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Effect of Rota Plant Extracts on the SIRT3 Sertoin-3 Concentration in the Alzheimer Patients' Blood

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ABSTRACT

We induced Alzheimer's disease in a group of mices, after which they were treated with extracts of the Ruta plant, which included a group of chemical compounds, including alkaloids, flavonoids, and the oil extract. The results of treatment with the Ruta plant extract showed a significant effect on the biochemical variables studied, as the Ruta extracts led to raising the concentration of the sirtuin3 enzyme to good levels with a significant difference from the affected group. As for the flavonoid extract, it was the most efficient by recording the lowest average of the enzyme between the groups. Infected.

Keywords : Ruta Plant, Alzheimer's Disease, Sirtuin3, Flavonoid, Preparation of Plant Extracts

I. INTRODUCTION

Plants in general contain a high percentage of antioxidant molecules (Such as carotenoids, polyphenols, unsaturated fatty acids, vitamins, enzymes and cofactors), that is able to capture free radicals. These compounds have gained particular attention for their potential use in preventive and therapeutic phytotherapy, Deterioration of food and food products results mainly from oxidation. In the literature, several assays have described the ability of redox compounds to scavenge free radicals to determine the antioxidant capacity of food and biological samples . Plants of the Rutaceae family (Citrus family) are known for their economic importance and also for their cultivated citrus fruits, woods and essential oils, being a potential source of many medicinal substances[1-3]. One of the genus of plants of the Rutaceae family that has been studied is the genus Ruta. Ruta (common name rue), belongs to the tribe Ruteae and is a genus of the family (4) (1), Ruta is a strongly scented sub-shrubs of the Mediterranean region. The genus Ruta includes ten species of perennial shrubs, among which are R. There are about 40 species of Ruta, (family Rutaceae), a genus of highly scented annual shrubs endemic to the Mediterranean basin. Ruta chalipensis and Ruta montana. The leaves of R. Graleolens have a foul odor,

and are small, oblong, divided, feathery, and glandular. The flower has four petals except for the central flower, which has five petals[4-6]. Ruta chalepensis L. and Ruta graveolens L. are species widespread in Algeria and used as medicinal plants to treat various diseases. It is a mitochondrial protein within the sirtuin family of NAD+-dependent deacetylases. This family consists of seven members, each with unique targets and locations within cells. SIRT3 acts as a histonedeacetylase, removing acetyl groups from proteins such as histones. It plays a crucial role in regulating energy demand during fasting and exercise through modulation of mitochondrial enzymes[7-8]. SIRT3 also has a protective role in preventing cancer cell development and apoptosis by eliminating reactive oxygen species (ROS). SIRT3 contains a conserved catalytic domain called the sirtuin core domain and targets lysine residues on various proteins, affecting mitochondrial function and cellular metabolism. Increasing evidence suggests that the sirtuin SIRT3 has neuroprotective effects in regulating oxidative stress and energy metabolism, both of which are involved in the pathogenesis of Alzheimer's disease. However, it is unclear whether SIRT3 is associated with cognitive performance and pathological changes in Alzheimer's disease. Acase-control study was conducted of postmortem brains of (n=16), mild cognitive impairment (n=13), and age and education matched cognitively normal (n, n = 11). We measured mRNA and protein levels of SIRT3 and evaluated their association with cognitive performance and pathology. In an ex vivo model of cortical neurons from transgenic mice carrying human tau, we modulated SIRT3 expression by genetic and Rapid methods to investigate the cause-and-effect relationship between SIRT3 and tau[9-10]. SIRT3 levels were reduced in the entorhinal cortex, middle temporal gyrus, and superior frontal gyrus of M subjects compared with those of N. This reduction was associated with lower neuropsychological assessment test scores and greater severity of tau pathology. Another study with genetic manipulation of SIRT3 revealed that amyloid increased levels through its modification of SIRT3. These data suggest that SIRT3 reduction is critically involved in the pathogenesis of Alzheimer's disease[10-15].

II. METHODS AND MATERIAL

2.1 Equipment used

- Centrifuge from the German company Heraeus-Christ Gmbh.
- Magnetic stirrer from the Irish company B.V.Cenco Instrument.
- Lyophilizer drying device from the English company Edwards.
- The sensitive balance is from the Japanese company (AD.HR- 200).
- Blender punching machine from the Japanese company National.
- Water bath from the English company Gallen Kamp.
- Micropipettes (fixed and adjustable) from the American company Oxford and the
- German company Slamed.
- pH meter from the Dutch company Philips.
- Swiss Perstaltic pump.
- Memmert incubator from the German company Karb-Klob.
- France Spectrophotometer Single beam 142 295 from the English company CECIL
- Instruments Limited.

