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FRUCTUS LIGUSTRI LUCIDI PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Fructus Ligustri Lucidi (FLL) is gaining popularity as a complementary medicine for the prevention and treatment of osteoporosis. *Fructus Ligustri Lucidi* is said to have anti-osteoporotic properties by correcting liver and kidney deficits and decreasing lower back pain. *Fructus Ligustri Lucidi* improves bone metabolism and quality in ovariectomized, growing, elderly, and diabetic rats via regulating signalling pathways, according to evidence from animal and cell research. This plant has yielded more than a hundred different chemicals. The effects of *Fructus Ligustri Lucidi* on bone oxidative stress and turnover indicators in diabetic rats are investigated in this study. Over an 8-week period, diabetic Sprague Dawley rats (n=6) were given one of three treatments through gavage: Saline (control), metformin (1000mg/kg bw), or *Fructus Ligustri Lucidi* (700mg/kg bw). As a normal control group, a healthy rat group was used. ELISA assays were used to detect insulin, oxidative stress, and bone turnover indicators in the blood. *Fructus Ligustri Lucidi* treatment substantially increased insulin and osteocalcin levels in diabetic rats compared to diabetic control rats. By increasing osteogenesis and decreasing bone oxidative stress, *Fructus Ligustri Lucidi* may be able to prevent diabetic osteoporosis. These data support the use of *Fructus Ligustri Lucidi* as an osteoporosis treatment in diabetics. *Fructus Ligustri Lucidi* offers a novel approach to the prevention and treatment of diabetic osteoporosis. More scientific data on its bone protective benefits and safety is expected from well designed clinical studies.

KEYWORDS

Fructus Ligustri Lucidi and Diabetic osteoporosis.

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INTRODUCTON

Fructus Ligustri Lucidi (*FLL*) has been shown to have anti-oxidant, anti-tumor, and anti osteoporosis properties in recent pharmacological investigations. Another study found that extracts had beneficial benefits in mice with non-alcoholic fatty liver disease. Type-2-diabetes (T2D) is characterised by

persistent hyperglycemia and elevated glucose levels, which can lead to glycometabolism and lipometabolism dysfunctions. T2D is responsible for 90% of all diabetes cases globally. This illness has been linked to an increased risk of Alzheimer's and depression[1-5]. Individuals with T2DM had a greater risk of fractures than non-diabetic patients for a given BMD. Because of micro architectural flaws in the bone, fragility fractures are more common in diabetics. These abnormalities are difficult to identify and usually have nothing to do with BMD. As a result, bone fragility in diabetics is an underestimated problem.

Diabetics have low bone turnover indicators, and their true fracture rates are higher than those predicted by fracture risk assessment methods.[6] Osteoarthritis and osteoporosis are caused by a disturbance in the delicate balance between these two processes. Several studies have shown that[7,8] STZ induced diabetes is a useful model for studying the pathophysiological processes of diabetes related bone loss[9]. *Fructus Ligustri Lucidi* extracts are widely used to treat postmenopausal osteoporosis. Despite the fact that *Fructus Ligustri Lucidi* has demonstrated significant anti-osteoporotic benefits in an osteoporosis model[10], its impact on diabetic osteoporosis prophylaxis is unknown. In STZ-treated rats, we chose to investigate the effects of *Fructus Ligustri Lucidi* therapy on bone oxidative stress and turnover markers.

MATERIAL AND METHODS

Fructus Ligustri Lucidi preparation

The dried *Fructus Ligustri Lucidi* were added with 70% ethanol for reflux extraction twice, with 1.5 h each time. Afterwards, the extracted solutions were combined, filtered, and then concentrated by a rotary evaporator under reduced pressure.

