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Therapeutic activity of fermented camel milk with *Artemisia absinthium* on alloxan induced diabetic rats

Mahmoud M. Motawee[1] and Hesham Z. Tawfeuk[2]

[1]*Nutrition evaluation and food Science Department, National organization for Drug Control and Research.*
[2]*Food Sci. & tech. Dept.; Fac. Agriculture and natural resources; Aswan University; Aswan, 81528, Egypt.*

Abstract

The aim of the present study was to investigate the therapeutic effect exerted by fortified fermented camel milk (FCM) with *Artemisia absinthium* (AA) on alloxan-induced diabetic rats. The anti-diabetic and antioxidant effect of FCM and AA aqueous extract were studied in diabetic rats model.

Fifty male Sprague-Dawley rats weighing 200±20 g were divided into five groups. The first group kept as negative control (-ve) group (n= 10 rats) which fed on basal diet only. The rest of rats were injected with a single intraperitoneal dose of alloxan (120 mg/kg bw) to induce diabetes. Diabetic rats were divided into 4 groups that were positive control +ve and 3 treated groups that were fermented camel milk(FCM) , *Artemisia Absinthium* aqueous extract(AA) and mixed with them (FCM +AA).

Results showed that consumption of (FCM) with aqueous extract(AA)group showed improvement of serum glucose, (ALT and AST), serum protein levels (total proteins, albumin, globulin and A/G ratio), oxidative stress marker (CAT, SOD MDA, GSH, NO and 8OHdG), Results showed that alloxan significantly (P≤0.05) disrupted glucose level, induced oxidative stress. In contrast treated group with FCM in combination with AA showed optimum protective effect to near control level compared to the individual treatments. The study suggested that, the mixture of FCM plus AA aqueous extract had a protective activity likely due to stimulation of pancreatic β-cells leading to insulin secretion. The study recommended that the combination of FCM and AA extracts rich polyphenols as antioxidant and activate insulin secretion.

Keywords: Fermented Camel Milk, *Artemisia absinthium*, Alloxan, Rats.

1. Introduction

Diabetes is an incessant issue of demented body metabolism of carbohydrate, fat, and protein [10, 29]. Diabetes mellitus (DM) is a lifetime condition caused by deficiency or diminished effectiveness of endogenous insulin that can be either inherited or acquired [46]. At least 250 million individuals worldwide suffer from diabetes and this number will be doubled by 2030. Increases in complications will undeniably follow increasing diabetes incidence rates [74] More than 80% of diabetes deaths take place in low- and middle-income countries [76]. Diabetic symptoms include increased urine output (polyuria), excessive thirst (polydipsia), excessive hunger (polyphagia), and fatigue [34]. Along with insulin there are several oral hypoglycemic drugs are available nowadays to decrease elevated glucose levels in the body, but with unavoidable side effects, that is why still this disease is a big challenge to the medical community. So it is essential to investigate and discover alternative drugs to overthrow the diabetic problems without any side effects. Herbal formulations and native plant derivatives are being used as another choice to treat the disease, where blood sugar swings wildly and its difficulties. Indian conventional medicinal entities like Ayurveda, Siddha and Unani system distinctly claim to cure the hyperglycemia and its symptoms with the use of natural drugs *Kirtikar and Basu* (2001) [43].

According to the World Health Organization (WHO) report, around four billion people (80% of the world´s population) use herbal medicine [31], with eleven different bioclimatic regions and around 7,500 different plant species. *Artemisia* is considered as an important medicinal plant species with high content of essential oils and flavonoids, and is thoroughly studied [75].