2.2 Materials used

The chemicals used for the manual measurement methods were supplied by various international companies, namely Hopkins and Williams, Sigma, Fluka, The British Drug House LTD Fluka and Difco Laboratories. Standard kits from the French company BIOLABO were also used to measure variables such as albumin and others.

Figure (1) Chemicals used for manual measurement methods

2.3 Specimens used

2.3.1 Control group

(45) blood samples were collected from healthy people whose ages ranged between (59-83) years for both genders and included 23 males and 22 females.

2.3.2 patient group

45 blood samples were collected from people with Alzheimer's aged between 60-83 years for both genders. The group included 23 males and 22 females. The patients' information was recorded according to a questionnaire form prepared for this purpose, Table (1).

2.3.3 Serum collection and preservation

Venous blood was drawn (5 ml) from a vein. Blood samples were collected in dry, clean tubes and placed in a water bath at 37°C for 15 minutes. Then the clotted part was separated from the clear solution using a centrifuge at 3000 rpm for a quarter of an hour, as represents blood serum that was divided into three parts in small, dry, clean plastic tubes and stored at a temperature (-10°C) until it is used to measure the variables specified in the research.

Table (2) Questionnaire form

Sample number	
Patient name	
Gender	Male ☐ Female ☐
Age	
Height	
Weight	
Disease type	
Smoking	
Other diseases	Diabetes ☐ pressure ☐
Drugs	

2.4 Human Sirtuin 3 (SIRT3) ELISA kit

2.4.1 The use

The kit is an enzyme immunoassay for in vitro quantification of SIRTS in human serum plasma. Tissue homogenates and other biological fluids.

2.4.2 Reagents and Materials Provided:

The Reagents and Materials that have been used in the study are listed in table (3) :

Table (3) Reagents and Materials list

Reagents	Quantity	Reagents	Quantity
Coated ELISA plate	12-well Tubes	Washing concentrate(30X)	20ml
Standard dilution	m	Instruction	1
Chromogen solution A	6ml	Seal plate membrance	2
Chromogen Solution B	6ml	Hermetic bag	1
Streptavidin-HRP	6ml	Stop solution	6 ml
Standard solution (48ng/ml)	0.5ml	Anti SIR3 antibodies labeled with biotion	1 m

2.4.3 Store collections

1- For unopened kits: Keep all reagents according to the labels on the vials. TMB substrate wash buffer (30× concentration) and should be stored at 4°C upon receipt while other materials should be at -20°C.

2- For open kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition.

2.4.4 Washing method

1. Hand Washing Method: Wash by hand: Shake the liquids in the holes of the ELISA plate; Place several absorbent papers on the test bed and force the ELISA plate down several times; Then inject at least 0.35 mL of the diluted wash concentration over 1-2 minutes. Repeat this process as needed.
2. Machine washing method: Wash by automatic dishwasher. If there is an automatic dishwasher, it should only be used for testing when you are fully familiar with its functions.

2.4.5 Sample collection and storage

3. Samples containing NaN3 should not be tested because it inhibits peroxidase (HRP) activity. After collecting the sample, extraction should be carried out immediately according to the relevant documents. After extraction, the experiment should be performed immediately as well. Otherwise, keep the sample at -20°C. Avoid repeated freezing and thawing cycles.
4. Serum: Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (2000-3000 rpm) for 20 minutes. Collect supernatants carefully. When sediment occurred during storage, centrifugation should be performed again.

2.4.6 Examination procedures

1- Dilution of standard solutions: (This kit contains the original concentration standard, which can be diluted in small tubes by the user independently following the instructions) figure (3)and table (4).

Table (4) the dilution of standard solutions:

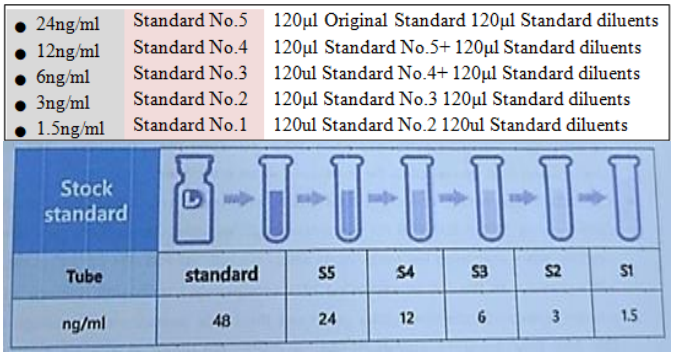

Figure (3) Instructions for diluting standard solutions

1- The number of strips required is determined by the samples to be tested and the standards added. It is suggested that each standard solution and each blank well be arranged with three or more wells as much as possible.

2- Sample injection

(1) into an empty well. Add Chromogen Solution A and B only, and stop the solution.

(2) Standard solution well: Add standard 50ul and streptavidin-HRP 50µl.

