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SclerocaryaBirrea Oil ModulatesHuman T-Lymphocyte Differentiation

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ABSTRACT

Sclerocaryabirrea (A. Rich.)Hochst. (Anacardiaceae) is one of the plants that played a role in feeding people in ancient times. English name Marula, is a savannah tree, belonging to the family Anacardiaceae, with a plum-like pale yellow fruit of 3–4 cm in diameter with a juicy mucilaginous flesh. Sclerocaryabirrea is deciduous and mainly dioecious, although there have been reports of monoecioustrees.The oil is extracted from the kernels that contain between 65% oil.This oil is composed with large amount of fatty acids which includes oleic acid (80-85%), linoleic acid (6.0-8.0 %), alpha-linolenic acid (0.2-0.9 %), palmitic acid (10-14%), stearic acid (7.0-9.0 %), arachidonic acid (0.5-0.8 %). Further tocopherols (26mg/100g), flavonoids are also found in Sclerocaryabirrea oil. Our results show that Sclerocaryabirrea oil exerted the most potent immunosuppressive effects on T cell proliferation and IL-2 mRNA expression.Sclerocaryabirrea oil-induced secretion of IL-4 by T-cells.The densitometric analysis of western blots revealed that Sclerocaryabirrea oilinhibited the phosphorylation of the three MAP kinases in T-cells.

Keywords: Fatty acids CD3 Jurkat T-cells Oil Sclerocaryabirrea.

ABBREVIATIONS

ERK, extracellular signal-regulated kinase; interleukin, IL; JNK, c-Jun N-terminalkinase; MAP, mitogen-activated protein; PUFA, polyunsaturated fatty acids.

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INTRODUCTION

Several plants have been used by traditional and ancestral medicine men in African countries for the treatment of several pathologies including digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhoea and insomnia [1,2]. The medicinal properties of this plant depend on the part of the plant concerned (root, leaf stalk and pulp or fruit) and the extract used (ethanolic, butanolic etc.). Butanol extracts of *Zizyphusspina-christi* leaves which are rich in saponin improved the oral glucose tolerance and potentiated glucose-induced insulin release in type II diabetic rats [3]. In a cross-sectional survey conducted in Dakar, 43% of patients attending consultancy at the hospital declared using *Sclerocaryabirrea*[4]. This shows the wide use of the plant for the treatment of various diseases, although investigations on biological effects are lacking. *Sclerocaryabirrea*stem-bark extracts have been shown to exert hypoglycaemic effects in animal models. In rats with streptozotocin-induced diabetes, acute administration of S. birrea stem-bark extract reduces blood glucose levels[5,6]. Chronically, the efficacy of *Sclerocaryabirrea* stem-bark extract administered for 5 weeks was shown to be similar to metformin treatment with regards to lowering of glycaemia [7]. In the consensus model of glucose-stimulated insulin secretion, glucose phosphorylation initiates its metabolism [8], ultimately leading to plasma membrane depolarisation[9] and the ensuing cytosolic calcium rise inducing insulin exocytosis[10]. Additional signals, generated by mitochondrial metabolism, amplify the calcium signal [11]. Elevation of plasma insulin was reported in stem-bark-treated diabetic rats [6]. Regarding the in vivo hypoglycaemic effect of *Sclerocaryabirrea*stem-bark, chronic treatments have been reported only with organic extracts [6,7], whereas aqueous extracts have been shown to be effective in acute conditions [5]. *Sclerocaryabirrea* is a widespread species throughout the semi-arid deciduous savannas of much of sub-Saharan Africa. The tree has been a part of civilization since ancient times with use of all parts of the tree. The fruits are utilized for food, juice, jelly, jam and beer, the bark for medicinal purpose, the kernels for food and oil and the wood for fuel wood. There has been a wide interest in the medicinal uses of the tree since it has been utilized for centuries to treat diseases. Recent studies revealed hypoglaecemic activity as well as other positive attributes such as anti-inflammatory and antibacterial properties. There have been numerous studies conducted to elucidate its valued components which contribute to its medicinal properties. Several initiatives are exploiting the commercial value of the tree such as the production of internationally acclaimed alcoholic drinks such as *Sclerocaryabirrea*liquer. Disorders in the immune system may be responsible for the onset of different pathological states. The

