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ROLE OF *SOPHORAE FRUCTUS* EXTRACT ON BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Krishnaraju Venkatesan[*1], Noohu Abdulla Khan[2], Ali Alqahtani[1], J. Muthu Mohamed[3], Fazil Ahmad[4], Premalatha Paulsamy[5], Kalpana Krishnaraju[6]

[1*]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia.
[2]Department of Clinical Pharmacy, College of Pharmacy King Khalid University, Abha, Saudi Arabia.
[3]Department of Pharmaceutical Technology, BIT Campus, Anna University, Tiruchirappalli, Tamil Nadu, India.
[4]Department of Anesthesia Technology, College of Applied Medical Sciences in Jubail, Imam Abdulrahman Bin Faisal University, P.O. Box 4030, Jubail, Saudi Arabia.
[5]King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia.
[6]Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, India.

ABSTRACT

The effects of *Sophorae Fructus* extracts on bone loss in diabetic rats caused by streptozotocin *(STZ)* were studied. For 8 weeks, rats were given *Sophorae Fructus* extracts orally. Diabetic Sprague Dawley rats (n=6) were administered one of three treatments through gavage for 8 weeks: saline (control), metformin (1000mg/kg/day), or *Sophorae Fructus* extract (500mg/kg/day). Diabetic rats treated with *Sophorae Fructus* had significantly greater insulin and osteocalcin levels than diabetic control rats. The benefits of *Sophorae Fructus* extracts on bone loss prevention or therapy in diabetic rats appear to be related to a decrease in bone turnover. These findings back up the usage of *Sophorae Fructus* in diabetic individuals as an osteoporosis therapy.

KEYWORDS

Sophorae Fructus extracts, Osteoporosis and Bone protective effect.

Author for Correspondence:

Krishnaraju Venkatesan,
Department of Pharmacology,
College of Pharmacy, King Khalid University,
Abha, Saudi Arabia.

Email: kvenkatesan@kku.edu.sa

INTRODUCTON

Matrix deposition, mineralization, and resorption are all important aspects of bone metabolism. Numerous studies have shown that dietary components and phytoconstituents can impact these processes by inhibiting bone resorption, resulting in skeletal benefits. In pharmacological models of osteoporosis, many traditional herbal formulas used in ayurveda and Chinese medicine have proved to be effective. *Fructus Sophorae (FS)* or Huaijiao, the dried ripe fruit of Sophora japonica Leguminosae, is

a well-known traditional Chinese medicine that has been used in China as a heat clearing and fire purging, cooling blood and hemostatic agent. In an ovariectomized rat model[1], extracts from Sophora japonica prevented bone loss. Flavonoids account for 20.2 percent of the total weight in *Fructus Sophorae*, according to earlier studies[2]. The medicinal components of *Fructus Sophorae* include flavonoids, which are mostly isoflavonoids and flavonols, as well as their glycosides[3]. The effects of *Fructus Sophorae* therapy on bone oxidative stress and turnover markers in STZ-treated rats were examined in the current study.

MATERIAL AND METHODS

Animals

The experiment was carried out with 24 male Sprague Dawley rats weighing 100-120g from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were housed in a temperature-controlled environment (21±°C with a 12 hour light/dark cycle) and given standard rat chow with full access to water. The animal ethics committee at King Khalid University approved the experiment methods, which included diabetes induction and sacrifice. They were carried out in compliance with the US National Institute of Health's standards for the care and use of laboratory animals (NIH Publication No.85-23, revised 1996).

Induction of diabetes

A single intraperitoneal injection of Streptozotocin (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 being administered STZ to avoid drug-induced hypoglycemia. After a week of injection, 20 animals were classified as diabetic if their fasting blood glucose levels were higher than 11mmol/L. The rats in the control group got the same amount of isotonic NaCl injection as the experimental group.

Experimental design

A total of 24 male rats (n=6) were split into four groups at random. Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rat groups received 1000mg/kg body weight of metformin, and 500mg/kg body weight of Eucommia ulmoides. Patients received oral gavage treatments once a day for a total of 56 days. At the completion of the trial, all of the animals were fasted overnight and blood glucose levels were tested. Before being killed at the end, the animals were administered ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia. The femur and tibia were cut apart at the stifle joint. The rats' blood samples (10-15mL) were taken by heart puncture into a simple red top tube with no anticoagulants. The serum was stored in aliquots at 80°C after centrifugation at 4000rpm for 15 minutes.