Corresponding authors: hesham_zakaria@agr.aswu.edu.eg

Artemisia absinthium, a perennial shrubby plant that belongs to the family Asteraceae, the species that is wide spreading Kashmir valley and have global distribution from Europe to North Asia, an ingredient in the liquor absinthe [19]. *Artemisia absinthium* (Wormwood) is an aromatic, perennial shrub that was used as alternative herbal medicine for several health disorders. Ethno pharmacological evaluation of *Artemisia absinthium* revealed its free-radical scavenging activity, anti-oxidative stress function, antioxidant activity and neurite outgrowth function [19], These strong principle characteristics of *Artemisia absinthium* provoked us to investigate whether this plant has any protective effect on alloxan induced diabetic rats.

On the other hand camel's milk is differed from other ruminant milk; it is low in cholesterol, sugar and protein but high in minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamins A, B_2, C and E, and contains a high concentration of insulin [44]. It also contains fat with a relatively large amount of polyunsaturated fatty acids and linoleic acids, which are essential for human nutrition [32]. Camel milk has a high biological value due to the higher contents of antimicrobial factors such as lysozyme, lactoferrin and immunoglobulins [27] and it has concentration of these components higher than cow and buffalo milk [25]. Several studies have been carried out at the Zayed Complex, UAE showed that camel milk has the IgA and IgG that have proved effective against several viral and bacterial Pathogens [41]. Camel milk is known to have medicinal properties since ancient times. Oral camel milk is well tolerated by lactase-deficient children who are allergic to cow milk [26], and it shows protective effects against heavy metal [9], chronic pulmonary tuberculosis [51] and viral and bacterial infections [27]. Additionally, Indians used camel milk for the treatment of multiple acute and chronic health problems, including asthma, anemia, jaundice, and spleen problems [62]. Interestingly, the low prevalence of diabetes in the Raica community was attributed to the regular consumption of camel milk [5]. This was further supported by the better glycemic control in diabetic patients and animals receiving camel milk [6, 7, 35].

Malik et al., 2012 [52] reported that Camel milk is traditionally fermented in order to be more sustainable, nutritious, and health promoting. Chal or Shubat is the homemade fermented camel milk (FCM) in Turkey, Kazakhstan, and Turkmenistan, [69] and by Turkmens in Iran. In Iran, a kind of pasteurized FCM similar to Chal has been produced industrially which was used in this study considering its pasteurization.

Agrawal et al., 2007b [3,4] reported that, there is a zero prevalence of diabetes in camel milk consuming due to CM has reach with insulin and insulin like protein.There is a growing interest in probiotic interventions for the management and treatment of diabetes [78]. Probiotics are defined as living microorganisms in food and dietary supplements that up on ingestion in sufficient amount scan improve the health of the host beyond their basic nutritional content *FAO/WHO (2001)* [30]. In animal studies, it has been confirmed that probiotics treatments inhibits β cells destruction in the islets of Langerhans in diabetic mice [11, 35, 77]. It was proposed that dairy product are more effective for administration probiotics [47] and the probiotic may be useful in therapy and in reducing serum total cholesterol(TC) ,triglycerides (TG), low density lipoprotein cholesterol (LDL- C), lipid peroxidation with increases High-density lipoprotein cholesterol levels(HDL-C) [73]. *Zhang et al.*, (2016) [79] reported that the probiotic may improve glucose metabolism with potential greater effect when the duration of intervention is ≥ 8 weeks.

So this study carried out to investigate the therapeutic effect exerted by fermented camel milk (FCM) inoculated with *Artemisia absinthium* (AA) on alloxan-induced diabetic rats.

2. Materials and Methods:

This study was carried out at animal physiology Lab., NODACR, Giza, Egypt starting from the first of August till the September 2020.

2.1.Materials. Camel milk samples were collected early in the morning from herd of camels by hand milking from Matroh, Behara governrate-Egypt in summer 2020. The samples were collected in sterile screw bottles, kept in cool box until transported to the laboratory, and stored at $5\pm1^{O}C$ for subsequent processing.

Starter culture:

1. Commercial starter culture: (YOFLEX- YC-X11), which contained Streptococcus thermophiles and Lactobacillus delbrueckii subsp. bulgaricus, were provided by Chr. Hansen

(Milwaukee, WIS., U.S.A.) and stored in deep freezer until used.