immunodeficient diseases when the immune system is less active than normal result in recurring and life-threatening infections. On the other hand, an autoimmune disease results from a hyperactive immune system attacking normal tissues as if they were foreign organisms [12]. Common autoimmune diseases include Hashimoto's thyroditis, rheumatoid arthritis, type I diabetes and lupus erythematosus. Further investigation in this field is expected to play a serious role in promotion of health and treatment of diseases. The T-lymphocytes are the principal mediators of immune-mediated diseases.Hence, a modification of T-cell activation will be a valuable tool to disrupt the disease progression. As far as the activation of immune system by *Sclerocaryabirrea* oil is concerned, not much is known on the subject[13]have shown that Zizyphus extracts alongwith other plants stimulated neutrophil functions and exerted hepatotoxic and immunomodulatory effects in guinea pigs. [14] assessed cell signaling mechanisms in T-cells and provided the evidence that a mixture of herbs containing Zizyphus extract induced the expression of mitogen-activated protein kinases (i.e. ERK, JNK and p38) in T-cells, indicating that the immunomodulatory effects of Zizyphus involve the activation of second messenger cascade. Since Zizyphus lotus L. (Desf.) has been shown to modulate different disorders[1,15, 16]. Moreover, all forms of diabetic have been linked to a pathological role of immune system and inflammation [17, 18]*Sclerocaryabirrea* oil, since antioxidants have been reported to modulate immune system [19].We investigated the role of *Sclerocaryabirrea* oil on human T-lymphocyte proliferation, expression of IL-2 mRNA and *Sclerocaryabirrea* oil-induced secretion of IL-4 by T-cells. Hence, we determined the contents of vitamin E, flavonoids which have been considered as anti-oxidant agent and fatty acids are known to modulate the metabolism of lipids.*Sclerocaryabirrea* oil-modulated T-cell activation by modulating MAP kinase phosphorylation.

MATERIALS AND METHOD

The culture medium RPMI 1640 and l-glutamine were purchased from Biowhitaker, Belgium. Fura-2/AM and bis-oxonol, bis-5(1,3-diethylthiobarbiturate)tirmethinoxonol, were procured from Molecular Probes, Eugene, OR. [3H]thymidine (sp act, 20Ci/mmol) was purchased from Amersham Radiochemicals, France.

Oil Extraction

The stored kernels were crushed and ground using a grinding mill (Petra electric, Burga,

Germany). The oil was extracted from the ground material by extraction with n-hexaneat 50–60°C in a Soxhlet apparatus for 6 following the AOCS method (20 AOCS 1996)]. The oil content was determined as a percentage of the extracted oil to the sample weight (w/w). The samples were

analyzed in triplicate, and then means and the standard deviations were calculated. The oil obtained was stored at 4°C for further investigation.

Fatty Acid Composition

The overall fatty acid composition of the investigated Sclerocaryabirrea kernels was determined following theISO draft standard [21]. In brief, one drop of the oil wasdissolved in 1mL of n-heptane, 50µL 2 M sodiummethanolate in methanol was added, and the closed tubewas agitated vigorously for 1 min. After addition of 100µL of water, the tube was centrifuged at 4,500g for10 min and the lower aqueous phase was removed. After that 50µl 1 M HCl were added to the heptane phase, the two phases were mixed for a short time and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulfate (monohydrate, extra pure, Merck,Darmstadt, Germany) was added, and after centrifugation 4,500x g for 10 min the top n-heptane phase was transferred to a vial and injected into a Varian 5890 gaschromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2µm). The temperature program was: from 155°C heated to 220°C (1.5°C/min.), 10 min isotherm; injector 250°C, detector 250°C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1µ L. The integration software computed the peak areas and percentages of fatty acid methyl esters (FAME) were obtainedas weight percent by direct internal normalization.

Tocopherols

For determination of tocopherols a solution of 250 mg of Sclerocaryabirrea kernel oil (SBKO) in 25 mL n-heptanewas directly used for the HPLC. The HPLC analysis wasconducted using a Merck-Hitachi low-pressure gradientsystem, fitted with a L-6000 pump, a Merck-HitachiF-1000 Fluorescence Spectrophotometer (detector wave-lengths for excitation 295 nm, for emission 330 nm) and aD-2500 integration system. Twenty microliters of thesamples were injected by a Merck 655-A40 Autosampleronto a Diol phase HPLC column 25 cm94.6 mm ID(Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/ min. The mobile phase used wasn-heptane/ tert - butylmethyl ether (99+1, v/v) [22].

