Insulin Autoantibodies (IAA) and the effect of royal jelly on Type 1 diabetic patients.

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A thesis submitted for the fulfilment of the requirements for Master degree in Zoology.

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May -2005

Dedication

To

SAJA

Parents

Brothers

AKNOWLEDGMENT

All thanks to **Allah** for his mercy, and beneficeny. Without his help, nothing can be accomplished.

I would like to express my sincere thanks and gratitude to my supervisor professor **Elmahdi Mohammed Ali Elmahdi,** Physician of Endocrinology, Department of Medicine, Faculty of Medicine, University of Khartoum, for his excellent supervision, keen guidance, and his good solving for the problems of this work.

I am greatly indebted to my Co-supervisor Dr. **Sadia Ahmed Younis**, Department of Zoology Faculty of Science, University of Khartoum, for her unlimited help, valuable advices and for providing her time especially during the practical of this study.

I would like to express my thanks to Professor **Siddig Ahmed Siddig**, Faculty of Science, Department of Zoology, University of Khartoum, and Miss **Notiela Mustafa** for their helping in the analysis of data.

Thanks also due to Professor **Mohammed Saeed Alsaraj**, Faculty of Agriculture, University of Khartoum, for his good suggestions during this work.

I would like to express my great thank for **Dr. Yusra A.Athem**, and Miss **Thoria M.A.Alhassan**, Department of

Biochemistry, Faculty of Medicine, University of Khartoum, for their good encouragement during this study.

My thanks also to the **International Center for Faith Research** for their supporting and funding.

My thanks are extended to all **staff,** and **patients** participated in this study, from Jaber-Abu Aleze Diabetic Center in Khartoum for their cooperation.

My deepest thank to my **family** and all my **friends** for their help, and support during the study.

Abstract

The main objective of measurement of insulin autoantibodies (IAA) in this study is to identify the level of these autoantibodies in patients under good insulin metabolic control and those under poor insulin metabolic control.

Results revealed that most of them (group B2) were under poor insulin metabolic control 64.3% patients compared to 35.7 % patients (B1) who were under good insulin metabolic control. The difference was statistically significant (p =0.00)

The second objective was to assess the effect of a daily dose of royal jelly for a period of 3 months on diabetic patients. The reference tests for diabetes mellitus were applied and that by detecting glucose level in blood and urine on fasting and postprandial fortnightly in two groups namely group A (control) and group B (study).

Results showed no significant difference between 2 groups with respect to these tests. Mean fasting plasma glucose level in group B which received royal jelly was 216.83± 63.20mg/dl compared to 276.94± 62.30mg/dl for group A(p =0.00).

Results of mean postprandial glucose level was significantly different for the study group (p = 0.00).

Similarly fasting urine glucose level for the study group was significantly different compared to control group (p=0.001).

ملخص الدراسة

في هذه الدراسة كان الهدف من قياس الاجسام المضادة الذاتية للانسولينIAA هو معرفة نسبة هذه الاجسام المضادة الذاتية لدى مجموعتين من مرضى السكري السودانيين المصابين بالمرض من النوع الاول.المجموعة الاولى كانت تحت درجة عالية من التحكم في المرض،والمجموعة الثانية ذات درجة تحكم ضعيفة. أظهر قياس هذه الاجسام المضادة الذاتيةIAA فرق معنوى كبير بين المجموعتين المشاركتين في الدراسة . اظهرت النتائج نسبة 35.7% من المرضى ذوي التحكم الجيد في المرض انخفاض ملحوظ في مستوى الاجسام المضادة الذاتية للانسولين في دمهم.في حين كان حوالى 64.3% من ذوي التحكم الضعيف اظهروا درجة ارتفاع عالية من هذه الاجسام المضادة الذاتية (p = 0.00).

هدفت الدراسة الثانية لمعرفة تأثير تناول غذاء ملكات النحل على مرضى السكري من النوع الاول.وذلك عند تناوله لمدة ثلاثة اشهر متواصلة .

ستة وثمانون مريضا(86) شاركوا في الدراسة ،ثلاثون(30) منهم اعتبروا كعينات ضبط وتحكم(لم يتناولو غذاء الملكات)ستة وخمسون تناولوا غذاء الملكات لمدة ثلاثة اشهر متواصلة.

