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Research Article

EFFECT OF ORAL ADMINISTRATION OF ETHYL ACETATE EXTRACT OF *ROSMARINUS OFFICINALIS* L. ON SERUM ENZYMES IN RATS AND EVALUATION OF α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITY

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Abstract

Background: Medicines made from herbs and other natural ingredients were utilised in traditional treatments. In the past few decades, researchers have placed a greater emphasis on herbs in the process of medication discovery due to the fact that herbs have fewer issues and fewer adverse effects. There has been a rise in the number of medical and pharmacological studies conducted all over the world in response to the evolving need. Extracts of rosemary, also known as *Rosmarinus officinalis* L. (Labiatae), have already been used in general traditional medicine to treat a well-known and diverse group of ailments, including serious ones, such as headaches, dysmenorrhea, epilepsy, rheumatic pains, all convulsions, annoying nervous excitations, and improving Memory function, depression and its side effects, and physical and mental exhaustion. **Methods:** After being dried and powdered to a fine consistency, samples of the aerial portion of the leaves of *Rosmarinus officinalis* were obtained. A brown residue was obtained by filtering the resultant solution and then evaporating it until it was completely dry under reduced pressure. This resulted in a brown residue based on the dry weight. An FTIR investigation was conducted on *Rosmarinus officinalis*, focusing on its inhibitory action against α-amylase and α-glucosidase. Experiments were carried out with albino rats weighing between 200 and 250 grammes, which is equivalent to 5 grammes. It was the animal breeding house that provided the animals for the project. **Results:** Experimental testing was conducted in vitro on laboratory rats to examine the effect of oral administration of *Rosmarinus officinalis* extract on the blood enzymes SGPT, SGOT, and ALP. The results of these tests were recorded 82.49±4.15, 96.80±5.02 and 30.16±2.19 respectively for *Rosmarinus officinalis* ethyl acetate extract while 132.08±7.05, 160.12±8.11 and 43.01±2.33 were recorded respectively for using Di-(2- ethylhexyl) phthalate and 54.21±3.79, 74.52±2.62 and 22.09±1.73 were recorded to Control (vehicle) (0.5 ml/kg Corn oil). **Conclusion:** In this particular research project, the findings that we have gathered demonstrate that various extracts of rosemary possess beneficial anti-inflammatory and anti-diabetic properties. This possible anti-inflammatory effect is most likely attributable to the presence of polyphenolic chemicals, which have the potential to offer numerous advantages in the treatment of oxidative stress and disorders associated to diabetes.

Keywords: Anti-inflammatory, Oral Administration, *Rosmarinus officinalis* L., Serum, α-amylase andα-glucosidase,

INTRODUCTION

It is clear that there are many well-known societies around the world that have used medicinal plants as products, and they are very important in treating diseases that affect humans, some of which are serious, and animals. Currently they have become the subject of much investigation because, compared to pharmaceutical drugs, they have practically much fewer adverse effects and more benefits [1]. As a clean and free treatment, it can also be used to speed up the progression of the treatment process. *Rosmarinus officinalis* L. is a well-known aromatic evergreen plant that is a member of the Lamiaceae family. It is characterized by its erect stems, its flowers having a distinct whitish-blue colour, and its leaves having a dark green colour. Rosemary is the common name for this plant, and it is known that its native habitat is countries located in the Mediterranean regions. It must be noted that the most important part of the plant is the leaves, which can actually be used as a spice, for example, or to produce the well-known herbal tea. Hence, both fresh and dried leaves are useful and acceptable for use. It has been shown through published studies that the most important active elements in this rosemary plant are phenolic diterpenes, triterpenes, and phenolic acids. On the other hand, the known chemical composition of rosemary sometimes varies depending on the extract used [2].