Marker of bone formation and bone resorption

All bone formation and resorption indicators were determined using serum. A Rat Mid Osteocalcin ELISA kit (IDS, UK) was used to measure the osteocalcin level, whereas a rat BALP ELISA kit was used to measure the BALP level (Qayee, Shanghai). Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) was used to determine bone resorption DPD (Qayee, Shanghai). All samples were run in triplicate, and the optical density was measured using a microplate reader (Bio Tek, USA) at 450nm.

Analysis of bone fatty acid composition

As stated by Nurdiana *et al*, total fatty acids were isolated from bone and identified and measured using a gas chromatography technique (2017). The percentages of total detected fatty acids are used to calculate the fatty acid proportions[4].

Statistical analysis

ANOVA was used to assess all of the data. Duncan's multiple comparison test was used to determine the significance. All of the analyses were done with a 95% confidence level.

RESULTS AND DISCUSSION

Fasting blood glucose and serum insulin

In comparison to the NC rats, the DC rats had high fasting blood glucose and low insulin levels (Table No.1). In diabetic rats, treatment with *Fructus Sophorae* dramatically lowered fasting blood glucose levels while considerably increasing serum insulin levels.

Bone turnover markers

Although blood osteocalcin were significantly lower after the STZ injection, serum DPD was significantly higher than in the NC group (Table No.2). After *Fructus Sophorae* treatment, serum

osteocalcin levels increased while DPD levels decreased, despite no significant differences in BALP values between the treated groups.

Bone fatty acid changes

In the femurs of DC rats, the total n-3 PUFA was considerably reduced, but the ratio of n-6 to n-3 PUFA was dramatically raised, as shown in Table No.3. In the MET group, similar observations were made. Surprisingly, in the *Fructus Sophorae* group, total bone n-3 PUFA increased but the n-6 to n-3 ratio dropped.

Discussion

Both clinical and preclinical research has demonstrated that oxidative stress influences the pathogenesis of osteopenia, osteoporosis, and osteoarthritis[5-7]. As a result, more investigation into the link between oxidative stress and bone quality is required. DC rats exhibited greater levels of oxidative damage markers, according to this study. By influencing the activity of osteoclasts and osteoblasts, oxidative stress and hyperglycemia have been shown to influence bone metabolism and architecture[6].

According to the findings of this study, blood DPD levels rose in DC rats, whereas serum osteocalcin and BALP activity fell. Zhukouskaya *et al*. (2015) discovered that bone turnover suppression is a major characteristic of T1DM related bone disease. Our findings are supported by previous observations of increased serum DPD in rats with osteoarthritis[8] and osteopenia[9]. Another intriguing finding from this study is that blood osteocalcin levels increased following *Fructus Sophorae* treatment while DPD levels decreased (Table No.2). A variety of herbs with osteoprotective characteristics have yielded similar results[10].

Despite the fact that osteocalcin is a specific osteoblast marker that closely correlates with histological alterations[11], blood OC levels tends to change with meal intake[10]. According to prior studies, osteocalcin does not appear to be a sensitive marker as BALP[12]. BALP activity is still low in *Fructus Sophorae* rats, suggesting that mineral metabolism is still impaired. BALP is a bone-specific alkaline phosphatase isoform that is generated by osteoblasts for bone remodelling, but it also reflects mineral metabolism[13]. The ratio of osteocalcin to DPD was nearly similar in the *Fructus Sophorae* and NC groups, suggesting that an equilibrium between bone formation and bone resorption was nearly achieved with *Fructus Sophorae* treatment.

The total bone n-3 PUFA and the n-6: n-3 ratio were significantly lower in the DC and MET rats with biochemical indications of bone loss and osteoarthritis like disease (Table No.3). Following *Fructus Sophorae* treatment, however, these alterations have significantly improved. Longo and Ward have discovered that a high intake of n-3 PUFA can enhance BMD and lower the incidence of fragility fractures[14]. Previous research has also shown that supplementing with n-3 PUFAs can preserve bone metabolism by lowering bone resorption indicators[15]. When compared to other STZ treated animals, the *Fructus Sophorae* rats had a considerably higher BMD and a much lower DPD (Table No.2). This discovery adds to the growing body of data that *Fructus Sophorae* therapy can help STZ treated rats to avoid bone loss.