في الدراسة الثانية كان هناك تأثير واضح لغذاء ملكات النحل بين عينات الضبط والمراقبة (متوسط قراءات لعينات الصيام حوالى (276.94 mg/dl± 62.30) في حين كان متوسط قراءات مجموعات الدراسة (216.83 mg/dl± 63.20) بعد تناولهم لغذاء الملكات.

كان هناك قرق معنوي عالى في نتائج تحليل عينات البول فى حالة الصيام وساعتين بعد الوجبة بين عينات الضبط ومجموعات الدراسة.

جمعت عينات الدم والبول من المرضى داخل مركز جابر ابو العز التخصصي لمرضى السكري في مدينة الخرطوم.

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CHAPTER ONE

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which there is an inability to metabolize carbohydrates due to disturbances in insulin reaction. This causes a blood glucose level above normal, with a fasting plasma level of 126 mg/dl or more, and a level of 200 mg/dl at 2 hours after glucose load (Peakman and Vergani, 1997). Insulin-dependent diabetes mellitus (IDDM) or type I diabetes is an autoimmune disease in which insulin deficiency is a consequence of immunological destruction of the pancreatic beta cells (Eisenbarth, *et. al.,* 1987).

People affected with type I diabetes mellitus produce antibodies to their own beta cell's, this happens years before the onset of symptoms , thus such autoantibodies represent the most important markers to identify individuals who are at increased risk for developing this condition.

Other important predictive markers for type I Diabetes mellitus are:

(1) Autoantibodies against glutamic acid decarboxylase (GAD-65) (DeAizpuria, *et. al*., 1992).

(2) Autoantibodies against Insulin (IAA) (Christie, *et. al*., 1994).

(3) Cytoplasmic islet cell Autoantibodies (ICA) (Verge, *et. al*., 1996).

4) Autoantibodies to tyrosine phosphate (IA-2).

These autoantigens are available as recombinant molecules; a progress that facilitated the development of highly sensitive assays for autoantibodies against these autoantigens.

Royal jelly has been used since a long time ago, and is still being used by many researchers to treat various diseases (Mahdi, 2000).In both type 1 and type 2 diabetes royal jelly decrease the plasma glucose level upon a daily dose over along period of time, however the mechanism is yet unclear(Krell,1996) The medical or pseudo-medicinal use of royal jelly is much more popular in Asia and Eastern Europe, where rules on medicinal formulations and applications are rather flexible compared to Western Europe and North America. In general royal jelly is one of the main ingredients in some most medical skin preparations and in cosmetics. It can also be provided as capsules, tablets or in injection form (Krell, 1996). In Africa, very little use of royal jelly has been reported, either as a food supplement or as medicine.

Royal jelly is a complex substance that cannot be synthesized by man (Fujiwara, 1990), it is secreted by the hypopharyngeal gland (sometimes called the brood food

gland) of young worker (nurse) bees, to feed young larvae and the adult queen bee and that as soon as it is secreted; i.e. it is not stored. This is why it has not been a traditional beekeeping product. The only situation in which harvesting becomes feasible is during queen rearing, when the larvae destined to become queen bees are supplied with an over abundance of royal jelly. The queen larvae cannot consume the food as fast as it is provided and royal jelly accumulates in the queen cells. (Donadieu, 1980). The principal constituents of royal jelly are water, protein, sugar, lipids and mineral salts (Takenake, 1984). Although these constituents occur with notable variations, the composition of royal jelly remains relatively constant when comparing different colonies, bee races and time of production.

Objectives of the study:

Accordlying the objectives of this work were:

1- To study the effect of royal jelly on type 1 Diabetes mellitus.

2- To estimate the plasma level of IAA on the degree of metabolic control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus:

Diabetes mellitus is a phrase used to describe a condition in which the body fails to sufficiently produce or utilize insulin. It has been known since the nineteenth century.

The Greek scientist Aryatyos was the first to coin the word "diabetes" to describe this condition.

In Greek "Diabetes" means "to flow through", the Latin word mellitus, which means "sweetened" or "honey like", was added later (Al Sukkari magazine, 2001). Symptoms are excess urine excretion, unusual thirst, extreme hunger, and unusual weight loss,but the condition may be symptomless.

Diabetes mellitus is a heterogenous groups of disorders all characterized by increased plasma glucose level, with a minimum fasting plasma glucose level of 126 mg/dl (7.0 mmol per l) or a level of 200 mg/dl 2 hours after glucose load (McCance ,*et. al.*, 1997).