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Carnosic acid and rosmarinic acid have been identified as the phenolic compounds that have actually been shown to have the most significant and realistic therapeutic effects. It has already been proven that these compounds actually possess antioxidant, anti-inflammatory and antibacterial properties [3]. The use of specific well-known and studied solvents and traditional processes also allows the extraction of medicinal plant extracts from a variety of plant parts: stems, roots, leaves, flowers, as well as fruits and seeds. Hence, established qualitative and quantitative investigations carried out in the laboratory on active and biologically active chemicals originally isolated from the studied plants depend on the real choice of the laboratory extraction process [4]. The aerial parts of *Rosmarinus officinalis* have already been used in a variety of international cultures for the purpose of preserving food and also serving as an important flavoring agent in a variety of food products, including cosmetics and natural beverages. A number of distinct therapeutic physical and chemical properties have already been documented to be associated with it. The most important of these properties are that it lowers blood sugar, actually lowers blood pressure, lowers cholesterol, is anti-inflammatory, is liver-protective, anti-depressant and anti-bacterial. It has also been experimentally proven to reduce symptoms of asthma, renal colic, and fatigue, both physically and mentally [5]. Diabetes mellitus is a chronic endocrine disease that can be caused by either an absolute or relative insulin secretion shortage, insulin resistance, or both. It has become a major and growing public health threat all over

the world. There are still certain limits in terms of efficacy and safety that are associated with clinical antidiabetic medicines at the present time. These limitations include gastrointestinal side effects, hypoglycemia, or weight gain. In addition to its use as a food additive and medicine, *Rosmarinus officinalis* is an aromatic evergreen plant that has been extensively utilised for the treatment of hyperglycemia [6]. A significant amount of pharmacological research demonstrated that rosemary extract and its phenolic constituents, particularly carnosic acid, rosmarinic acid, have the potential to significantly improve diabetes mellitus by regulating glucose metabolism, lipid metabolism, anti-inflammatory, and anti-oxidation processes. This demonstrates that rosemary extract has an extremely high research value.

Diseases that are characterised by inflammation are commonly recognised as the primary contributor to morbidity among people all over the world. Chronic rheumatoid arthritis, multiple sclerosis, known inflammatory bowel disease, known autoimmune inflammatory diseases, and serious neoplastic changes are diseases that can develop rapidly as a result of inflammation that is not already well managed. In addition to this, chronic inflammation is linked to several phases of carcinogenesis, which makes it a potential risk factor for the development of specific forms of cancer [7]. There is a tendency for chronic disorders to present itself as persistent low-grade inflammation. Given the dearth of drugs that are both safe and effective, treatment continues to be a struggle for several of these conditions. In the laboratory, many laboratory animal models have been developed in order to examine the therapeutic anti-inflammatory properties of various drugs used. This work is a real response to the challenges already faced in the process of identifying safe, important and effective therapeutic alternatives in controlling pathological inflammation. Here we must mention that the process of selecting this laboratory animal model suitable for the laboratory experiment is a clear problem that must actually be overcome to complete this research project. This is very necessary to determine the level of in vitro effectiveness of the drug actually used and to actually translate its therapeutic properties to humans [8]. Although there are a very large number of inflammation models that have already been studied in vivo and have already been established in order to investigate the pathological anti-inflammatory potential of therapeutics used in vitro, it is necessary to choose the most appropriate animal model for in vitro study. The purpose of this study was to evaluate in vitro the inhibitory activity of α-amylase and α-glucosidase, as well as to analyse the potential anti-inflammatory benefits shown by *Rosmarinus officinalis* in preclinical models of inflammation in vivo.

MATERIALS AND METHODS

Producing the Ethanol Extracts and the Plant Material Used in the Process

Laboratory samples were collected from the leaves of the upper aerial part of the *Rosmarinus officinalis* plant, after which they were dried and ground well. One hundred grammes of *Rosmarinus officinalis* was extracted with ethanol at room temperature for a period of two days. A brown residue was obtained by filtering the resultant solution and then evaporating it until it was completely dry under reduced pressure. This resulted in a brown residue based on the dry weight.

An examination of *Rosmarinus officinalis* using Fourier transform infrared spectroscopy (FTIR)

The data from an FTIR instrument (Model/Make: IFS 25, Bruker, Germany) was operated and processed using a PC-based programme in order to capture the FTIR spectra of both native and defatted GLVs. This was done in order to record the FTIR spectra of both types of GLVs. In order to get ready for FTIR analysis, a tiny quantity of powdered leaf samples was transformed into pellets by using KBr, and a thin film was created by applying pressure to the mixture. In order to complete the process of collecting information about the transmittance of infrared light in the laboratory, the required data were collected in a specific wave number range including 4000 cm−1 to 500 cm−1. At this time, all three samples studied were subjected to separate tests, with untreated KBr pellets serving as a control during this study [9]. The laboratory-studied spectral data and the standard reference were actually compared to determine which functional groups were actually present in the sample.