Total Flavonoids

The total flavonoid content was determined following hang et al.[23] Briefly, 5 mL of 2% aluminum trichloride (AlCl3) (Labosi, Paris, France) in methanol (FlukaChemie, Switzerland) was mixed with the same volume of oil (0.01 or 0.02 mg/mL). Absorption readings at 415 nmwith a spectrophotometer (Shimadzu Corp. Kyoto, Japan)were taken after 10 min against a blank sample consistingof a 5 mL oil solution with 5 mL methanol without AlCl3.The total flavonoid content

was determined using a standard curve with rutin (Sigma-Aldrich Chemie, Steinheim,Germany) (0–50 mg/L) as the standard. The mean of three readings was used and expressed as mg of rutin equivalents (RE)/100 g of oil.

Cell culture

The human (Jurkat) T-cells were routinely cultured in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine, 50 µg/ml penicillinstreptomycin and 20 mM HEPES at 37°C in a humidified chamber containing 95% air and 5% CO2 [24].Cell viability was assessed by trypan blue exclusion test. Cell numbers were determined by hemocytometer.

T-cell proliferation assay

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate (Nunc, Roskilde, Denmark), then cells were incubated for 4 h with increasing concentration of *Sclerocaryabirrea* oil (0 µM - 100µM), then stimulated with anti-CD3 antibodies (30 µg/ml). Cells were distributed in six replicates as follows: 160 µl of cell suspension, 20 µl of *Sclerocaryabirrea* oil and 20 µl of anti-CD3 antibodies as described elsewhere [25]. After 36 h, 20 µl of [3H] thymidine (20 Ci/mmol, 0.5 µCi/well) was added and, 12 h later, the cells were harvested with a cell harvester (Dynatech, Burlington, MA, USA), trapping their DNA onto glass filter mats. Dried filter circles were placed in plastic minivials (Pakard, Downers Grove, IL, USA), 2 ml of Optifluor-O (Pakard) was added, and the radioactivity was recorded in a scintillation counter (Beckman, Fullerton, CA, USA).

ELISA

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate (Nunc, Roskilde, Denmark), then cells were incubated for 4 h with or without *Sclerocaryabirrea* oil then stimulated with anti-CD3 antibodies (30 µg/ml) as described elsewhere [24]. Cells were distributed in six replicates as follows: 160 µl of cell suspension, 20 µl of *Sclerocaryabirrea* oil.Cell culture supernatants were collected for quantitative evaluation of immunoactive IL-4 by ELISA, as described elsewhere [25].

RNA isolation and real time quantitative PCR

Cells were cultured as described above in the presence of *Sclerocaryabirrea* oil and stimulated with anti-CD3 antibodies for 2 h [26]. Total RNA from cells was extracted using trizol and underwent DNase treatment using the RNase-free DNase Set (Qiagen). One µg of total RNA was reverse transcribed with Super script II H-reverse transcriptase using oligo (dT) according to the manufacturer's instructions.Real time PCR was carried out on the iCycleriQ real time detection system and amplification was undertaken by using SYBR Green I detection. Oligonucleotide

primers were as follow: beta-actin forward: 5'-ATGATATCGCCGCGCTCGTCGTC-3', beta-actin reverse 5'-AGGTCCCGGCCAGCCAGGTCCAG-3'; IL-2 forward 5'-CACTAATTCTTGCACTTGTCAC-3', IL-2 reverse 5'- CCTTCTTGGGCATGTAAAACT-3'. IL-2. The Amplification was carried out in a total volume of 25 µl containing 12.5 µl SYBR® Green supermix, i.e., PCR buffer [50 mMKCl, 20 mM, Tris-HCl (pH 8.4), 3 mM MgCl2], 0.2 mM each dNTPs, 0.63 U iTaq DNA polymerase, SYBR green 1,10 nMfluoresein, and 12.5 µl containing 0.3 µM each primer and diluted cDNA.The conditions of amplification consisted of an initial denaturation step at 95°C for 5 min as a "hot start" followed by 40 cycles at 95°C for 30 s/60°C for 30 s with a single fluorescence detection point at the end of the relevant annealing or extension segment. At the end of the PCR, the temperature was increased from 60 to 95°C at a rate of 2°C/min, and the fluorescence was measured every 15 s to construct the melting curve. The standard curves were generated for each gene using serial dilutions of positive control template in order to establish PCR efficiencies. All determinations were performed, at least, in duplicates using two dilutions of each assay to achieve reproducibility.Results were evaluated by iCycleriQ software including standard curves, amplification efficiency (E) and cycle threshold (Ct). Relative quantification of mRNA in different groups was determined as follows: $\Delta\Delta Ct = \Delta Ct$ of gene of interest - ΔCt of beta actin. ΔCt = Ct of treated cells - Ct control cells. Relative quantity (RQ) was calculated as follows: $RQ = (1+E)^{(-\Delta Ct)}$.