Table No.1: Effects of *Sophorae Fructus (FS)* 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	4.82 ± 0.30a	4.91 ± 0.11a	2.70	4.14 ± 3.13c
2	DC	19.00 ± 3.24b	30.11 ± 2.65b	50.61	1.55 ± 0.13a
3	MET	28.30 ± 3.60c	19.73 ± 3.74c	−32.22	1.76 ± 0.24a
4	*Fructus Sophorae*	26.87 ± 6.02c	17.27 ± 4.87c	−37.03	2.39 ± 0.18b

Values with different superscripts down the column indicate significant difference at ($p < 0.05$).

Table No.2: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± SD)

S.No	Groups	Bone formation markers		Bone resorption marker
		Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
1	NC	136.76 ± 6.9^{c}	100.49 ± 7.59^{b}	167.08 ± 5.13^{b}
2	DC	13.34 ± 0.87^{a}	65.06 ± 4.72^{a}	164.10 ± 0.11^{c}
3	MET	56.40 ± 8.14^{b}	81.38 ± 0.35^{a}	151.16 ± 4.48^{ab}
4	*Fructus Sophorae*	153.64 ± 4.10^{d}	76.30 ± 8.21^{a}	145.53 ± 0.31^{a}

Values with different superscripts down the column indicate significant difference at ($p < 0.05$).

Table No.3: Fatty acid composition (percentage of total identified fatty acids) of the bone (data represent mean ± SD)

S.No		NC	DC	MET	*Fructus Sophorae*
1	Myristic acid (C14:0)	1.50 ± 0.27^{c}	0.40 ± 0.05^{a}	1.10 ± 0.08^{bc}	0.61 ± 0.03^{ab}
2	Palmitic acid (C16:0)	24.38 ± 4.58	24.88 ± 3.47	28.17 ± 2.88	23.34 ± 12.78
3	Stearic acid (C18:0)	7.07 ± 1.01^{a}	8.88 ± 0.52^{b}	7.13 ± 0.80^{a}	9.43 ± 1.72^{b}
4	Palmitoleic acid (C16:1)	2.52 ± 0.51	1.57 ± 0.38	1.68 ± 0.41	1.76 ± 0.64
5	Oleic acid (C18:1n9)	20.62 ± 7.99	25.02 ± 4.73	27.61 ± 3.12	23.66 ± 3.00
6	Linoleic acid (C18:2n6)	3.04 ± 1.37	3.27 ± 0.25	4.19 ± 0.42	2.69 ± 0.39
7	Arachidonic acid (C20:4n6)	1.00 ± 0.08	1.68 ± 0.04	1.04 ± 0.06	2.12 ± 0.14
8	α- Linolenic acid (C18:3n3)	1.55 ± 0.45	1.16 ± 0.29	1.24 ± 0.52	1.36 ± 0.96
9	Eicosapentaenoic acid (C20: 5n3)	0.62 ± 0.15	0.14 ± 0.05	0.27 ± 0.05	0.68 ± 0.15
10	Docosapentaenoic acid (C22: 5n3)	0.38 ± 0.02	0.57 ± 0.04	0.50 ± 0.24	0.38 ± 0.15
11	Docosahexaenoic acid (C22: 6n3)	0.65 ± 0.16	0.14 ± 0.01	0.28 ± 0.03	0.31 ± 0.03
12	total SFA	32.94 ± 5.86	34.16 ± 4.32	36.39 ± 3.53	33.38 ± 13.01
13	total MUFA	23.13 ± 7.51	26.59 ± 4.72	29.29 ± 2.76	25.43 ± 3.44
14	total n-6 PUFA	4.04 ± 1.96	4.96 ± 0.79	5.23 ± 0.98	4.81 ± 1.39
15	total n-3 PUFA	2.81 ± 0.60^{c}	1.44 ± 0.18^{a}	1.78 ± 0.24^{ab}	2.34 ± 0.47^{bc}
16	n-6 : n-3	1.44 ± 0.69^{a}	3.44 ± 0.23^{d}	2.94 ± 0.34^{cd}	2.03 ± 0.20^{ab}

Values with different superscripts down the column indicate significant difference at ($p < 0.05$).

CONCLUSION

Our findings suggest that *Fructus Sophorae* demonstrates a significant, but less pronounced, bone protective effect in STZ-treated rats. *Fructus Sophorae* seem to stimulate osteoblastogenesis and bone formation by increasing bone mineral density.

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CONFLICT 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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