In Vitro Assay for Alpha-amylase Inhibition

In the laboratory, the usual practical methodology was used to evaluate the α-amylase inhibitory activity of the used extract and fractions [12]. It must be mentioned that there were some minor laboratory modifications that had already been made to the methodology. In the laboratory, twenty liters of extract and parts with different concentrations of 0.5 milligrams per milliliter were combined with fifty liters of laboratory phosphate solution containing a concentration of 100 mM and a pH of 6.8. Ten actual liters of alpha-amylase at a calculated concentration of two units per milliliter were also included in the mixture used. After transferring it to a 96-well plate, in the meantime, the mixture was subjected to a pre-incubation period for twenty minutes at a temperature of 37°C. For the purpose of performing the pre-incubation procedure, the temperature was already maintained at 37°C. After that, a substrate consisting of twenty liters of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added to the mixture.

The laboratory mixture is then reintroduced to the incubator for thirty minutes at a temperature of 37 degrees Celsius. After adding one hundred liters of DNS color reagent, we actually heated the liquid for 10 minutes while maintaining a constant and calculated pressure. Through the use of the Multiplate Reader (Multiska Thermo Scientific, version 1.00.40), a laboratory absorbance reading was already obtained at a wavelength of 540 nm. The measurement was performed laboratory-based so that the absorbance of the resulting mixture could actually be determined. The use of acarbose in concentrations ranging from 0.1 to 0.5 mg/ml was employed so that it could serve as a standard. For the purpose of serving as a control, a substance that had not been subjected to any experimental methods (extracts and fractions) was created simultaneously but in parallel. In addition, each experiment was repeated three times to ensure accuracy.

α-Glucosidase Inhibitory Assay

In order to ascertain the amount of α-glucosidase inhibitory activity exhibited by the extract and fractions, an analysis was administered. The analysis was carried out by employing the conventional procedure, with a few minor adjustments [13]. Preincubation of a reaction mixture was performed at 37

degrees Celsius for fifteen minutes in a plate with 96 wells containing the mixture. There were fifty litres of phosphate buffer with a concentration of one hundred millimolar and a pH of six.8; ten litres of alpha-glucosidase with a concentration of one unit per millilitre; and twenty litres of various extracts and fractions with a concentration of half a milligramme per millilitre. All of these components were included in the reaction mixture. For the preincubation, the temperature was maintained at 37 degrees Celsius throughout the process. With the assistance of a multiplate reader and a wavelength of 405 nm, this test was carried out in order to carry out an actual evaluation of the absorption of freshly released nitrophenol. In the meantime, acarbose was discovered in the sample that was being tested at a concentration of 0.5 mg/mL, and at the same time, it was utilised as a standard measurement.

Experimentation on Real Animals

The males that participated in the studies were albino rats that were in good health and weighed between 200 and 250 grammes. Polypropylene cages were used to house them [10], and they were provided with the standard laboratory diet. Additionally, the temperature in the room was maintained at a controlled level of 23 ± 2 degrees Celsius. Before beginning the studies, the rats were allowed to become accustomed to their environment in the laboratory for a minimum of eight hours.

Drug administration and the use of animals

Experiments were carried out with albino rats weighing between 200 and 250 grammes, which is equivalent to 5 grammes. They came from the animal breeding home, which was the source of the creatures. They were kept in cages that had enough ventilation, and they were kept in regular climatic conditions (23±3 degrees Celsius, 55-70% relative humidity, 12 hours of darkness and light cycle). Additionally, they were given conventional rat feed. The rats numbered twelve and were separated into three groups, each consisting of four animals. To act as a normal control, animals belonging to group I were given maize oil, which was then employed as a vehicle control. In every single trial that involved Apium graveolens methanol fraction, the positive controls consisted of 100 mg/kg of Di-(2- ethylhexyl) phthalate when it was administered. Group 1 was composed of Di-(2-ethylhexyl) phthalate at a dose of 100 mg/kg, whereas Groups 2 and 3 were composed of fractions of *Rosmarinus officinalis* at concentrations of 0.50 mL/kg and 0.75 mL/kg, respectively.