Preparation of immunoblot analysis

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate (Nunc, Roskilde, Denmark), then cells were incubated for 4 h with increasing concentration of *Sclerocaryabirrea* oil20 µM, then stimulated with anti-CD3 antibodies (30 µg/ml). Briefly, cells were washed twice with ice-cold PBS containing 1 mM PMSF, resuspended in 250 µl buffer A (10 mM HEPES, pH 7.8, 0.1 mM EDTA, 10 mMNaCl, 3 mM $MgCl_2$, 300 mM sucrose, 10 µg/ml aprotinin, 100 µM leupeptin, 1 mM DTT, and 1 mM PMSF), and incubated on ice for 10 min. Then, 25 µl of 1% Nonidet P-40 was added and mixed carefully. Cells were collected by centrifugation at 800 x g for 1 min at 4°C and washed with 200 µl buffer A. Nuclei were then resuspended in 50 µl buffer B (20 mM HEPES, pH 7.8, 3 mM $MgCl_2$, 420 mMNaCl, 0.2 mM EDTA, 25% glycerol, 10 µg/ml aprotinin, 100 µM leupeptin, 1 mM DTT, and 1 mM PMSF) and incubated for 15 min on ice. Nuclear debris was removed by centrifugation at 16,000 x g for 1 min. The supernatant was then removed, and protein content was determined by Bradford Assay. Equal amounts of nuclear protein were subjected to 10% SDS-PAGE and polyvinylidenedifluxoride membrane (Millipore, Bedford, MA) [27]. The MAP kinases in the

cytosolic fractions were detected by the antibodies raised against phosphorylated forms of p38, c-Jun N-terminal kinase (JNK), and ERK1/2 (Ozyme/Cell Signaling, Beverly, MA). Membranes were incubated with these antibodies for 1hr and then washed, and incubated with goat anti-rabbit HRP conjugate (1/2000 dilution in TBS-Tween) for 45 min at room temperature. After washing, bands were visualized using a chemiluminescence kit, according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistical analysis

Statistical analysis of data was carried out using Statistical (version 4.1, Statsoft, Paris, France). The significance of the differences between mean values was determined by analysis of variance one way, followed by a least significant- difference (LSD) test. For all the tests, the significance level chosen was $p < 0.05$. The Spearman's rank test was employed for the correlation coefficients.

RESULTS AND DISCUSSION

Chemical Composition

Sclerocaryabirrea oil is extracted from nuts of the *Sclerocaryabirrea* tree which is basically from the Anacardiaceae family. The oil is extracted from the kernels that contain between 65% oil.This oil is composed with large amount of fatty acids which includes oleic acid (80-85%), linoleic acid (6.0-8.0 %), alpha-linolenic acid (0.2-0.9 %), palmitic acid (10-14%), stearic acid (7.0-9.0 %), arachidonic acid (0.5-0.8 %). Further tocopherols (26mg/100g), flavonoids are also found in *Sclerocaryabirrea* oil. A number of studies have investigated the effects of n-3 PUFA in auto-immune diseases. These reports have demonstrated that n-3 PUFA exerted beneficial effects and inhibited T-cell proliferation.

***Sclerocaryabirrea* oil inhibits T-cell proliferation**

Sclerocaryabirrea oil exerted an inhibitory effect on T-cell proliferation (Figure 1). The*Sclerocaryabirrea*-induced inhibitory effects at 20 M were not statistically different from 50 M and 100 M of this oil. Therefore, in the further experiments, we employed *Sclerocaryabirrea* oil at 20 M. The inhibitory effects of *Sclerocaryabirrea* oil at 20 μM were not due to its cytotoxicity as we checked the cell viability by trypan blue exclusion test in these assays. Figure 2 shows that *Sclerocaryabirrea* oil-induced secretion of IL-4 by T-cells.

