

Ameliorating Effect of Frankincense on Red Blood Cells of Alloxan Induced-Diabetes in Rat

Anwar Masoud¹, Mohammad Al-Ghazali², Fatima Al-Futini³, Samar Alzagruri⁴, Eman Al-Ansi⁴, Ayman Salama⁴, Haron Al-Selwi⁴

¹Biochemical Technology Program, Department of Chemistry, Thamar University, Thamar, Yemen, P.O.Box (87246)

²Department of Pharmacy, Faculty of Medicine and Health Sciences, Thamar University, Thamar, Yemen, P.O.Box (87246)

³Department of Biology, Faculty of Science, Sana'a University, Sanaa, Yemen.

⁴Department of Pharmacy, Faculty of Medicine and Health Sciences, Al-Nasser University, Sanaa, Yemen.

ABSTRACT: Oxidative stress has been implicated in Diabetes mellitus (DM) pathology which affects many people around the world, leading researchers to look for antioxidant therapy of DM. The antioxidant capacity of frankincense (FRN) made it a good candidate for studies as an antioxidant on DM hence the present study has been designed. Twenty eight Female Albino rats were segregated into four groups and were assigned as control, FRN (500 mg of FRN/Kg for 5 weeks), Alloxan (single dose of 150 /kg i.p) and Alloxan + FRN (single dose of alloxan 150 /kg i.p followed by FRN 500 mg/Kg for 5 weeks). After 5 weeks, RBCs were separated and the biochemical assays were measured. Significant increase ($p < 0.05$) on thiol contents (TSH, GSH and PSH) have been observed after administration of FRN to the rats with DM (FRN+ alloxan group) as compared to diabetic rats only (alloxan group), concomitantly, a recovery on catalase activity ($p < 0.05$) has also been seen. Uric acid level was increased more than 2 folds in the RBCs of FRN+ alloxan group as a result of FRN administration as compared to diabetic group accompanied with increase of protein and albumin ($p < 0.05$) and decreases the activity of RBC's LDH in FRN+alloxan group as compared to alloxan group. The present study clearly demonstrates that the administration of FRN extract enhance the antioxidant defense system of RBC's in alloxan-induced DM on rats.

KEYWORDS- Frankincense; Diabetes Miletus; RBC; Antioxidant; Oxidative stress.

I. INTRODUCTION

Diabetes mellitus (DM) affected more than 350 million people which is expected to rise to 552 million by 2030. It is characterized by relative or absolute deficiency of insulin secretion and/or insulin resistance that causes chronic hyperglycemia and impaired carbohydrates, lipids, and proteins metabolism (1). Increased extracellular glucose level is the main feature of DM, which advance glycation end-products and will subsequently produce reactive oxygen species (ROS) which can cause irreversible damage to the body system (2). The imbalance between free radical formation and the ability of the organism's natural antioxidants causes oxidative stress (3). There are many sources of oxidative stress in DM including enzymatic, non-enzymatic and mitochondrial pathways. Increasing oxidative stress in DM occurs due to multiple factors, glucose auto-oxidation is the most dominant factor that results in the development of free radicals. Other factors include; imbalance cellular reduction/oxidation and reduced antioxidant defenses (1). One of the models used to induce DM in animal is by administration of alloxan which consistently produced the main characteristics of DM including weight loss, hyperglycemia, polydipsia, polyuria and decreased insulin levels (4). Thus, it allows elucidation of antihyperglycemic agents in the treatment of DM. Frankincense (FRN) is an aromatic resin obtained from trees of the genus *Boswellia* (Burseraceae family). *Boswellia sacra* is found and used traditionally in many countries and has a variety of pharmacological effects, particularly anti-hyperglycemia effect, antioxidant effects, and anti-inflammatory effects (5-10). Preparations that contain FRN extract reduce blood sugar in STZ-induced DM (11-12) and alloxan-induced DM (13). These effects of FRN made it a good candidate for studies as an antioxidant on DM hence the present study has been designed to evaluate the antioxidant properties of FRN on RBC's following DM induction on rats.