(3) A good sample to be tested: Add 40 µL of sample, then 10 µL of SIRT3 antibody, 50 µL of streptavidin-HRP and then cover with a seal plate membrane. Gently shake to mix them. Incubate at 37 °C for 60 minutes.

3- Prepare the washing solution: Dilute the washing concentration (30X) with distilled water for later use.

4- Washing: Carefully remove the sealing plate membrane, drain the liquid and dispose of the remaining liquid. Fill each well with washing solution. Drain the fluid after standing for 30 seconds. Then repeat this procedure five times and wipe the plate.

5- Color development: Add 50 µL of chromogen solution A first to each well and then add 50 µL of chromogen solution B to each well as well. Gently shake to mix them. Incubate for 10 minutes at 37°C away from light to develop color.

6- Stop: Add 50 µL of stop solution to each well to stop the reaction (the blue color changes to yellow immediately at that moment).

7- Assay: Take a blank well at zero, and measure the absorbance (OD) of each well one by one under the wavelength of 450nm, which should be done within 10 minutes after adding the stop solution.
8- Depending on the concentrations of the standards and their corresponding values, the linear regression equation for the standard curve is calculated. Then according to the OD value of the samples, calculate the corresponding sample concentration. Special calculation software can also be used.

2.5 Test principle

This kit uses an enzyme-linked immunosorbent assay (ELISA) based on the Biotin double antibody sandwich technology to screen for Human Sirtuin 3 (SIRT3). Add Sirtuin 3/SIRT3) to the wells, which are pre-coated with a Sirtuin 3 (SIRT3) monoclonal antibody and then incubate. Next, add biotin-labeled anti-SIRT3 antibodies to combine with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution turns blue and turns yellow under the action of acid. Solution shades and Human Sirtuin 3 (SIRT3) concentration are positively correlated.

2.6 Calculate the results

Make the concentration of the standards abscissa and the OD value the abscissa. Draw the standard curve on the coordinate sheet. According to the OD value of the sample, locate its corresponding concentration (which is the sample concentration); Or calculate the linear regression equation of the standard curve according to the standard concentration and OD value. Then replace the sample's OD value to calculate its concentration figure (4).

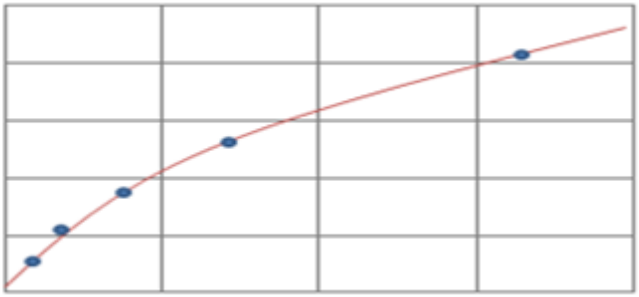

Figure (4) Standard curve of a linear regression equation

2.7 The quality

This test has high sensitivity and excellent specificity for detecting SIRTS. No significant cross-reactivity or interference was observed between SIRT3 and its analogues.

2.8 Accuracy

Intra-assay precision (in-assay precision): 3 samples with low, medium and high SIRT3 levels were tested 20 times on one plate, respectively. Inter-assay precision: 3 samples with low, medium and high SIRTS levels were tested on 3 different plates, 8 replicates in each plate.

CV(%)=SD/meanX100
Internal examination, CV <8%
Inter-assay: CV <10%

2.9 stability

The stability of an ELISA kit is determined by the rate of loss of activity. The loss rate of this batch is less than 5% within the expiration date under appropriate storage conditions.

III. RESULTS AND DISCUSSION

3.1 Animal experiments

3.1.1 Biochemical study

Sirtuin enzymes are known to act as causative agents of Alzheimer's disease and play a crucial role in modulating the immune system. Therefore, we measured the concentration of the enzyme sirtoin,

and found that Alzheimer's was associated with an increase in this pro-disease marker.

Shown in Table (3-8) are the results of the biochemical tests that were estimated in the blood serum of laboratory animals, which included estimating indicators associated with the progression of induced Alzheimer's and their response to treatment with extracts prepared from the ruta plant. The results showed a significant effect of treatment with ruta plant extracts on the biochemical variables studied.

It is noted that with regard to the sirtuin enzyme - the group of infected and untreated animals recorded the lowest averages, which amounted to 0.313 ± 4.420 U/L, and that the extracts of the ruta plant led to raising the concentration of this enzyme to good levels, with a significant difference from the infected group, and the flavonoid extract was the most efficient by recording it. The lowest average of the enzyme among the infected groups amounted to 0.564 ± 8.540 U/L, while the healthy group recorded a significantly higher concentration than the untreated infected group, which amounted to 0.578 ± 9.384 U/L table (5).