2. Probiotic bacteria Culture: Lactobacillus rhominosus, Lactobacillus gasseri and Bifidobacterium breve distributed by Wal-Mart Stores, Inc., Bentonville. AR. 72716 Canada.

Alloxan monohydratewas purchased from Sigma chemical Company (St Louis Mo, USA) and *Artemisia absinthium* was obtained from local market, Aswan, Egypt.

Animal's (*Sprague Dawley)* rats weighting 200±20 g were obtained from the central animal housing, NODCAR, Egypt. The animals were maintained in standard plastic cages at temperature of 22 ± 1^{O}C and light–dark cycle of 12/12 h in the Animal Housing of Biochemistry Division to acclimatize two weeks before experiment. The animals were fed with commercial pellets and given free access to fresh water ad libitum. The experimental protocols were approved according to the Guide for the care and use laboratory animals of local Ethics Committee, NODCAR, Egypt.

2.*2. Methods.*

*2.2.1. Maintenance of probiotic strain*s: Probiotic bacteria were adopted according to [42, 72] and [60].

2.2.2.Preparation of starter culture: The day prior to fermented camel milk manufacture, starter culture was made by addition of 2% of Lactobacillus delbrueckii ssp. Bulgaricus and streptococcus thermophillus mother culture into camel milk in different separated container with or without *Artemisia absinthium*. Then, stirred well and incubated at 43ºC for 6 hours at the end of fermentation.

2.2.3.Extract preparation Approximately 100 g of *Artemisia absinthium* leaf were extracted twice with 70% ethanol using a 2 h reflux extraction, and the extract was concentrated under reduced pressure. The concentrate was filtered, lyophilized, and subsequently stored at 4∘C. The yield of the dried extract from starting crude materials was 14.62% (w/w).

2.3 Induction of hyperglycemia

Rats were induced with a single intraperitoneal dose of alloxan (120 mg/kg body weight), after an 18 hour overnight fast. Thereafter, the animals were allowed free access to food and water. Plasma glucose levels were determined using the glucose oxidase method [28]. The level of blood glucose considered to be normal in rates novergicus ranges from 50 mg/dl – 135 mg/dl., *Shah and Khan (2014)* [67].

2.4. Experimental design

A total of fifty rats (*Sprague Dawley)* were utilized in this study. The rats had an initial weight 200±20 g (9-12 weeks old). Rats were randomly divided into five groups; each selected six rats appear the best results for diabetic induction model. The study was conducted for one month plus two weeks before diabetic model.

Group I served as normal control was fed with commercial diet.

Group II to V were treated with a single intraperitoneal dose of alloxan (120 mg/kg body weight) at day 15th of experiment then divided into 4 groups started with **group II** control (+ve). **Group III** served as diabetic treated with fermented camel milk (**Dia+FCM**). **Group IV** served as diabetic treated with AA (200 mg/kg b.w p.o) (**Dia+AA**). **Group V** served as diabetic treated with fermented camel milk plus AA (**Dia+FCM+AA**). The study period was 30 days from starting experiment.

All blood samples were collected within one hour period between 8:00am and 9:00 am. Twelve hours fasted blood samples were collected under light ether anesthesia by retro orbital puncture. Blood samples were collected after 30 days of the treatment period. These blood samples were used for glucose level, liver function. Then in the morning of the 30th day animal groups were sacrificed by cervical dislocation after general anesthesia.

Biochemical analyses of blood and 10% of homogenate liver were assayed according to the methods mentioned in table 1.

2.5.Statistical analysis

The values were expressed as the mean ± SD for the 10 rats in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using *SAS (2004)* [65] software for Windows (version 13.0). Statistical analysis of the obtained data was performed using the general linear model (GLM). Significant differences among means were evaluated using Duncan's (1955) **.** Multiple Range Test.