II. MATERIALS AND METHODS:

Experimental Animals: Twenty eight Female Albino rats were obtained and housed in the animal house unit, Sana'a University. They were fed diet (Aman Veterinary Manufacturing Comp., Sana'a, Yemen), while water was provided *ad libitum*. They were divided randomly into four groups having 6 -8 animals; first group was

served as control group which fed lab diet, second group assigned as FRN group, however, third group was Alloxan group and the fourth group was both Alloxan+ FRN group. The study was conducted for 5 weeks and followed the guidance of animal care and approved ethically and the doses were given as follows:

Group A: Normal control rats (n=6) given DMSO solution. Group B: FRN group (n=6) received ethanolic extract of FRN (500 mg/Kg) dissolved in DMSO solution for 5 weeks. Group C: Alloxan group (n=8) given Alloxan (single dose of 150 /kg interaperitoneally (i.p) to induce diabetes). Group D: Alloxan + FRN group (n=8) given Alloxan (single dose of 150 /kg i.p to induce diabetes) and followed by ethanolic extract of FRN (500 mg/Kg) dissolved in DMSO solution for 5 weeks.

Induction of diabetes : Experimental Diabetes was induced in rat with freshly prepared Alloxan monohydrate (Sigma- Aldrich, USA) given as a single dose of 150 mg/kg dissolved in normal saline (0.9% w/v NaCl) and injected i.p to induce hyperglycemia. The blood glucose level was monitored in blood sample collected by tail tipping method using Accu-Check Glucometer. Rats with blood glucose level of greater than 250g/dl considered diabetic and were selected for the study.

Preparation of the Extract : Plant was obtained from Hadramout Governorate, Yemen and was collected freshly. FRN was manually grinded by mortar and pestle and then was suspended in ethanol (96%) and extracted on a shaker and this solution was gently shaken for 4 h. The mixture was filtered and the residue was re-suspended in aqueous ethanol (96%) for additional 4h and re-filtered to obtain a clear aromatic suspension. After filtration of the drying agent, the ethanol was evaporated under vacuum in a rotary evaporator and dried in oven (14). This extract was dissolved for better solubility of olibanum in dimethyl sulfoxide (DMSO). The dose 500 mg/kg was given orally as it is reported as a safe dose (15).

Sample preparation and isolation: After five weeks of experiment, 5 ml of blood each was collected from all the rats into heparinized bottles and RBCs were separated and the biochemical assays were measured.

III. BIOCHEMICAL ANALYSES

Total thiols : Total thiol groups were quantified in the erythrocyte according to the method of (16) as modified by (17). Briefly, reaction mixture containing 0.2 M Tris-HCl and 0.02 M EDTA (pH 8.2), erythrocyte lysate and 0.01 M DTNB (in methanol) was incubated for 15 minutes at room temperature then was centrifuged at 1,200 x g for 5 minutes. The supernatant was collected and the absorbance was read at 412 nm. Results were expressed as nmoles of T-SH/mg protein using molar extension coefficient of DTNB ($13,600 \text{ cm}^{-1}\text{M}^{-1}$).

Low molecular weight thiols : Low molecular weight thiols, LMW-SH (primarily glutathione, GSH) were measured in the erythrocyte according to the method of (16). Briefly, proteins in the erythrocyte were precipitated by 4 % (w/v) sulphosalicylic acid followed by centrifugation at 1200 x g for 5 minutes. To the supernatant, 0.1 mM DTNB in 0.1 M phosphate buffer (pH 8.0) was added and the absorbance was read at 412 nm after 2 minutes. Results were expressed as nmoles of LMW-SH /mg protein using molar extension coefficient of DTNB ($13,600 \text{ cm}^{-1}\text{M}^{-1}$).

Protein thiols (P-SH) : Protein thiols were measured by subtracting the low molecular weight thiols from total thiols. Results were expressed as nmoles of P-SH/mg.