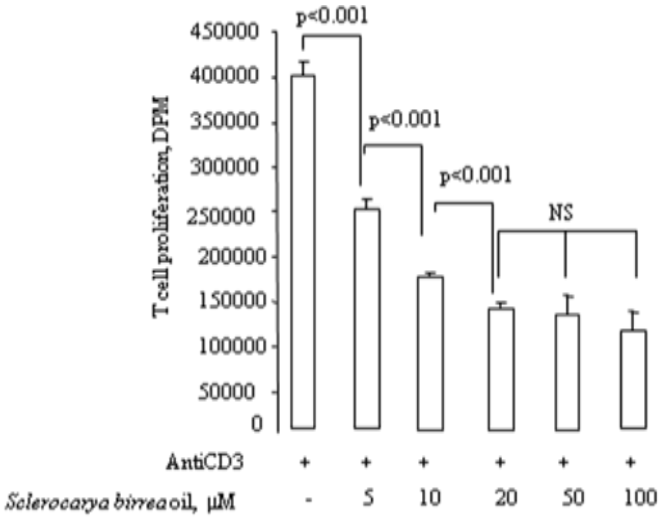

Figure. 1. Sclerocaryabirrea oil inhibits T-cell proliferation.

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate. Then cells were incubated for 4 h with increasing concentration of *Sclerocaryabirrea* oil (0 µM - 100µM), then stimulated with anti-CD3 antibodies (30 µg/ml). Values differ significantly compared with anti-CD3-stimulated cells $p < 0.001$ (*). Each value represents the mean of six determinations.Data were analyzed by two-way ANOVA **.**

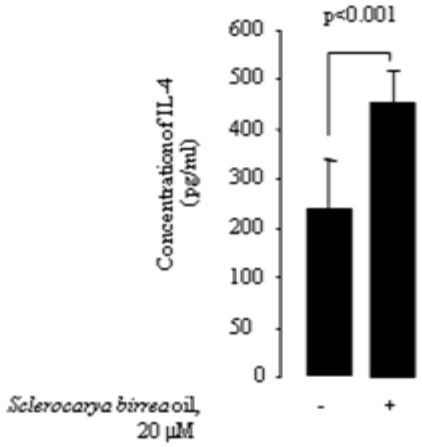

Figure. 2. Sclerocaryabirrea oil modulates secretion of IL-4.

Jurkat T-cells (0.1 × 106 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate. Then cells were incubated for 4 h with or without Sclerocaryabirrea oil then stimulated with anti-CD3 antibodies (30 µg/ml). Cell culture supernatants were collected for quantitative evaluation of immunoactive IL-4 by ELISA. Values differ significantly compared with anti-CD3-stimulated cells $p < 0.001$ (*). Each value represents the mean of six determinations. Data were analyzed by two-way ANOVA.

***Sclerocaryabirrea* oildownregulatesIL-2 mRNA expression**

Since *Sclerocaryabirrea* oil modulated the secretion of cytokines into the extracellular environment, we measured the expression of IL-2 mRNA in T-cells. We observed that *Sclerocaryabirrea* oil diminished the expression of IL-2 mRNA (Figure 3). Similarly, we also observed that *Sclerocaryabirrea* oil exerted the most potent immunosuppressive effects on T cell proliferation and IL-2 mRNA expression.*Sclerocaryabirrea* oil-induced secretion of IL-4 by T-cells.

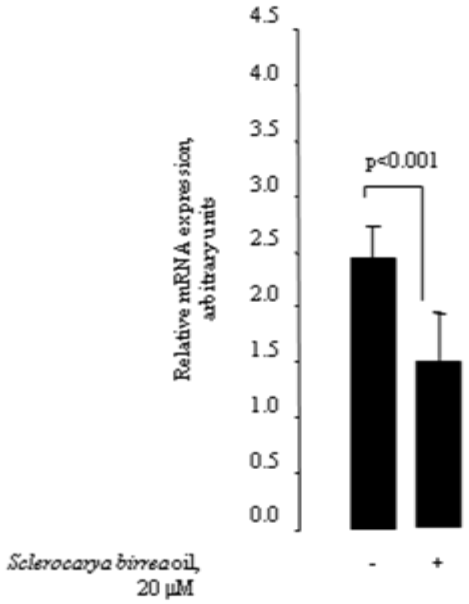

Figure. 3. Sclerocaryabirrea oil downregulatesIL-2 mRNA expression .