The following linear model was applied:
Yij = μ + αi + ξij
Yij = Observation measured
M = Overallmean
αi = Effect of treatment
ξij = Experimental error assumed to be randomly distributed (σ2 = 0).

Table 1. Methods and kits used to quantify the different biochemical analyses of blood and liver homogenate

Parameters	Method	Company	Reference
Glucose	colorimetric	Quimica Clinica Aplicada	Shin et al., (2011)
AST	Enzymatic-colorimetric	Quimica Clinica Aplicada S.A.(Amposta, Spain)	Reitman and Frankel (1957).
ALT	Enzymatic-colorimetric	Quimica Clinica Aplicada S.A.(Amposta, Spain)	Reitman and Frankel (1957).
MDA (nmol/g tissue)	HPLC	Standard of 1, 1, 3, 3 tetraethoxypropane (Sigma)	Karatep (2004)
GSH	HPLC	Standard of 1, 1, 3, 3 tetraethoxypropane (Sigma)	Jayatilleke and Shaw (1993)
NO (nmol/g tissue)	HPLC	Standard of nitite and nitrate (Sigma).	Papadoyannis et al., (1999).
8-OH-dG (nmol/g tissue)	HPLC	Standard of 8 -hydroxy-2 – deoxyguanosine (Sigma).	Lodovici et al., (2000).
SOD	colorimetric	Against pyrogalol	Marklund and Marklund (1974).
CAT	colorimetric	Against hydrogen peroxide	Aebi (1984).
TP	colorimetric	Quimica Clinica Aplicada	Bradford (1976).
Alb	colorimetric	S.A.(Amposta, Spain)	Gustafsson (1976).
Glob	colorimetric	S.A.(Amposta, Spain)	Gustafsson (1976).

3.Results

Results in Table (2) showed effect of fermented camel milk, *Artemisia Absinthium* aqueous extract and there combination on glucose level in Alloxan induced diabetic rat, these data illustrated that the highest value in glucose level recorded by Dia group (222.6 mg/dl) followed by Dia+FCM group (188.6 mg/dl), in another side the lowest value in glucose recorded by Dia+AA group (143 mg/dl) when compared with control group (106.6 mg/dl).

Also, data in Table (2) show the effect of fermented camel milk, *Artemisia Absinthium* aqueous extract and there combination on trans amines (ALT and AST) on rats, Aloxan caused an increase in ALT and AST values from 32.7 and 31.1 U/L to 50.7 and 41.2 U/L.; respectively. Ingestion *of Artemisia Absinthium* aqueous extract caused significant decrease in both parameters was found to be 35.7, and 26.4 U/L.; respectively in comparison with Dia group (50.7, 41.2 U/L) and Dia+FCM group (53.5, 35.5 U/L) [64].

Table 2. Effect of fermented camel milk and Artemisia Absinthium aqueous extract on glucose level and some liver function in Alloxan induced diabetic rat.

Groups	Parameters		
	Glu mg/dl	**ALT U/L**	**AST U/L**
Control (-ve)	106.6 ± 3.025^{d}	32.7 ± 0.893^{cd}	31.1 ± 0.833^{c}
Dia (control +ve)	222.6 ± 5.982^{a}	50.7 ± 1.357^{a}	41.2 ± 1.173^{a}
Dia+FCM	188.6 ± 5.277^{b}	53.5 ± 1.448^{a}	35.5 ± 0.978^{b}
Dia+AA	143 ± 3.949^{c}	35.7 ± 0.949^{bc}	26.4 ± 0.725^{d}
Dia+FCM+AA	164.9 ± 4.615^{bc}	39.8 ± 1.121^{ab}	33.8 ± 0.937^{bc}

Values are expressed as mean ± SD for 6 rats /group. a: significant difference from control group at $P < 0.05$, b: significant difference from Diabitic group (control +ve) at $P < 0.05$. c: significant difference from Diabitic+ fermented camel milk (Dia+FCM) at $P < 0.05$. d: significant difference from Diabitic+ *Artemisia Absinthium* group (Dia+AA) at $P < 0.05$