Glucose is converted within the cell into energy, and is used as a major fuel for the body (Pickup; Williams, 1998). It is important that the body has storage forms of glucose (glycogen) to call upon when needed; the body can also break down muscle protein to form glucose under extreme

conditions. Glucose can not enter the cell until the insulin hormone unlocks it, thereby allowing the cell to receive the glucose and burn it for energy (Clark, 1999).

Insulin is produced by beta cells of the pancreas, and it is necessary for both storage and reconversion of glucose (Guyton and Hall,1996).The beta cells were originally identified by Pool Langerhans in 1867 as a type of endocrine glands in the pancreas, later these cells were found to produce insulin(Al Sukkari magazine, 2001).

If beta cells do not make sufficient insulin or the body's cells are resistant to its activity of promoting glucose, or the pancreas is unable to make specific hormones, then this will lead to the development of Diabetes mellitus (Bell, 1996).

Diabetics whose pancreas fail to make sufficient insulin, need insulin injections for survival and without this, they will slowly starve to death despite abnormally high blood glucose levels (Laackso; Pyorala, 1985).

2.2 Types of Diabetes:

In June 1997, an international expert committee released a report with new recommendations for the classification and diagnosis of diabetes mellitus as type 1 diabetes, type 2 diabetes, and other specific types of diabetes (Diabetes care, 1997).

Type 1 is insulin-dependent; type 2 is non insulin-dependent.

There is also gestational Diabetes mellitus which refers to women who develop the condition during pregnancy (National Diabetes data group, 1995).It affects about 4% of pregnancies and such mothers have great risk of developing diabetes in the future.

2.2.1 Type 2 diabetes mellitus:

Type 2 diabetes mellitus is the most common type ,it is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the beta cells (Ganong,2003),where by the pancreas still produces insulin but it is not enough to meet the body's needs, and sometimes the liver and muscle cells are resistant to its action. This type of diabetes is highly associated with family history of diabetes, older age, obesity and lack of exercise and is refered to as noninsulin dependent diabetes mellitus (NIDDM).

2.2.2 Type 1 Diabetes mellitus:

It is the most severe type of diabetes leading to life long dependence on daily dosage of insulin (IDDM). Type 1 diabetes mellitus (Previously known as juvenile Diabetes) represents approximately 5-10% of Diabetes patients. Type 1 Diabetes is characterized by beta cell destruction caused by an autoimmune process; usually leading to absolute insulin

deficiency (Schranz, 1998).It is generally diagnosed in children and young adults. Over 95 percent of persons with type 1 Diabetes mellitus develop the disease before the age of 25, with an equal incidence in both sexes, but usually there is no age restriction (Schranz, 1998), it can appear at any age.

The period of time to develop type 1 diabetes may take months in young children to years in young adults (Gorsuch, 1981).

In type 1 diabetes the beta cells in the pancreas are unable to make insulin hormone, because the immune system mistakingly recognize these insulin producing cells (beta-cells) as " foreign", and destroys them, this leads to absolute insulin deficiency (McCane, *et. al*., 1995).Unfortunately the clinical onset of type 1 diabetes does not occur until 80% to 90% of these cells have been destroyed (Sepe, 1996). This means that the presence of insulin auto antibodies in the serum of affected patients provide early evidence of autoimmunity to pancreatic insulin-producing beta cells, and the measurement of these autoantibodies can be useful in assisting the physician to predicting the disease,and to proper diagnosis, and management of patients (Bingley, 1997).

Autoantibodies which are directed against other prominent autoantigens of islet cells frequently appear and

can also be detected years before the onset of symptoms (Bottazzo, 1974), (Verge, *et. al.*, 1996).

2.3 Autoantibodies predictive of type 1 diabetes mellitus:

2.3.1 Autoantibodies against glutamic acid decarboxylase (GAD-65):

GAD is an enzyme that is produced primarily by islet cells; it is present in the cytoplasm of the human β-cell. However, GAD is not a β-cell specific antigen and is widely distributed in the cerebellum, ganglion cells and testis (Peakman and vergani, 1997).

The presence of GAD autoantibodies has been shown to be a strong predictive marker for the eventual onset of IDDM, identified in 70% of patients of IDDM onset (Sepe, 1996).It is involved in reactions that ultimately control the release of insulin from secretory granules in the β-cells. Autoantibodies against this islet cell protein were detected in 1982 by Bakkeskov, et, al., However the antigen targeted was identified as GAD in 1990 (Bakkeskov, *et.al*., 1990).