Statistical analysis

In the laboratory, the statistical package GraphPad Prism 5 (GraphPad Software, USA) was used to complete various statistical analysis procedures. One-way analysis of variance (ANOVA) was actually used to analyze the studied data, and at the same time a Bonferroni test was performed on the calculated results. For triplicate determinations, in vitro IC50 results were represented as the mean plus or minus the standard error of the arithmetic mean. Indeed, free radical scavenging activities were indicated as a percentage, and at the same time the quantification of the studied phytochemicals was expressed as an average plus or minus the standard deviation actually associated with them. For in vitro statistical significance, a p value of less than 0.05 was assessed.

RESULTS

Since ancient times, rosemary, also known as *Rosmarinus officinalis* L., has been utilised as a herbal medicine for the treatment of a wide range of chronic ailments all over the world. As a result of its anti-hyperlipidemic and anti-hyperglycemic properties, rosemary has recently been proven to have the potential to be used in the treatment of obesity and diabetes mellitus. Peak (Wave number cm-^{1}), (Type of Intensity, Bond and Functional group assignment) were 960.55, (Strong, =C–H, Alkenes), 1029.99 (Strong, C-F, alkyl halides), 1097.50 (Strong, C-F, alkyl halides), 1141.86 (Strong, C-F, alkyl halides), 1321.24 1373.32 (Strong, C-F, alkyl halides), 1373.32 (Strong, C-F, alkyl halides), 1456.26 (Medium, C=C, Aromatic), 1616.35 (Bending, N-H, Amide), 1723.65 (Strong, C=O, Aldehyde), 2852.72 (Strong, C-H, Alkane), 2922.16 (Strong, C-H, Alkane). Experimental testing was conducted in vitro on laboratory rats to examine the effect of oral administration of *Rosmarinus officinalis* extract on the blood enzymes SGPT, SGOT, and ALP. The results of these tests were recorded 82.49±4.15, 96.80±5.02 and 30.16±2.19 respectively for *Rosmarinus officinalis* ethyl acetate extract while 132.08±7.05, 160.12±8.11 and 43.01±2.33 were recorded respectively for using Di-(2- ethylhexyl) phthalate and 54.21±3.79, 74.52±2.62 and 22.09±1.73 were recorded to Control (vehicle) (0.5 ml/kg Corn oil) Figure 2, 3, 4. Both the ethyl acetate and methanol fractions derived from *Rosmarinus officinalis* L. exhibit inhibitory efficacy against α-amylase and α-glucosidase, as depicted in Figures 1 and 2, respectively.

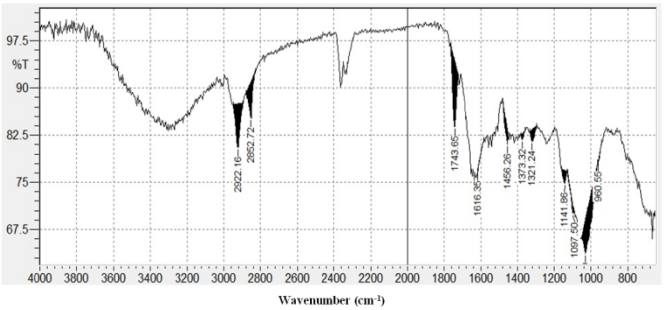

Figure 1.Fourier-transform infrared spectroscopic profile solid analysis of *Rosmarinus officinalis* L

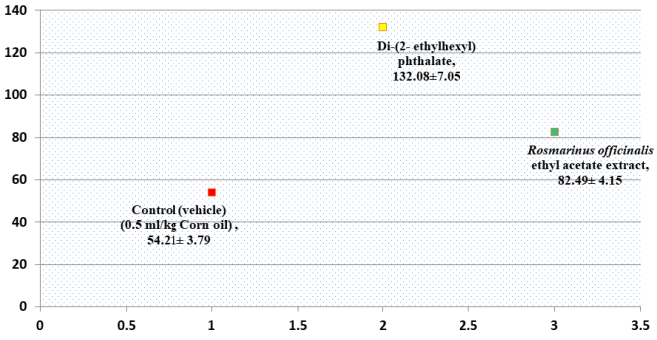

Figure 2. Effect of oral administration of bioactive secondary metabolites of *Rosmarinus officinalis* (ethyl acetate extract) , Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes SGPT

According to the findings of the enzymatic inhibitor assay, the inhibition activities of the *Rosmarinus officinalis* L. fractions against α-amylase and α-glucosidase were found to be dependent on both the dose and the fraction. The highest dose investigated exhibited a significant level of inhibition, while the lowest dose exhibited the least amount of inhibition.