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate. Then cells were incubated for 4 h with or without *Sclerocaryabirrea* oil then stimulated with anti-CD3 antibodies (30 µg/ml). The expression of mRNA was quantitatively analyzed at 6 h postactivation by employing RT-PCR as described in materials and methods section. Values differ significantly compared with anti-CD3-stimulated cells $p < 0.001$ (*). Each value represents the mean of six determinations.Data were analyzed by two-way ANOVA**.**

***Sclerocaryabirrea* oilmodulate MAPK phosphorylation**

The densitometric analysis of western blots revealed that *Sclerocaryabirrea* oilinhibited the phosphorylation of the three MAP kinases in T-cells (Figure 4). T-cell abnormalities are believed to be the major cause of autoimmune diseases like type 1 diabetes. In type 2 diabetes also, the inflammation leading to the activation of monocytes is postulated to be important for enhancing insulin resistance and contributing to the loss of insulin secretary function by islet cells.The densitometric analysis of western blots revealed that *Sclerocaryabirrea* oilinhibited the

phosphorylation of the three MAP kinases in T-cells. These observations suggest that *Sclerocaryabirrea* oil might shift the T_H1 cells to T_H2 phenotype. These immunosuppressive effects of *Sclerocaryabirrea* oil on T-cell proliferation corroborate the observations of several investigators who have reported that docosahexaenoic acid inhibits the lymphoproliferative responses in vitro and in vivo [28, 29]. Beneficial effects of plants may be related to the presence of biologically active compounds [30, 31].

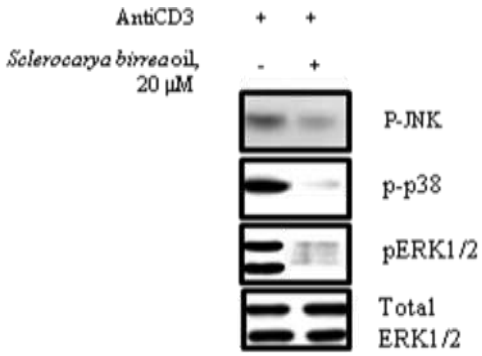

Figure.4. Sclerocaryabirrea oil modulate MAPK phosphorylation.

Jurkat T-cells (0.1×10^6 cells/160 µl) were suspended in RPMI-1940 without serum and seeded in 96-well plate. Then cells were incubated for 4 h with or without *Sclerocaryabirrea* oil then stimulated with anti-CD3 antibodies (30 µg/ml). The phosphorylated forms of MAPK were analyzed by Western blot as described in materials and methods section. Each value represents the mean of six determinations.Data were analyzed by two-way ANOVA**.**

These immunosuppressive effects of *Sclerocaryabirrea* oil on T-cell proliferation corroborate the observations of several investigators who have reported that, Zizyphus lotus L. (Desf.), a plant known for its antidiabetic properties is a good source of antioxidant agents and its decoction exerts immunosuppressive activities [32]. It is possible that the antioxidant activity might be due to the presence of vitamins. The Spearman's correlation coefficient (Rs) between antioxidant activity and vitamins or fatty acids are as follows: vitamin A vs antioxidant Rs = 0.95; vitamin C vs antioxidant Rs = 0.82; vitamin E vs antioxidant = Rs 68; n-fatty acids vs antioxidant Rs = 0.27. Furthermore, Lenucci et al. [33]have demonstrated that antioxidant activity is likely due to the presence of ascorbic acid, tocopherol and pigments. This argument also supports the highest antioxidant activity of *Sclerocaryabirrea* oil which contains substantial quantity of tocopherols, fatty acids, further, flavonoids. These investigators further assessed the antioxidant capacity of *Sclerocaryabirrea* oil and finally concluded that the oil, rich in polyphenols, was responsible for the antioxidant property. We observed that *Sclerocaryabirrea* oil exerted an inhibitory effect on T-cell