Animals

Twenty four male Sprague Dawley rats weighing 100-120g were used in the study, which were procured from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were kept in a temperature controlled facility (22±°C, 12 hour light/dark cycle) and were fed normal rat chow with unlimited access to water. The experiment protocols, which included diabetes induction and sacrifice, were authorised by King Khalid University's animal ethics committee and were carried out in accordance with the US National Institute of Health's guidelines for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

A single intraperitoneal injection of 60mg/kg STZ dissolved in 10mm citrate buffer was used to chemically produce diabetes like hyperglycemia in rats (pH 4.5). The rats were given 5% glucose water for two days after STZ injection to avoid drug induced hypoglycemia. After a week of injection, rats with fasting blood glucose levels more than 11mmol/L were classified as diabetic[11]. The rats in the control group received the same amount of isotonic NaCl injection as the experimental group.

Experimental design

Four groups of twenty four male rats (n = 6) were formed. Normal control rats (NC) received saline, diabetic control rats (DC) received 1000mg/kg body weight of metformin (MET), and diabetic rats received 700mg/kg body weight of *Fructus Ligustri Lucidi*. For 56 days, oral gavage treatments were administered once a day. All of the animals fasted overnight at the end of experiment, and their blood glucose levels were assessed. The animals were given ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia before being sacrificed. Cutting at the stifle joint separated the femur and tibia. Blood samples (10-15mL) were collected from the rats through heart puncture and placed in a simple red-top tube with no anticoagulants. After centrifuging the blood samples at 4000rpm for 15 minutes, the serum was divided into aliquots and kept at -80°C.

Measurements of bone oxidative stress and antioxidant activities

A mortar and pestle were used to grind the femur bone fragments. Bone tissues were homogenised with a Teflon pestle in a 10% (w/v) homogenising buffer (50mm Tris-HCl, 1.15 percent KCl pH 7.4). To remove nuclei and debris, the homogenates were spun at 9000rpm for 10 minutes in a chilled centrifuge (4°C). A TBARS assay kit was used to monitor lipid peroxidation, a glutathione peroxidase

(GPx) assay kit for GPX activity and a superoxide dismutase (SOD) assay kit for SOD activity in the generated supernatant. The protein content was calculated using the technique[12], which used bovine serum albumin as a reference.

Marker of bone formation and bone resorption

Serum was used to assess all indicators of bone formation and resorption. The osteocalcin level was measured using the Rat Osteocalcin ELISA kit, whereas the BALP level was obtained using the rat BALP ELISA kit. DPD was tested using a Rat deoxypyridinoline (DPD) ELISA Kit to evaluate bone resorption. According to Abdul-Majeed *et al*[13], all samples were run in triplicate, and the optical density was measured at 450nm using a microplate reader.

Statistical analysis

ANOVA was used to analyse all of the data. Duncan's multiple comparison test was used to evaluate the significance of the means. The findings were reported using the average minus one standard deviation. All of the analyses were carried out using a 95% confidence level.

RESULTS

Fasting blood glucose and serum insulin

The DC rats exhibited higher fasting blood glucose and lower insulin levels than the NC animals (Table No.1). Treatment with *Fructus Ligustri Lucidi* significantly reduced fasting blood glucose levels while significantly raising serum insulin levels in diabetic rats.

Oxidative stress marker and antioxidant enzymes in bone

Table No.2 summarises the effects of *Fructus Ligustri Lucidi* on bone lipid per oxidation and antioxidant enzyme activity. The DC rats had a considerable increase in MDA levels as compared to the NC rats, but no significant changes in GPx or SOD activity. A similar observation is found with the *Fructus Ligustri Lucidi* treated rats.

Bone turnover markers

The STZ injection significantly reduced blood osteocalcin, but serum DPD was significantly higher than in the NC group (Table No.3). Despite no significant differences in BALP values across the treatment groups, blood osteocalcin levels increased while DPD decreased following *Fructus Ligustri Lucidi* therapy.

