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Biological Evaluation of Aqueous Herbal Extracts and Stirred Yoghurt Filtrate Mixture Against Alloxan-Induced Oxidative Stress and Diabetes in Rats

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Abstract: The aim of the present study was to investigate the biological effects of aqueous herbal extracts mixed with stirred yoghurt filtrate against alloxan-induced oxidative stress and diabetes in rats. Aqueous extracts of six medicinal plants: fenugreek, greater burdock, goat's rue, colocynth, chicory and lupine were mixed with stirred yoghurt filtrate and used in the experiments. Blood glucose and alanine and aspartate aminotransferase (ALT and AST) activities were estimated before and after alloxan-induced oxidative stress and diabetes in rats. Obtained results showed that blood glucose levels in sera of treated rats fed on aqueous extract of medicinal plants and stirred yoghurt filtrate mixture decreased with mean values of 135.0±26.85 mg/100 mL serum compared with the treated rat fed on basal diet (positive control) with mean value of 237.66±14.43 mg/100 mL serum. Data showed that ALT and AST activities in sera of treated rat fed on aqueous extract of medicinal plants and stirred yoghurt filtrate mixture were nearest to the level of un-treated rats fed basal diet (negative control). The means values of ALT and AST level in treated group fed on aqueous extract of medicinal plants and stirred yoghurt filtrate mixture were 57.33±20 and 189.33±48.85 compared with the positive control 90±31.76 and 260.00±57.27 and negative control 44.66±9.5 and 180.66±23.58 U L^{-1}, respectively. Data concluded that mixture of medicinal plant extracts and stirred yoghurt filtrate may play a role in protection against alloxan-induced oxidative stress and diabetes in rat.

Key words: Biological effects of aqueous herbal extracts, yoghurt, oxidative stress and diabetes

INTRODUCTION

Diabetics are prone to develop number health problems and cardiovascular diseases are the primary cause of their premature death. Fortunately, relatively small improvements in blood glucose, lipid levels and blood pressure can decrease the risk of developing cardiovascular problems (NIH National Diabetes Education Program Publication, 2004). Health recommendations for diabetics emphasize frequent eating of small balanced meals, taking medications and exercising. An optimal diet should include a variety of whole unprocessed foods, limited saturated fats, refined carbohydrates and caffeine-free beverages to help maintain healthy weight.

For various reasons in recent years the popularity of complimentary medicine has increased. Plant therapies are widely practiced in many countries (WHO, 1999). Surveys conducted in USA and Australia indicated that 34 and 48.5% of the population used one form of unconventional therapy including herbal medicine (Eisenberg *et al.*,1993; Maclennan *et al*., 1996). The World Health Organization recommended the evaluation and the effectiveness of plants in conditions where no safe modern drugs are available (Upadhayay and Pandey, 1984; WHO, 1999, 2003).

The primary objective of this study is to investigate the biological effects of popular herbal extracts when stirred with yoghurt filtrate against diabetes and oxidative stress.

Great burdock (*Arctium lappa*) is a coarse biennial herb native to Europe and Asia. The entire plant has both nutritive and medicinal uses (Lin *et al*., 2001). The roots contain as much as 45% inulin, as well as alkaloids, essential oils, flavonoids, glycosides, resins and tannins. (Hoffmann, 2003). Whereas Goat's rue (*Galega officinalis*) is a diaphoretic, galactagogue and hypoglycemic (Bailey and Day, 1989). Galegine in goat's rue has been associated with marked reductions in blood sugar levels (Chevallier 1996). On the other hand Fenugreek (*Trigonella foenum-graecum*) and lupine (*Lupinus albus*) have a long history of medical use in ayurvedic and Chinese medicine. It has

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been used for numerous indications, including labor induction, aiding digestion and as a general tonic to improve metabolism and health. Preliminary animal and human trials suggested possible hypoglycemic and antihyperlipidemic properties of oral fenugreek seed powder. Fenugreek and lupine seeds were found to have good results and effects after development of experimental diabetes in rats (Basch *et al*., 2003; Riyad *et al*., 1988; Swanston-Flatt *et al*., 1990). Colocynth (*Citrullus colocynthis*), has wide medical uses in ayurvedic medicine as a cathartic, anti-diabetic. Violent purge (pulp) is diuretic, anti-epileptic, anti-blenorrhoeic, strong cathartic when mixed with Arabic gum, to mitigate the effects). While the emptied fruit, filled with water and honey, is drunk for rheumatism, gout, dropsy, ascites (Sincich, 2002). Chicory (*Cichorium intybus*) leaves are tasty and frequently used in salad to clean the blood and gallbladder in cases of jaundice. Most of the active principles are concentrated in the root. The fresh root is used in a decoction, dried or roasted, to treat diabetes.

Oxidative stress plays an important role in chronic complications of diabetes and is associated with increased lipid peroxidation (Elangovan *et al*., 2000).