Table 3. Effect of fermented camel milk and *Artemisia Absinthium* aqueous extracts on serum protein level (TP, Alb, Glob and A/G) in Alloxan induced diabetic rat

Groups	Parameters			
	TP g/dl	Alb g/dl	Glob g/dl	A/G
Control (-ve)	7.4 ± 0.208^{b}	4.4 ± 0.119^{a}	3 ± 0.081^{ab}	1.4 ± 0.041^{b}
Dia (control +ve)	6.3 ± 0.176^{c}	3.8 ± 0.081^{b}	2.5 ± 0.085^{b}	1.52 ± 0.035^{a}
Dia+FCM	6.5 ± 0.119^{c}	3.9 ± 0.084^{b}	2.6 ± 0.039^{b}	1.5 ± 0.036^{a}
Dia+AA	7.7 ± 0.208^{ab}	4.3 ± 0.123^{ab}	3.3 ± 0.089^{a}	1.3 ± 0.035^{c}
Dia+FCM+AA	7.8 ± 0.206^{a}	4.6 ± 0.129^{a}	3.2 ± 0.084^{a}	1.4 ± 0.039^{b}

Values are expressed as mean ± SD for 6 rats /group. a: significant difference from control group at $P < 0.05$, b: significant difference from Diabitic group (control +ve) at $P < 0.05$. c: significant difference from Diabitic+ fermented camel milk (Dia+FCM) at $P < 0.05$. d: significant difference from Diabitic+ *Artemisia Absinthium* group (Dia+AA) at $P < 0.05$.

Table 4. Effect of fermented camel milk and *Artemisia Absinthium* aqueous extracts on superoxide dismutase (SOD) enzyme activity and catalase (CAT) in the liver tissue in Alloxan induced diabetic rat [2]

Groups	Parameters	
	SOD U/g liver	CAT U/g liver
Control (-ve)	42.7 ± 1.137^{ab}	31.1 ± 0.831^{b}
Dia (control +ve)	31.9 ± 1.022^{d}	17.1 ± 0.484^{d}
Dia+FCM	33.3 ± 1.012^{c}	14.4 ± 0.391^{e}
Dia+AA	43.8 ± 1.168^{a}	33.3 ± 0.922^{a}
Dia+FCM+AA	43.1 ± 1.134^{a}	24.7 ± 0.705^{c}

Values are expressed as mean ± SD for 6 rats /group. a: significant difference from control group at $P < 0.05$, b: significant difference from Diabitic group (control +ve) at $P < 0.05$. c: significant difference from Diabitic+ fermented camel milk (Dia+FCM) at $P < 0.05$. d: significant difference from Diabitic+ *Artemisia Absinthium* group (Dia+AA) at $P < 0.05$.

Table 5. Effect of fermented camel milk and *Artemisia Absinthium* aqueous extracts on (GSH, NO, MDA and 8OHdG) level on liver in Alloxan induced diabetic rat

Groups	Parameters			
	GSH µmol/g liver	NO µmol/g liver	MDA nmol/g liver	8OHdG pg/g liver
Control (-ve)	14.2 ± 0.4^{c}	11.7 ± 0.332^{c}	20.8 ± 0.569^{c}	145.3 ± 3.835^{d}
Dia (control +ve)	9.7 ± 0.267^{e}	19.7 ± 0.546^{a}	42.5 ± 1.125^{a}	226.8 ± 6.004^{a}
Dia+FCM	11.3 ± 0.299^{d}	22.4 ± 0.594^{a}	37.3 ± 1.026^{ab}	183.3 ± 4.912^{ab}
Dia+AA	19.6 ± 0.535^{b}	13.3 ± 0.378^{bc}	34.7 ± 0.986^{ab}	155.6 ± 4.354^{cd}
Dia+FCM+AA	20.2 ± 0.543^{a}	14.3 ± 0.407^{b}	32.5 ± 0.903^{bc}	174.6 ± 4.937^{bc}