Catalase activity : Catalase activity was assayed in the erythrocyte following the method of (18). Appropriate amount of erythrocyte was added to 12.5 mM H_2O_2 in 0.067M phosphate buffer (pH 7.0). The decrease in absorbance was followed at 240 nm for 3 minutes. Results were expressed as $\mu\text{moles of H}_2\text{O}_2$ decomposed/min/mg protein using molar extinction coefficient of H_2O_2 ($71 \text{ M}^{-1} \text{ cm}^{-1}$).

Other tests : The other biochemical tests includes assays of glucose, total protein, albumin, LDH and uric acid (UA) were estimated following the instructions of commercial kits provided by Spinreact, Spain.

Statistical analysis : Data were expressed as mean \pm S.D. and were analysed by one way ANOVA. Differences between groups were considered significant when $P < 0.05$. All analyses were performed using the sigma-stat software (version 3.5).

IV. RESULTS

As shown in tables 1-3 typical differences in the parameters studied have been observed in alloxan group as compared to control group, however, slight and mostly non-significant changes in these parameters have been seen in FRN group as compared to controls. The antioxidant effects of FRN have been studied on the

RBCs of alloxan induced DM in rats. Significant increase ($p < 0.05$) on thiol contents (TSH, GSH and PSH, table 1) have been observed after administration to the rats with DM (FRN+ alloxan group) as compared to diabetic rats only (alloxan group), concomitantly, a recovery on CAT activity ($p < 0.05$) has also been seen (table 2). Uric acid level was increased more than 2 folds in the RBCs of group D as a result of FRN administration as compared to diabetic group (table 2). Administration of FRN raises the levels of protein significantly ($p < 0.05$) in FRN+ alloxan after the decreases in alloxan group (table 2). As shown in table 3, FRN has increased significantly levels albumin following its administration to diabetic rats ($p < 0.05$) and decreases the activity of RBC's LDH in FRN+alloxan group as compared to alloxan group.

V. TABLES

Table (1): The levels of thiols in the RBC of diabetic and FRN rats.

	Total thiols	Glutathione	Protein thiol
Control	23.97 \pm 0.94 ^a	5.98 \pm 0.42 ^a	17.99 \pm 0.52 ^a
FRN	21.26 \pm 0.85 ^a	4.74 \pm 0.17 ^b	16.52 \pm 0.68 ^a
Alloxan	6.75 \pm 0.10 ^b	2.18 \pm 0.05 ^c	4.57 \pm 0.05 ^b
Alloxan + FRN	20.89 \pm 1.35 ^a	5.38 \pm 0.72 ^a	15.51 \pm 0.63 ^c

Results are expressed as mean \pm S.D.; n= 6. Superscript alphabets are significantly different from their corresponding group, $p < 0.05$.

Table (2): The activity of catalase and levels of UA and total protein in the RBC of diabetic and FRN rats.

	Catalase	U A	Total Protein
Control	19.01 \pm 2.15 ^a	1.47 \pm 0.07 ^a	1.3 \pm 0.15 ^a
FRN	24.32 \pm 3.06 ^b	1.38 \pm 0.14 ^a	0.83 \pm 0.07 ^b
Alloxan	3.38 \pm 0.46 ^c	1.79 \pm 0.19 ^b	0.56 \pm 0.03 ^c
Alloxan + FRN	22.06 \pm 1.84 ^b	3.12 \pm 0.51 ^c	1.07 \pm 0.10 ^d

Results are expressed as mean \pm S.D.; n= 6. Superscript alphabets are significantly different from their corresponding group, $p < 0.05$.

Table (3): The level of albumin and activity of LDH in the RBC of diabetic and FRN rats.

	Albumin	LDH
Control	0.71 \pm 0.07 ^a	18.66 \pm 4.13 ^a
FRN	0.16 \pm 0.01 ^b	78.08 \pm 11.16 ^b
Alloxan	0.15 \pm 0.01 ^c	101.61 \pm 22.27 ^c
Alloxan + FRN	0.18 \pm 0.02 ^d	53.85 \pm 4.11 ^d

Results are expressed as mean \pm S.D.; n= 6. Superscript alphabets are significantly different from their corresponding group, $p < 0.05$.