Table 1. Fourier-transform infrared spectroscopic profile solid analysis of *Rosmarinus officinalis* L.

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	960.55	78.231	0.287	962.48	941.26	2.086	0.024	Strong	=C–H	Bending	Alkenes	650-1000
2.	1029.99	63.707	5.480	1051.20	964.41	14.080	1.756	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1097.50	70.089	1.063	1128.36	1093.64	4.754	0.073	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1141.86	74. 848	2.020	1159.22	1130.29	3.482	0.181	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1321.24	81.543	1.842	1332.81	1296.16	3.080	0.224	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1373.32	81.727	1.028	1381.03	1357.89	1.941	0.060	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1456.26	81.044	2.502	1481.33	1450.47	2.360	0.201	Medium	C=C	Stretch	Aromatic	1400-1600
8.	1616.35	76.701	0.352	1618.28	1583.56	3.496	0.015	Bending	N-H	Stretch	Amide	1550-1640
9.	1723.65	83.817	10.556	1764.87	1722.43	2.176	1.080	Strong	C=O	Stretch	Aldehyde	1720-1740
10.	2852.72	85.206	5.797	2877.79	2827.64	2.625	0.584	Strong	C-H	Stretch	Alkane	2850-3000
11.	2922.16	80.517	6.985	2947.23	2897.08	3.840	0.933	Strong	C-H	Stretch	Alkane	2850-3000

According to the type of extract (Ethanol fraction, Ethyl acetate fraction, Water fraction and Acarbose as standard) recorded (35.47 ± 2.39, 60.42 ± 4.96, 72.30 ± 5.16, and 30.25 ± 1.94) respectively inhibitory potency against α-amylase. While recorded (50.14 ± 3.61, 32.13 ± 2.10, 25.53 ± 1.07, and 14.16 ± 0.13) respectively inhibitory potency against α-glucosidase activity Figure 5 and 6.

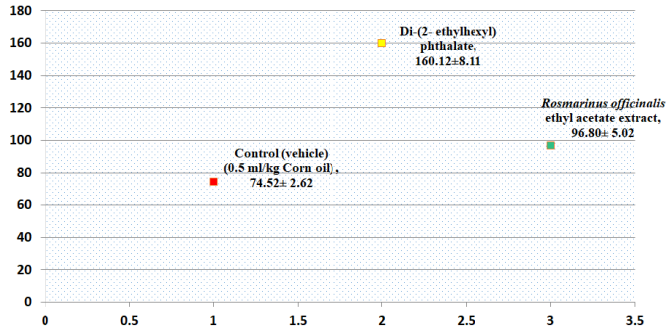

Figure 3. Effect of oral administration of bioactive secondary metabolites of *Rosmarinus officinalis* (ethyl acetate extract) , Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes SGOT

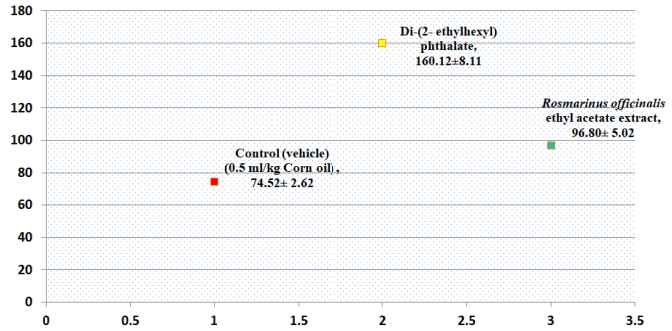

Figure 3. Effect of oral administration of bioactive secondary metabolites of *Rosmarinus officinalis* (ethyl acetate extract) , Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes SGOT

The apparent antidiabetic effect of rosemary can actually be attributed to a number of different mechanisms, including a rise in insulin levels, activation of B cells in the pancreas, or inhibition of the intestinal amylase enzyme from working at all. Hence, we know that the proposed method by which the natural plant extract mediates its antidiabetic effect is by increasing the pancreatic secretion of insulin from the remaining cells already present in the islets and accelerating the process of glucose transit into the blood [11].

This is one of the most important mechanisms that can be imagined. According to the results of this study, the ethanolic extract of R. officinalis showed clearly effective hypoglycemic activity, which actually included an apparent significant reduction in blood glucose levels.