2.3.2 Cytoplasmic islet cell Autoantibodies (ICA):

ICA were first diagnosed in 1974 in IDDM patients with other autoimmunities (Bottazzo, 1974).

ICA present the best validated serological markers for preclinical type 1 diabetes according to Verge *et al*., 1996.Autoantibodies to ICA were found in 90% of newly

diagnosed IDDM patients (Lendrum, *et al*., 1975).ICA consist of a group of antibodies with different specificities directed against β-cell specific components (Dozio et.al., 1994).They decrease soon after clinical onset of diabetes probably due to destruction of β-cell themselves (Dotta and DiMario, 1996).

2.3.3 Autoantibodies to tyrosine phosphate (IA-2):

Autoantibodies to Islet cell tyrosine phosphatase-like protein were found in 50% to 75% of type 1 diabetics prior to disease onset (Christie, *et. al.,* 1993).

2.3.4 Autoantibodies against insulin (IAA):

Insulin autoantibodies (IAA) were the first islet autoantigen to be identified (Palmer, *et. al*., 1989). IAA was studied and found to be of IgG class similar to true insulin autoantibodies from diabetic patients treated with insulin (Naserke, *et. al*., 1999)(Ziegler, *et. al.*, 1989).These were the first islet autoantibodies to be identified. However. Serial autoantibody studies in infants indicated that IAA is the first islet autoantibody to develop during progression to type 1 diabetes and can be detected several years before the onset of the clinical condition. Association between specific Human Leucocytes Antigens and level of IAA have been demonstrated, predisposition is strongly associated with HLA-Q and DR (Chromosome 6) also with the insulin gene on chromosome

11. Also a sex related difference have also been reported, with higher levels in females (Dotta and DiMario, 1996).Age related differences have been suggested with higher values in young individuals (Sepe, 1996).

The role of IAA in the autoimmune mechanism of IDDM was first- suggested by Palmer, etal, in 1983, whereby IAA were found in 50% of newly diagnosed IDDM patients

IAA is more strongly correlated with the age of the patient than the other autoantibodies (Sepe, 1996; Ziegler, *et. al.*, 1989). IAA can be detected in 90-100% of patients less than 5 years of age, whereas the prevalence in children >12 years is about 40% and are almost absent in adult onset of type I diabetes (Christie, *et. al*., 1994).In addition the prevalence of autoantibodies to insulin in untreated patients is much higher than in insulin-treated patients. Therefore autantibodies to insulin should be sought before the administration of exogenous insulin because after 5-7 days of exogenous insulin treatment the level of insulin autoantibodies will fall (Ganong, 2003).

In this study only (IAA) will be considered.

Other important autoantibodies which are specific for various islet cell proteins have been reported such as autoantibodies to proinsulin, carboxypeptidase H, insulin

receptor, and glucose transporter (Sepe; Dotta and DiMario 1996).

2.4 Insulin, and insulin receptors:

Insulin is a polypeptide containing two chains of amino acid linked by disulfide bridges ,with molecular weight of 5808 Dalton, and synthesized in the rough endoplasmic reticulum of the β-cells(Guyton and Hall,1996).

Like other polypeptide hormones and related proteins that enter the endoplasmic reticulum, insulin is synthesized as a part of larger prehormone.

The half- life of insulin in circulation in the human is a bout 5 minutes (Ganong, 2003).

The physiologic effects of insulin are far-reaching and complex; the best known is the hypoglycaemic effect, but there are additional effects on amino acid and electrolyte transport, and on many enzymes, and on growth. The net effect of this hormone is storage of carbohydrate, protein and fat. Therefore, insulin is a appropriately called the "hormone of abundance" (Ganong, 2003).

Human insulin produced in bacteria by recombinant DNA technology is now widely used.

The gene for the insulin receptor in humans is located on chromosome 19. The insulin receptor is made up of two α

and two β glycoprotein subunits (340KD) bound to each other by disulfide bonds (Ganong, 2003).

Insulin receptors are found on many different cells in the body, including cells in which insulin does not increase glucose uptake.

When insulin binds to its receptors, they aggregate in patches and are taken into the cell by receptor mediated endocytosis, eventually, the insulin-receptor complexes enter lysosomes, where the receptors are broken down or recycled. The half-life of the insulin receptor is about 7 hours.