proliferation. A plausible explanation for the diminished T-cell activation anti-CD3-stimulated T-cell proliferation is not available. Hence, we can hypothesize that the diminished in vitro T-cell proliferation may be due to the fact that these cells are already activated in vivo in these pathologies, although some studies have demonstrated both high mitogenic lymphocyte responses and high secretion of cytokines in infants of diabetic mothers [34] compared with newborns of healthy mothers [35]. Our hypothesis can be supported by the observations of several authors who have reported a significant increase in the percentage of in vivo-activated T lymphocytes in diabetic rats [36, 37]. It has been suggested that maternal diabetes during pregnancy is an important risk factor for fetal overnutrition [38, 39, 40] and development of obesity in the offspring at 1 month of age [41, 42]. These alterations in macrosomic infants persist postnatally[43, 44]. Because dietary fatty acids are known to modulate the metabolism of lipids [45, 46] and exert immunomodulatory effects, the present study was conducted to elucidate the role of *Sclerocaryabirrea* oil in T-cell activation.Silva et al. [47] have reported that the oil of Zizyphusmistol was rich in n-3 fatty acids (18:3 n-3) and, therefore, modulated tumor growth in animal models [48]. It has been well established that n-3 fatty acids exert immunosuppressive and anti-inflmmatory activities bothin experimental and clinical studies [49]. Indeed, the extracts of Zizyphus lotus L. (Desf.) have been shown to possess anti-inflammatory properties [15]. It is noteworthy that the Zizyphus extracts in the absence of anti-CD3 antibodies failed to inhibit cell proliferation, suggesting that Zizyphus extracts under normal conditions do not modulate T-cell proliferation. These observations are in analogy to the n-3 fatty acids which, being authentic immune suppressors, failed to influence normal T-cell proliferation in healthy subjects [50]. In fact, the best immune suppressors might interfere, principally, with abnormal T-cell activation, as seen in the autoimmune diseases, without influencing the same in healthy situations [51]. As far as the mechanism of action of fatty acids is concerned, it has been well established that they interfere with cell signalling, particularly with the cascade of MAP kinases like ERK1/2 and p38 [52, 53]. In fact, Chan et al.[54] have also demonstrated that a mixture of herbs containing Zizyphus extracts also interfere with the phosphorylation of ERK1/2 and p38 in T-cells. The induction of IL-2 gene transcription is a critical event for T-cell proliferation and effector functions. We observed that *Sclerocaryabirrea* oil inhibited T-cell blastogenesis and IL-2 mRNA expression.However, this oil increased the secretion of IL-4, a cytokine secreted by T_H2 cells. The *Sclerocaryabirrea* oil-induced secretion of IL-4 by T-cells. These observations suggest that *Sclerocaryabirrea* oil might shift the T_H1 cells to T_H2 phenotype. It is possible that less IL-2 secretion may account for less T-cell proliferation in as T-cell blastogenesis is an IL-2-dependent phenomenon.The differentiation of naive T-cells into T_H1 and

T_H2 subsets is tightly regulated through the activities ofspecific signaling pathways and transcription factors[55].Several reports have well-shown that activity of the MAP kinase is associated with T_H1 Tcell differentiation and IFN-γ production [56]. We observed that *Sclerocaryabirrea* oil diminished the expression of the three MAPK in T-cells. We have previously shown that *Sclerocaryabirrea* oil exerts immunosuppressive effects via its inhibitory action on ERK1/2 in T-cells. To sum up, we can state that *Sclerocaryabirrea* oil bear potential therapeutic properties in diabetes possess antioxidant, anti-hyperglycemic and immunosuppressive activities. To our knowledge, no study has, as yet, been carried out on the effects of *Sclerocaryabirrea* oil in autoimmune diseases. Further studies are required to elucidate the effects of *Sclerocaryabirrea* oil in the progression of autoimmune diseases or organ transplantation.

CONCLUSION

Our study demonstrates that *Sclerocaryabirrea* oil plays a novel role in the transcriptional regulationof IL-2 gene expression in T-cells and increased the production of IL-4. The ability of *Sclerocaryabirrea* oilto negatively regulate the activation-induced expression ofIL-2 in T-cells may influence the timing of the switch fromT_H1 and T_H2 phenotypes. The abilityof *Sclerocaryabirrea* oil to suppress activation of the MAP kinasesmay allow activated $CD4^+$ T cells to sense and to respond to environmental factors that serve to influence the actions of T-cells following stimulation. Our study also shows that *Sclerocaryabirrea* oil may induce the T_H2 differentiation consequently, modulate the progression of immune-mediated diseases.

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