Fermented milk and cultures containing dairy products were found to be effective antioxidants and their potential hypoglycemic and hypocholesterolemic effect is the focus of current research (Bengmark and Martindale, 2005).

It has been found that in adult humans, after milk or yogurt ingestion, many peptides derived from α_{s1}, β or κ-caseins were detected in stomach, including the κ-caseinoglycopeptide, an inhibitor of platelet aggregation. Smaller peptides derived from casein and lactoferrin were recovered from duodenum. Two long peptides, the κ-caseinoglycopeptide and the N-terminal peptide of α_{s1}-casein, were absorbed and detected in plasma. These results support the concept that food-born peptides could have physiological activities in man.

MATERIALS AND METHODS

Milk samples: Fresh cow milk samples (3.10% fat) were purchased from healthy animals in private farms (Saudi Arabia).

Yoghurt cultures: Starter cultures of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from Chr. Hansen`s Lab., Copenhagen Denemark.

Medicinal plant samples: Plants used in this study are showed in Table 1. Fenugreek, greater burdock, goat's rue, colocynth, chicory and lupine were collected from local market, Qassim, Saudi Arabia. This study was conducted during the period extending between March, 2006 and September, 2007.

Table 1: English, botanical and family name of plants used in the experiments

English name	Botanical name	Family	Part used
Greater Burdock	*Arctium lappa*	Asteraceae	Root, leaves and seeds (fruits)
Goat's rue	*Galega officinalis*	Leguminosae	Leaves
Colocynth	*Citrullus colocynthis*	Cucurbitaceae	Fruits
Fenugreek	*Trigonella faenum-graecum*	Leguminosae	Seeds
Lupine	*Lupinus albus*	Leguminosae	Seeds
Chicory	*Cichorium nntybus*	Asteraceae	Roots

Preparation of traditional yoghurt and its filtrate: Traditional yoghurt milk was prepared according the method described by Tamime and Robinson (1999) and Al-Wabel *et al.* (2007) with slight modifications. Yoghurt containing probiotic strains (1.0×10^9) of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* strains were stirred in electric blender and filtered through filter paper (Whatman No. 1) and stored at 4°C for mixing with aqueous extracts of medicinal plants (fenugreek, greater burdock, goat's rue, colocynth, chicory and lupine) to be used in the experiments for feeding.

Preparation of aqueous extracts of fenugreek, greater burdock, goat's rue, colocynth, chicory and lupine: Aqueous extraction of fenugreek, greater burdock, goat's rue, colocynth and lupine were carried out according to methods described by Melendeza and Caprilesa (2006). The extracts were then filtered and preserved in sterile dark bottles (500 mL) in a cool environment (4°C) until further mixing with yoghurt filtrate before feeding.

Animals: Thirty-six Male albino Wistar rats weighting about 100-120±19.8 g were randomly divided into three-test groups (each containing 18 rats). Animals were placed in cages and were given initially the basal diet.

Basal diet: The composition of basal diets used in this study is as follows: milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (64%).

Treatments: The first group (G1) was kept as untreated, control group fed on the basal diet only (negative control).

The other two groups (G2, G3) were both treated with alloxan as inducer of diabetes. Group (G2) was fed on the basal diet only and used as a positive control.

The third group (G3) was fed on basal diet + aqueous herbal extracts mixed with stirred yoghurt filtrate in drinking bottles.

The feeding experiment was continued for 6 weeks and at the end of the experiment, animals were anesthetized by exposure to an atmosphere of 100% diethyl ether and then they were killed by decapitation. Blood was collected in dry tube and left to clot, then centrifuged at 3000 rpm for 10 min. Serum was separated and stored frozen at -20°C for subsequent analyses.

Biochemical analysis: At the end of the experimental period, animals were anesthetized by exposure to an atmosphere of 100% diethyl ether then they were killed by decapitation. Blood samples were taken into plain tubes. Serum was harvested after centrifugation (3000 rpm) for 15 min frozen at -20°C for subsequent analyses.

Blood glucose concentration was determined by the Haemo-Glukotest 20-800-R and measurements read with a Reflolux test (Boehringer-Mannheim) (Brodrick *et al*., 1987).

Determination of Glutathione-S-Transferase (GST) in serum: The enzyme activity of Glutathione-S-Transferase (GST) was determined according to Habig *et al*. (1974).

Determination of ALT and AST activities: ALT and AST activities were determined according to the method described by Reitman and Frankel (1957).

Statistical analysis: Mean and standard Error of the obtained data from each different experimental group were calculated and analyzed.

Chemical compositions analysis were carried out according to the official method AOAC (2000) and sensory evaluation was adopted from NASA (1999).