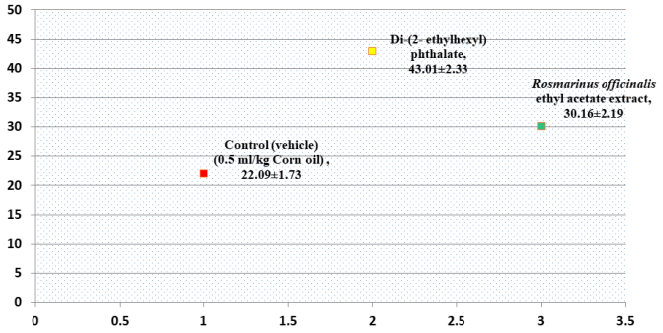

Figure 4. Effect of oral administration of bioactive secondary metabolites of *Rosmarinus officinalis* (ethyl acetate extract) , Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes ALP

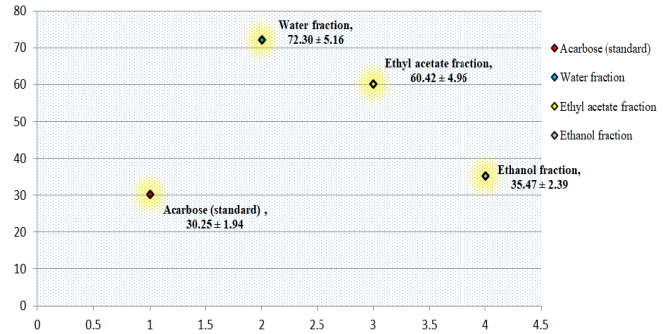

Figure 5. IC50 values of α-amylase inhibition by *Rosmarinus officinalis* L. (Ethyl acetate fraction, Ethanol fraction, Water fraction, and Acarbose (standard)) fractions.

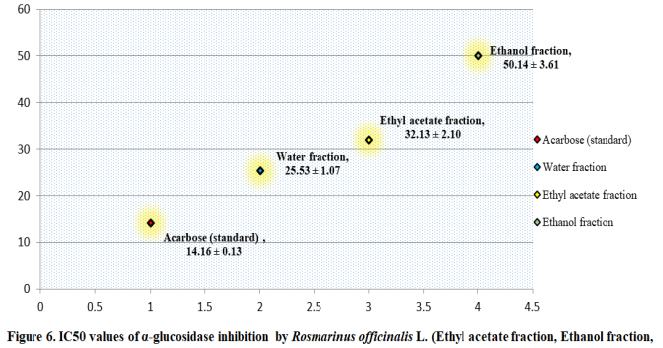

Figure 6. IC50 values of α-glucosidase inhibition by *Rosmarinus officinalis* L. (Ethyl acetate fraction, Ethanol fraction, .Water fraction, and Acarbose (standard)) fractions

It has been laboratory proven that the presence of natural and effective insulinotropic compounds in the plant extract actually protects the remaining beta cells in the pancreas from further degeneration and damage and also stimulates beta cell regeneration [12]. The anti-inflammatory activity of natural compounds extracted from R. officinalis extract significantly protects against the harmful effects of alloxan on beta cells as well as the pathogenic effects of oxidative stress on various known diabetic problems [13, 14]. Furthermore, these compounds also prevent the development of diabetic complications. An instantaneous onset, a rapid increase in intensity over a short period of time, and the possibility that symptoms will continue for a few days are all characteristics of acute inflammation in humans. Inflammation that is subacute is the time that occurs between acute and chronic inflammation and can last anywhere from two to six weeks. For this reason, chronic inflammation is defined as inflammation that continues for a period of time that is greater than six weeks [15]. Another characteristic of chronic inflammation is that it is characterised by a gradual and persistent inflammation that lasts for extended periods of time, ranging from several months to years.

Conclusion

Even though the powerful anti-inflammatory activities of Rosemary, *Rosmarinus officinalis* extract have been well recognised in this study, there is still a need for additional trials that are dependable in the future. In this particular research project, the findings that we have gathered demonstrate that various extracts of rosemary possess beneficial anti-inflammatory and anti-diabetic properties. There is a high probability that the presence of polyphenolic chemicals, which may have numerous advantages in the treatment of oxidative stress and disorders associated to diabetes, is responsible for this anti-inflammatory potential. In order to effectively manage a variety of pathological illnesses, it is essential to conduct additional research on the safety and effectiveness of *Rosmarinus officinalis* and its primary active components.

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