Values are expressed as mean ± SD for 6 rats /group. a: significant difference from control group at $P < 0.05$, b: significant difference from Diabitic group (control +ve) at $P < 0.05$. c: significant difference from Diabitic+ fermented camel milk (Dia+FCM) at $P < 0.05$. d: significant difference from Diabitic+ *Artemisia Absinthium* group (Dia+AA) at $P < 0.05$.

Parameters of serum protein level (total proteins, albumin, globulin and A/G ratio) were evaluated in control, diabetic, Dia+FCM, Dia+AA and Dia+FCM+AA animals Table (3). Diabetic animals showed significant marked decline in serum total proteins, albumin and globulin relative to the corresponding controls.

Treatments of diabetic rats with *Artemisia Absinthium* aqueous extract (Dia+AA) and there combination with fermented camel milk (Dia+FCM+AA) resulted in modulation of the measured serum protein profile parameters to the normal when compared with control group.

Table (4), represents liver activities of antioxidant enzymes as (SOD and CAT) in different groups. The activities of these enzymes were significantly decreased in liver tissue of Dia (31.9, 17.1 U/g) and Dia+FCM (33.3, 14.4 U/g).; respectively in treated rat model there are a significant difference from control group at $P < 0.05$. Treatments of diabetic rats with *Artemisia Absinthium* aqueous extract (Dia+AA) and there combination with fermented camel milk (Dia+FCM+AA) increased these enzymes activity and were brought back to near normal when compared with control group.

Data of glutathione in Table (5) show decrease in glutathione (GSH) in diabetic rat's liver (9.7µmol/g) compared to control group (14.2µmol/g). Treated with fermented camel milk, *Artemisia Absinthium* aqueous extracts and there combination were increased significantly $p < 0.05$ of glutathione levels as compare with diabetic group (11.3, 19.6 and 20.2 µmol/g).; respectively [36].

Also data in this table showed effect of fermented camel milk, *Artemisia Absinthium* aqueous extracts and there combination on nitric oxide (NO) level on liver in Alloxan induced diabetic rat model. We found increase in nitric oxide (NO) in diabetic rat's liver (19.7µmol/g) as compared to control group (11.7µmol/g). Treated with *Artemisia Absinthium* aqueous extracts and there combination with fermented camel milk were decreased significantly $p<0.05$ of nitric oxide levels as compare with diabetic group (13.3, 14.3 µmol/g), respectively.

Liver MDA and 8OHdG decreased in the Dia+AA (34.7 nmol/g and 155.6 pg/g), respectively, and Dia+FCM+AA (32.5 nmol/g and 174.6 pg/g), respectively at the end of the experiment as compared to the Dia group (42.5 nmol/g and 226.8 pg/g).

4.Discussion

In recent years, much attention has been focused on using natural products as an alternative therapy for treatment of many diseases including diabetes mellitus. In the present study, we aimed to evaluate the efficacy of fermented camel milk, *Artemisia Absinthium* aqueous extract and there combination.

The results of our study showed a significant effect of fermented camel milk and extraction of A. absinthium on blood glucose in alloxan induced diabetic rats. The hypoglycemic effect of fermented camel milk could be probably due to the high levels of insulin or insulin-like proteins in camel milk. These results are in agreement with those of *Agrarwal et. al.* (2007c) [4]. Moreover camel milk contains high levels of vitamins A, B_2, C and E and high mineral content (sodium, potassium, iron, zinc, copper and magnesium). These vitamins play the role of antioxidants, thus eliminating free radicals, useful in the prevention of tissue damage caused by toxic agents [1]. Vitamin C has been found to play a significant role in decreasing the high levels of blood hydroperoxide, glucose in diabetic rats [14]. The antidiabetic action of camel milk and the enhancement of β-cell activity may be due also to its role in regulating the immune system and preserving β-cell destruction [66].