2.5 Pathological consequences of diabetes mellitus:

2.5.1 Diabetes Retinopathy:

The pathological changes in the retina are of two kinds, namely background retinopathy and proliferative. Retinopathy the former consists of changes which appear early in the disease and is represented by microhemorhages, increase in capillary permeability of retinal tissue and retinal edema. The condition worsen as the disease progresses, the high serum glucose levels are maintained. Proliferative retinopathy involve the development and proliferation of new blood vessels on the surface of the retina also growth of new connective tissue. These changes are followed by hemorrhage and retinal detachment .The condition is dangerous and may

end in blindness depending on age of onset and duration of diabetes (Tang, 1995).

2.5.2 Diabetes nephropathy:

This is the most serious of pathological changes associated with diabetes. Renal failure may appear early in type 2 diabetics but in type 1 it may appear up to 15 years after clinical onset. Renal failure is responsible for about 50% of death among diabetics (Felig, *et.al*., 1987).

Histological changes such as thickening of basement membrane result in increased glomerular permeability which finally results in hypertension and impaired renal function.

2.5.3 Diabetes neuropathy:

This condition which occurs in type 1and type 2 diabetes is manifested by disordered function of peripheral and autonomic nerves. Signs include loss of peripheral sensations, abnormal bladder function, weight loss, vomiting and general unclear pain (Felig, *et al*., 1987).

2.5.4 Diabetes angiopathy:

Significant microvascular complications may develop in some patients of type 1 or type 2 diabetes after many years of disease onset even if they are under good control. Acute macrovascular lesions such as myocardial infraction which are diagnosed very late (Skrhla, 1998).Other microvascular complications due to autoimmunity include immune

mechanism involved in the pathogenesis of cataract.Autoantibodies to lens protens have been detected in 60% in cataract of diabetics (Angunawela, 1987).

2.6 Prevalence of Diabetes mellitus:

Diabetes is becoming increasingly common, especially in developing countries. Recently compiled data (WHO, 2002) show that approximately 150 million people have diabetes mellitus worldwide, and this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary life style.

According to the study done by the ministry of health in Sudan in 1976 for hospital admission all over the country, diabetic patients constituted about 1.0% to 2.5%of the total population. Now it can be estimated from hospital records that the number of diabetic patients is increasing in all socioecononomic classes of the Sudanese population (Elhabeby, 1998).

The majority of these diabetics are in poor glycaemic control. This has been attributed to poor compliance to drugs, poor diet and the problem associated with insulin injection (Elmahdi, *etal*., 1989) Insulin-dependent diabetes mellitus (IDDM) among children 7-14 years of age is not rare in Sudan, and showed a steady rise in prevalence over a 4 years

period (Elamin, *et. al.*, 1989; 1992).According to WHO report(1994)the incidence of IDDM in Sudan was approximately 6 per 100.000 person per year, with poorly controlled diabetes with a minimum of diabetic care. Both acute and long- term complications are common and associated with high mortality rates among children (Elamin, *et. al*., 1992).

2.7 Royal Jelly:

2.7.1 Physical characteristics of Royal Jelly:

Royal Jelly is a homogeneous substance, it is whitish in colour with yellow or beige tinges, has a pungent phenolic odour and characteristic sour flavour, it has a density of approximately 1.1 g/cm^3 (Lercker, *et. al.*, 1992).It is partially soluble in water, it's viscosity varies according to water content and age. It slowly becomes more viscous when stored at room temperature or in a refrigerator at 5°C. Changes in viscosity have also been related to the phenomon which regulates caste differentiation in a bee colony (Takenaka, *et. al.*, 1986).

Certain debris in royal jelly is a sign of purity as, for example, the fragments of larval skin, and wax fragments too.

2.7.2 Chemical Composition and properties:

Investigations on the chemical nature of royal jelly began as early as 1852 (Wetherill, 1852).Now royal jelly is

known to be chemically very complex. 66% of royal jelly is water; but it is rich in other elements.

It is rich in B vitamins; the average contents of the vitamins B1, B2, B3, B6, were 5.6, 6.1, 169.3, 12.3 mg/dl, respectively. Worker jelly samples contained 117.0-128.1ug/g of B3, whereas royal jelly contained 149.1-184.8 ug/g (Sera and Escola, 1991). The B vitamins are very important for the efficient processing of nutrients in the human body (Saenz, 1984).Royal jelly also contains vitamins C and D and E but in low proportions (Sera and Escola, 1991).