Table (5): Estimation of the concentration of the sirtuin enzyme for experimental animals with induced Alzheimer's disease and treated with extracts of the ruta plant:

Average ± standard deviation SIRT3 U/L	(groups)
*0.578 ± 9.384	control (proper)
*0.313 ± 4.420	Mice with induced Alzheimer's disease (untreated)
*0.593 ± 6.282	Mice with induced Alzheimer's treated with the oil extract
*0.564 ± 8.540	Mice with induced Alzheimer's disease treated with flavonoids
*0.717 ± 7.284	Mice with induced Alzheimer's disease treated with alkaloids

* Indicates that there is a significant difference at the probability level ($p<0.05$) between the control group and the other groups separately.

** It indicates that there is no significant difference at the probability level ($p<0.05$).

The table (6) showed that the concentration of the enzyme decreased from 12.09 for the control group to 2.40 for the patient group in general. When comparing control females with female patients, we find that there is a clear significant difference in the concentration of the enzyme, as its concentration decreased from 10.19 to 1.68, respectively, and we notice this decrease. Also, when comparing control males with sick males, the enzyme concentration decreased from 13.91 to 3.15.

It is worth noting that there are many factors that lead to a significant decrease in the concentration of the sirtuin enzyme, including changes in gender and age. We also note from the table that changing gender had a clear effect on the concentration of the sirtuin enzyme, as its concentration was 1.62 for female patients, then rising to 3.15 for male patients. As for the effect of the age factor, the concentration of the sirtuin enzyme decreased significantly, as its concentration in the control group was approximately 12.15, then decreased to 2.44 for the patient group.

the group of untreated mice with Alzheimer's showed a significant increase in the activity level of the SIRT3 enzyme (0.319±2.016 U/L) compared to the healthy control group (0.069±1.23 U/L). This is consistent with the results showed that there was a significant

increase in the activity of the SIRT3 enzyme in rats in which Alzheimer's disease was induced.

The reason for the decrease in the effectiveness of the SIRT3 enzyme in groups treated with natural products of the Rota plant is due to the fact that these products contain active substances that work in different ways to reduce the effectiveness of the enzyme, each according to a different mechanism. For example, the effect of flavonoids may be attributed to their containment of quercetin, which was determined using HPLC technology, and this substance plays a role in. It plays an important role in reducing the effect of free radicals that attack the synovial wall of the joint. It also plays an important role in treating Alzheimer's by inhibiting the SIRT3 enzyme in an indirect way, which is by inhibiting the inflammatory cytokines that stimulate the secretion of the SIRT3 enzyme. Quercetin helps in treating Alzheimer's through Preventing the formation of what is known as synovial pannus, which is a characteristic of this injury.

The results shown in Table (3-1) showed a significant decrease in the level of SIRT3 in the serum of individuals diagnosed with PD (2.38 ± 0.070) compared to the level of SIRT3 in the control group (7.61 ± 0.076), with a probability level of ($P<0.05$).). This decrease can be attributed to mitochondrial dysfunction and poor response to oxidative stress, as it is believed that the decrease in sirtuin SIRT3 levels contributes to causing an imbalance in cellular energy metabolism and increasing susceptibility to oxidative stress.

Since Alzheimer's disease is more prevalent among older adults, age-related decline in SIRT3 may play a role in its decreased incidence in patients with this condition and a decline in SIRT3 levels may also be linked to dysfunctional cellular signaling. SIRT3 plays a critical role in modulating multiple cellular signaling pathways that influence cellular metabolism, stress response, and human lifespan.

Table (6) Effect all parameters on the concentrations of the Sirtuin3 enzyme

Group of patients (45)		Control group (45)	
Standard deviation	the average	Standard deviation	the average
0.79	2.40	1.96	12.09
Patients group Female (22)		Control group Female (22)	
Standard	the average	Standard deviation	the average
0.17	1.62	0.52	10.19
Patient group Male (23)		Control group Male (23)	
Standard	the average	Standard deviation	the average
0.20	3.15	0.55	13.91
Patient group Male (23)		Patient group Female (22)	
Standard	the average	Standard deviation	the average
0.20	3.16	0.17	1.62
Patients group: Ages (59-70) years		Control group: Ages (59-70) years	
Standard	the average	Standard deviation	the average
0.77	2.44	1.96	12.15

IV. CONCLUSION

The group of untreated mice with Alzheimer's disease shows a significant increase in the activity level of the SIRT3 enzyme (0.319±2.016 U/L) compared to the healthy control group (0.069±1.23 U/L). This is consistent with the results that showed that there was a significant increase in the activity of the enzyme. SIRT3 in rats induced to develop Alzheimer's. The flavonoid extract was the most efficient, recording the lowest average enzyme among the infected groups, which amounted to 0.564 ± 8.540 U/L, while the healthy group recorded a significantly higher concentration than the untreated infected group, which amounted to 0.578 ± 9.384 U/L.

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