Discussion

STZ injection has been linked to a reduction in chondrocyte counts and an increase in tidemark roughness in the femoral articular cartilage[14]. These findings point to the possibility that diabetic rats develop osteoarthritis like symptoms. In both T1DM and T2DM animals, osteoarthritis-like symptoms have been seen[12,15]. These alterations are considered to be aided by the activation of oxidative stress. *Fructus Ligustri Lucidi* is commonly utilised in Asia to treat renal and liver disorders as well as build bones. *Fructus Ligustri Lucidi* might potentially be utilised as an antioxidant and anti neoplastic therapy, according to research.

In animal investigations, oxidative damage indicators were shown to be higher in STZ-induced diabetic control rats. Furthermore, oxidative stress in combination with hyperglycemia has been demonstrated to alter bone metabolism and shape by changing the activity of osteoclasts and osteoblasts[11]. Blood DPD levels in DC rats increased, but serum osteocalcin and BALP activity decreased, according to the findings of this study. This conclusion corresponds to the findings[16] that a decrease in bone turnover is a critical feature of T1DM-related bone deterioration. BALP (Bone-Specific Alkaline Phosphatase) is a bone-specific alkaline phosphatase isoform that is generated by osteoblasts for bone remodelling but depicts mineral metabolism more precisely[17]. The *Fructus Ligustri Lucidi* groups had virtually the same ratio of osteocalcin to DPD as the NC groups, suggesting that *Fructus Ligustri Lucidi* therapy successfully balanced bone production and resorption.

Table No.1: Effects of *Fructus Ligustri Lucidi* on fasting blood glucose level and serum insulin in STZ induced diabetic rats (data represent mean ± 1SD)

S.No	Groups	Fasting blood glucose (mmol/L)		% Changes	Serum insulin (μIU/mL)
		Before	After		
1	NC	5.70 ± 0.30a	4.93 ± 0.21a	3.61	3.56 ± 3.03c
2	DC	22.00 ± 3.24b	28.03 ± 2.69b	51.55	1.58 ± 0.16a
3	MET	26.30 ± 4.70c	20.73 ± 3.75c	−32.32	1.98 ± 0.34a
4	*Fructus Ligustri Lucidi*	27.87 ± 7.03c	18.17 ± 4.97c	−38.03	2.71 ± 0.18b

Different values a, b, c in a column differed significantly at ($p < 0.05$).

Table No.2: Oxidative stress marker and antioxidant enzymes of various experimental groups (data represent mean ± 1SD)

S.No	Groups	Oxidative stress marker	Antioxidant enzymes	
		TBARS (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
1	NC	32.73 ± 0.50a	45.65 ± 0.78ab	0.60 ± 0.01
2	DC	58.74 ± 0.66b	44.40 ± 0.80bc	0.327 ± 0.04
3	MET	74.51 ± 9.20c	42.06 ± 0.98b	0.34 ± 0.04
4	*Fructus Ligustri Lucidi*	76.79 ± 0.14c	46.41 ± 0.46bc	0.66 ± 0.18

Different values a, b, c in a column differed significantly at ($p < 0.05$).

Table No.3: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± 1SD)

S.No	Groups	Bone formation markers		Bone resorption marker
		Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
1	NC	145.68 ± 7.82c	100.79 ± 7.49b	166.08 ± 5.23b
2	DC	13.35 ± 0.87a	69.06 ± 4.60a	164.10 ± 0.21c
3	MET	57.42 ± 8.34b	83.38 ± 0.45a	156.16 ± 4.18ab
4	*Fructus Ligustri Lucidi*	153.66 ± 4.01d	77.30 ± 8.21a	148.53 ± 0.31a

Different values a, b, c in a column differed significantly at ($p < 0.05$).

CONCLUSION

Fructus Ligustri Lucidi has the ability to prevent bone loss in STZ-treated rats, according to our data. After *Fructus Ligustri Lucidi* treatment, fasting blood glucose levels were lower, DPD activity was higher, and insulin secretion was higher.

CONFLICTS OF INTEREST

"The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings".

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