RESULTS AND DISCUSSION

The results of this study indicated that feeding aqueous herbal extracts of six medicinal plants-fenugreek, greater burdock, goat's rue, colocynth, chicory and lupine mixed with stirred yoghurt filtrate against alloxan-induced oxidative stress and diabetes in rats resulted in beneficial effects. In this study the chemical composition of the tested yoghurt was investigated. It has been found that the total solid, carbohydrate, fat, protein and ash values were 13.2, 5.5, 3, 1, 3.80 and 0.85%, respectively. Also, the sensory evaluation properties were found to have good scores and were acceptable for consumers.

The blood glucose level in diabetic rats was found to be 246 mg/100 mL. Treatment with herbal extracts mixed with yoghurt filtrate lowered serum glucose level in diabetic rats from 246 to 150 mg/100 mL indicating hypoglycemic effect.

Table 2: Comparison of serum glutathione-s-transferase activities between control and Herbal extracts with yoghurt filtrate

GST	Min.	Max.	Mean	SD	CV (%)	A / 9.6*1000
Negative control	0.080	0.120	0.10	0.02	20.00	10.42
Positive control	0.120	0.126	0.12	0.05	2.84	12.71
Herbal extracts with yoghurt filtrate	0.175	0.240	0.21	0.04	16.77	21.10

Table 3: Comparison of serum AST activity between control and herbal extracts with yoghurt filtrate (U L^{-1})

AST	Min.	Max.	Mean	SD
Negative control	156	203	180.66	23.58
Positive control	141	360	260.00	57.27
Herbal extracts with yoghurt filtrate	133	220	189.33	48.85

Table 4: Comparison of serum ALT activity between control and Herbal extracts and yoghurt filtrate (U L^{-1})

ALT	Min.	Max.	Mean	SD
Negative control	35	54	44.66	9.50
Positive control	63	125	90.00	31.76
Herbal extracts with yoghurt filtrate	42	80	57.33	20.00

The activities of the liver enzymes (AST and ALT) were decreased and the activity of serum GST increased due to the treatment (Table 2-4).

Diabetic patients were found to exhibit abnormal antioxidant status due to auto oxidation of glucose and excess glycosylated proteins (Ceriello *et al*., 1991; Mak *et al*., 1996). OS in diabetes leads to tissue damage and complications including retinopathy, nephropathy and coronary heart disease (Jennings *et al*., 1991; Lyons, 1991).

Oxidative stress is an important factor that may induces liver fibrosis and represents a key feature of hepatitis induced by various conditions (Poli *et al*., 1987; Wasmuth *et al*., 2003). In this study, treatment with herbal plants and yogurt markedly reduced the activities of AST and ALT and increased the activity of GST after diabetes induction by alloxan. The herbal plants used in this study included burdock root and chemicals in this root were found to help the body to regulate the blood sugar and/or the release of insulin from the pancreas (Silver and Krantz, 1931; Swanston-Flatt *et al*., 1990). Inulin is a helpful type of sugar, beneficial to diabetics and have hypoglycemic effect because it does not trigger quick insulin production from the pancreas. This root contains numerous other constituents (chemicals) which either directly or indirectly affect the blood sugar level (Brinker, 2001; Swanston-Flat *et al*., 1990; Lapinina and Sisoeva, 1964; Silver and Krantz, 1931).

The phytochemical galegin present in *Galega* officinalis lowers the blood sugar level and this explains why goat's rue is used in treatment of diabetes mellitus (Bailey and Day, 1989). Studies have shown that extracts from goat's rue inhibit the transport of glucose into cells. These studies do not recommend the use of goat's rue for self medication of diabetes because diabetes is a serious condition and it is difficult to standardize the strength of the active component levels (UKPDS Group, 1989).

Chicory (*Cichorium intybus*) is mainly cultivated for its roots that contain high amounts of inulin (a frupolymer) (Westerdijk, 2000). The chicory components were found to positively affect various physiological functions. They affect the bioavailability of minerals, reduce the risk of intestinal infectious diseases, cardiovascular diseases, non-insulin-dependant diabetes, obesity and osteoporosis.

Glutathione depletion in liver and kidneys is seen during severe oxidative stress in goats induced by lead administration (Mousa *et al*., 2002) and in diabetes. The depletion of GSH content also may lower GST enzyme, because GSH is required as a substrate for GST activity (Rathore *et al*., 2000)

Glutathione is a tripeptide synthesized in the body from free amino acids, cysteine, glutamic acid and glycine. In many cancer epidemiological studies, it has been found that the major milk proteins caseins and whey have protective effect against cancer (McIntosh, 1993). In this connection we can assume that milk proteins plus microbial proteins that are synthesized by probiotics can enrich amino acids pool in the body and consequently increase glutathione, main bio-antioxidant and other protein precursors and synthesis. Similar assumption were proposed by McIntosh (1993).

It can be concluded that feeding diabetic rats aqueous herbal extracts and stirred yoghurt filtrate mixture have beneficial effects as manifested by lowering of blood glucose level and enhancement of the antioxidant status. This protocol can be used as a new nutritional and therapeutic products that may be administered orally and replace the laborious administration of insulin.

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