Also, the significant antihyperglycemic activity of extraction of A. absinthium may be due to the presence of active components of A. absinthium like α-and β-thujones, thujyl alcohol, azulenes, bisabolene, cadinene, sabinene, pinene, and phellandrene [9, 22, 49, 56]; the exact mechanism of action in diabetes of most of these components are unknown. The plant extracts may also contain some biomolecules that may sensitize the insulin receptor to insulin or stimulate the β-cells of islets of Langerhans to secret insulin which may lead to enhancement of carbohydrate metabolizing enzymes in the direction of the re-organization of normal blood glucose level. It is possible that A. absinthium extracts may act by undeter-mined ways to stimulating insulin production from the pancreatic islets of Langerhans besides effect of thujone.

In addition, administration of AA and FCM caused marked amelioration of serum glucose concentration of alloxan diabetic rats, besides elevating insulin and C-peptide concentrations which were reduced by alloxan administration. The anti-hyperglycemic effects of FCM and AA are possibly linked to their antioxidant properties, which could counteract the toxic and pro oxidant effects of alloxan. Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles [39, 58]. Flavonoids are known to regenerate the damaged β cells in the alloxan diabetic rats [20]. Phenolics were also found to be effective anti-hyperglycemic agents [37], [38]. Our data are in good agreement with other investigators [18, 57] who stated that the positive effects of specific plant extracts on insulin activity suggest a possible role of these plants in improving glucose and insulin metabolism.

It is evident that increased hepatic glucose output in diabetes mellitus may be derived either from glycogenolysis or from gluconeogensis or both [63, 68]. This was confirmed by our results which showed a marked increase of the detected gluconeogenic serum enzymes; Alanine transaminase (ALT) and Aspartate transaminase (AST) compared to those of the non-diabetic ones. Our study demonstrated that, the administration of AA and FCM resulted in the attenuation of liver injury induced by alloxan treatment as indicated by the activities of ALT, and AST. These results are in accordance with those of [63], who found that the decrease of transaminases activities with treatment may be attributed to improved liver function with the return of gluconeogenesis towards its normal rate.

An overall significant reduction in serum total protein, albumin and globulin in diabetic animals consequents with slight non-significant elevation in A/G ratio were observed in the present study Table (3). Insulin generally has an anabolic effect on protein metabolism as it stimulates protein synthesis and retards protein [55]. Reduction of serum total protein in diabetic animals may be due to the hypoinsulinemia induced by treatment of animal with alloxan. Hypoinsulinemia increases the rate of protein catabolism and might have induced a direct adverse effect on the synthesis and secretion of albumin and globulin. In the current study, the elevated levels of serum total proteins, albumin and globulin in diabetic rats treated with fermented camel milk and extraction of A. absinthium may be related to the recovery of serum insulin levels. This effect is due to the antihyperglycemic activity of the A. absinthium, which might have increased the uptake of glucose by the tissue and its utilization and correct kidney function. Very close results were obtained by [17,23, 33].

Antioxidant enzymes such as SOD, CAT are primary enzymes that are involved in the direct elimination of free radicals [48, 57]. SOD enzyme catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), and consequently represents an important defense mechanism against superoxide radical toxicity [13]. hence, *Singh et al.* (2011) [71] reported that an increase in catalytic activity of superoxide-dismutase is expected as part of the system defense.

