁴⁸. The reduction in total protein may be due to decrease in the hepatotoxicity induced by the cholesterol which was mixed with the basal diet.

The serum levels of the LDH had been reduced after G2, G3, G4, G5 and G6 in contrast to the present results. Kubena *et al.*⁴⁹ reported that the high serum LDH activity could be an indication of tissue damage (especially the liver) if accompanied by an increase in the activity of other serum enzymes, especially aminotransferases. The activities of all the other enzymes measured were within the reference range.

The significant differences were detected in myocardial markers enzymes as CK. Data in Table (9) showed that the Camel milk supplemented with 5 % *Echinacea* increased significantly the serum levels of Alpha amylase and calcium compared with group received high fat basal diet (G1). The alpha amylase acts at random locations along a starch chain, yielding Maltotriose, maltose and limit dextrin from amylase. Maltose, glucose and limit dextrin from amylopectin, alpha amylase unable to function in absence of (Ca²⁺)⁵⁰.

Rats fed on date pulp fruits lead to decrease the levels of levels of malondialdehyde had been increased compared with the high fat diet group (Table 9), these data similar to data documented by Saafi *et al.*,⁵¹ they proved that the rats fed on date pulp fruits lead to decrease the levels of the malondialdehyde which had been increased in rats treated with dimethoate alone.

Data in Table (10) showed that restored GSH levels to normal in rats administered with mixture of camel's milk and date pulp with 5% *Echinacea* compared with positive control group (G1). Also, the serum GSH level was not affected significantly by different types of food supplementation. This is in agreement with study performed by Abdalla⁵², who concluded that high level of glutathione (GSH) is needed for cellular functions, signal transduction and protection against certain carcinogens.

The present data in Table (10) agree with results had been documented by Al-Farsi *et al.*,⁵³ and Mansouri *et*

al.⁸ which they proved that the presence of any harmful material could be included in the diet leading to deplete serum glutathione levels the family of enzyme utilize glutathione in reactions leading to the detoxification of any harmful material could be included the diet could be lead to diminish in the serum glutathione levels. Probably the body will sort out this deficiency by using alternative mechanisms.

The immune system is an important defensive system to protect the human body from invasion by various pathogens such as allergens, viruses and bacteria⁵⁴. *Echinacea* aerial part contained alkylamides and anandamide it have a potentially inhibited lipopolysaccharide-induced inflammation in human whole blood and exerted modulatory effects on cytokine expression⁵⁵. In the present study, using camel's milk, date pulp fruit supplemented with 5 % *Echinacea* increased the mixture in addition to basal diet could be stimulate IgG, and IgM and IL-2 production significantly in the serum of rats comparing with positive group fed on high fate in basal diet, (Table, 11). Thus, this dietary supplementation might alleviate inflammatory reactions in the immune system components and immunoglobulin production is class-specifically regulated by various types of lymphokines⁵⁶. Phenolic antioxidants inhibit the NF- κ B (nuclear factor κ B) and AP1 which participate in the production of proinflammatory cytokines interleukin1 α (IL-1 α) and tumor necrosis factor α (TNF- α)¹⁶.

CONCLUSION

The camel milk was successfully supplemented with different ratio of the *Echinacea* and dates pulp obtained functional foods, antibiotic, a good a sources of the natural antioxidants, which stimulate antioxidant potential effect. Finally, it had been stimulate bioactive necessary components, which play a great a role in stimulating the immune system effect. Additionally, Feeding on diets containing the high dose of the *Echinacea* (5%) activated some various components of the immune system as IgG, IgM and IL-2 levels to stimulate the ability of the body against pathogen to resist any pathogen while it could be suppressed others as IgE and IL-1 α . This may be due to the presence of phenolic and flavonoid compounds in the *Echinacea* and date pulp fruits as well as camel's milk have rich of phenolic which were confirmed by the GC/Mass analysis. The improvement in the liver and kidney functions and other metabolic indicators, it might be reflected in stopping greatly the destructive changes in the cellular tissues of the liver and kidney. However, more research is necessary and further studies are recommended warranted before a final conclusion can be made.

Also, this study highly recommends that camel's milk supplemented with dates pulp and high dose of the *Echinacea* (5%) should be included among the

medications used for treatment of liver diseases and further research is needed to confirm our work.

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Abbreviations:

Camel milk	CM
IL-1 α	Interlukin-1 α
IL-2	Interlukin-2
Ig	Immune globuline
MAD	<u>Malondialdehyde</u> (lipid peroxide)
GSH	Glutathione reduced
ALT	Alanine aminotransfrase
AST	aspartate aminotransfrease
GGT	Gamma glutamyl transferase
T.B	total bilirubin
Alb	albumin
TG	Triglycerides
CHOL	Total Cholesterol
ALB	Albumin
T.P	Total protein
ALP	Alkaline phosphatase
GGt	γ -glutamyl transferase
CK	Creatine kinase
LDH	lactate dehydrogenase
AMY	α -amylase
HF-HC	High fat high Cholesterol