VI. DISCUSSION

FRN is an aromatic resin obtained from trees of the genus *Boswellia* and has been used traditionally in the treatment of rheumatoid arthritis and anti-inflammatory, antibacterial, antifungal and anticancer activities (6, 14, 19). We assessed the antioxidant capacity of FRN in RBCs following development of alloxan-induced DM on rats. All the parameters studied were significantly differ in alloxan group as compared to control indicating the development of DM, the plasma glucose level used as a biomarker of DM induction (data not shown), on the other hand, slight and mostly non significant changes of all the parameters have been observed when FRN group compared to control which might be due to the higher dose used (500 mg/kg for five weeks). The antioxidant effects of FRN in RBCs on alloxan induced DM have been reported here where our data showed recovery on the antioxidant defense system of RBC following administration of FRN.

The use of FRN as antidiabetic has been studied which is found to decrease the glucose levels in diabetic animals induced by alloxan (13) or streptozotocin (11-12). Oxidative damage due to the generation of superoxide radicals, glucose auto-oxidation and protein glycation have been reported in the pathogenesis of DM induced by alloxan (20-22). This stress results from an imbalance between the production of free radicals and the effectiveness of the antioxidant defense system. Our data show significant decreases on the level of thiols, albumin and total protein and activity of CAT of RBCs in alloxan group compared to control which indicates more oxidative stress as a mechanism of DM mechanism, moreover, UA another oxidative stress marker, has been observed to be higher in alloxan group. UA is the final product of catabolism of the purine nucleotides in human system (23), its levels serve as valuable indicators for certain clinical conditions (24). Being an endogenous antioxidant it's able to protect human body from different reactions involving free radicals. These results are in agreement with those of Shabeer (25) and Punitha and Manoharan (26) they suggested that hyperglycemia induces a depletion of the antioxidant system due to the increased lipid peroxidation and formation of free radicals. The treatment of RBCs of alloxan-induced diabetic rats with the FRN extract administered to the diabetic animals was confirmed by the recovery of RBC's antioxidant system. It has been reported that FRN can be used as antioxidant (11, 27).

This recovery may be due to the activation of enzymes by FRN, resulting in higher CAT activity (an antioxidant enzyme important at higher H₂O₂ concentration, (28) and higher thiol levels which lead to a reduce ROS level and hence reduce oxidative stress by preventing the generation of free radicals and, thus inhibiting the development of DM. These data demonstrate the important role of the FRN as an important antioxidant. The non-enzymatic and enzymatic antioxidants help cells to detoxify the ROS (3). Thiols, the organic sulfur derivatives, are characterized by the presence of sulfhydryl groups (-SH) they are classified as large molecular weight (protein) thiols and low molecular weight thiols (GSH, cysteine and homocysteine). GSH is an important water soluble antioxidant that is central to cellular defense against oxidative stress and potentially toxic chemicals acting as a redox buffer (29). It directly quenches ROS and other oxygen-centered free radicals (30). Low levels of GSH could be due to enhanced generation of ROS which are scavenged by GSH or decreased activity of glutathione reductase enzyme, which converts oxidized glutathione (GSSG) to its reduced form. Glutathione depletion was reported to induce apoptotic cell death which occurs through the upregulation of novel protein kinase C and activator protein-I (31). Our study showed increase LDH activity in the DM group which is in agreement with those of Celik *et al.*, (32), however, administration of FRN has recovered the LDH activity of RBC as shown in our data.

VII. CONCLUSION

To our knowledge, our data evaluate for the first time, the antioxidant effects of FRN on the RBC's of rat model of DM induced by alloxan. In Conclusion, the present study clearly demonstrates that the administration of FRN extract enhance the antioxidant defense system of RBC's in alloxan-induced DM on rats. For future studies we suggest the use of different doses less than 500 mg/kg as this dose give slight changes in the FRN group as compared to controls.

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