As revealed in Table (4) activities of enzymatic antioxidants (SOD and CAT) decline significantly ($P<0.05$) in liver tissue of diabetic group when compared with normal control animals. The decreased concentration of enzymatic antioxidants in the organs of diabetic rats might be attributable in part to the reduced synthesis of this antioxidant enzyme. Hyperglycemia is a main cause for elevated free radical levels, followed by production of reactive oxygen species (ROS), which can lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in biological system [15]. there were an increased activities of serum SOD and CAT, following administration of *Artemisia Absinthium* aqueous extract (Dia+AA) and there combination with fermented camel milk (Dia+FCM+AA) increased these enzymes activity and were brought back to near normal when compared with control group. According to *Kostadinovic et al*. (2015) [45] showed that addition of Artemisa absinthium to broiler diet led to higher SOD activity in hemolysed blood and liver homogenates. An imbalance between oxidation and antioxidant status has been shown to play an important role in mediating oxidative stress [53, 61, 70]. So we can say that *Artemisia Absinthium* aqueous extract (Dia+AA) and there combination with fermented camel milk (Dia+FCM+AA) scavenges free radicals and inhibits superoxide radical production as well as enhance the activity of antioxidant enzymes.

Antioxidant enzymes, such as glutathione (GSH) has the capacity to break down free-radical reactions using a chain reaction mechanism [16]. Glutathione is among the major antioxidant defenders, wherein GSH play a major role in reduction of the acute toxicity of xenobiotics and products of lipid peroxidation as a substrate for GShPx [50]. Subsequently, with the increased risk of lipid peroxidation in the liver, there is an increase in the enzymatic activity of GShPx. Depletion of GSH, causes the cells to be more resistant to free radical toxicity, indicating that GSH is involved in the reduction. Also Malondialdehyde is used as a sensitive indicator to determine the level of oxidative stress in the organism. The determination of malondialdehyde has attracted widespread interest, since it appears to offer a simple means of assessing lipid peroxidation in biological materials [24, 40, 59].

Data of glutathione and malondialdehyde in Table (5) show decrease in glutathione (GSH) and increase in malondialdehyde in diabetic rat's liver compared to control group. Treated with fermented camel milk, *Artemisia Absinthium* aqueous extracts and there combinations were provided a significant improvement in the antioxidant system, namely an increment in GSH levels and a reduction in MDA levels. Considering that fermented camel milk, *Artemisia Absinthium* aqueous extracts are a rich source of polyphenolic compounds which have inhibitor potential of glucose absorption from the intestines, so we can be concluded that the antidiabetic effect of fermented camel milk, *Artemisia Absinthium* aqueous extracts is based on its hypoglycemic and antioxidant properties. *Cherlan et al.* (2013) [21] reported that addition of *Artemisia annua*, also known as sweet wormwood, had an influence on lipid oxidation products measured as TBA reactive substances (TBARS) which were lower in the breast and thigh muscle of birds fed different doses of sweet wormwood diets, compared with the control groups. The investigation of *Aouadi et al. (*2014) [12] with the use of *Artemisia herba* alba and Rosmarinus officinalis essential oils (EOs) to evaluate the impact of the dietary administration of EOs on the overall antioxidant capacity of the muscle and on the oxidative stability of meat over a period of refrigerated storage, showed that MDA content was significantly lower in treatments treated with medical herbs in concentrations of 400 mg/kg each, compared to control treatments. On the basis of these mentioned studies it may be concluded that medical herbs play an important role in animal nutrition in terms of the oxidative stability of organisms.

Also data in this table showed effect of fermented camel milk, *Artemisia Absinthium* aqueous extracts and there combination on nitric oxide (NO) and 8OHdG level on liver in Alloxan induced diabetic rat model. We found increase in both in diabetic rat's liver as compared to control group. Treated with *Artemisia Absinthium* aqueous extracts and there combination with fermented camel milk were decreased significantly $p < 0.05$ as compare with diabetic group may be due to the high content of antioxidant and antidiabetic factors.

5. Conclusion

From above results the study suggested that, FCM fortified with AA aqueous extract had a protective activity likely due to stimulation of pancreatic β-cells leading to insulin secretion.

The study recommended that the combination of FCM and AA extracts rich polyphenols as antioxidant and activate insulin secretion.

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