It also contains specific fatty acids (Weaver, *et. al.*, 1968), which are responsible for most of the recorded biological properties of royal jelly.

It has also been shown that royal jelly contains organic amino acids such as 10-hydroxydeconoic acid (Blum, *et.al.*, 1959) which exhibits antibiotic activity against many bacteria and fungi.

All amino acids essential for humans are present in royal jelly and a total of 29 amino acids and derivatives have been identified. Proline and lysine were found in the greatest quantities.Arginine; histidine, tryptophan, serine, and cystine were present in moderate quantities (Osman and Ismail, 1977). Takenaka (1984) analyzed eighteen samples of royal

jelly. The mean composition was 66.8% water, 12.3% protein, 11.6% sugar, 5.1% ether-soluble compounds, 1% ash, and other minor components. Fresh royal jelly contained 2% nitrogen, 89% of that was water-soluble. Proteins contributed 73.9% of total nitrogen, free amino acids 2.3% and peptides 0.16%.Five proteins were found in the water-soluble fraction. Among the free amino acids, proline (58% of total) and lysine (15%) were present in the highest concentrations. Carboxylic acids, present in the ether-soluble fraction, constituted 5.8% of royal jelly; of the 13 acids identified 10-hydroxy decanoic acid constituted 13.8% of total acids and water soluble gluconic acid 24.0% .The presence of 3-hydroxyoctanic acid was reported for the first time. The main components of royal jelly also include minerals namely, in order of importance, Potassium , followed by calcium ,sodium ,magnesium whereas zink,coper ,iron, and manganese are present at lowerr concentration(Benfenati,1986).The sugar content of royal jelly consist of fructose, glucose and sucrose(Takenaka,1982).A number of enzymes are also present including glucose oxidase (Nye, *et. al***.,** 1973), phosphatase and cholinesterase (Ammon and Zoch, 1957). Furthermore an insulin-like substance has been identified by Kramer, *et. al***.** (1977; 1982).It has been found that changes in

composition due to long term storage do occur such as higher acid titre, a large unsoluble protein fraction, less free amino acids, and some more (Sasaki, etal., 1987). Kramer et al. (1982) used affinity chromatography for final purification after separation of insulin-like peptides from *Apis mellifera* royal jelly, by acid extraction, ion-exchange chromatography and gel filtration, the quantity detected per gram of royal jelly was equivalent to 25 ug.

2.7.3 Proposed actions of royal jelly:

It has been observed that royal jelly revives and stimulates the function of cells and secretion of glands; it also stimulates the metabolism (Martinetti and Caracristi, 1956), and the circulatory system (Chmidt's, 1956). Royal jelly can be used as a hormone balancer, and can speed up the work of fats. It has been observed that daily doses of royal jelly normalize the cholesterol and triglycerides (Saenz, 1984). Similarly it normalizes a low or high blood pressure (Destrem, 1956).

The antibiotic action of royal jelly has been proven by Lavie, (1968) and Yatsunami and Echigo (1985). Antiviral effects have also been described by Derivici and Petrescu (1965).

2.8 Royal jelly and Diabetes:

Studies on the effect of royal jelly on diabetes are not completely clear.

It has been proven that royal jelly decreases blood glucose level after being used daily for a long time (Mahdi, 2000). However, the mechanism of this is still unknown. It has been observed that royal jelly in long term has contributed to the decrease of glycaemia and lipaemia in a more rapid way than conventional treatments (Saenz, 1984).It has also been shown that royal Jelly in both type 1 and type 2 diabetes mellitus is able to reduce the daily dose of exogenous insulin hormone (Internet site, 2003) although it should never be used as a substitute.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Patients:

The population in this study consisted of eighty six Sudanese type I diabetes mellitus patients diagnosed according to WHO criteria (1985) (Appendix 1). All diabetic patients in this study were using insulin injection twice daily, and all patients had no family history of the disease and had no complications. Age, sex, and the degree of diabetes control was considered.

They were divided in to three groups; group A included 30 diabetic patients who did not receive royal jelly. These acted as a control against group B (56 patients) who received a daily dose of royal jelly for a period of three months.

Group B was further divided into two sub groups: B1 included 20 patients who received royal jelly and were under good insulin metabolic control of diabetes whereas B2 included 36 patients who also received royal jelly, but were under poor insulin metabolic control of the disease. The serum level of IAA was compared in the 2 groups (B1, B2). All patients were regularly visiting the out patients' clinic of Jaber-Abulez Diabetes center in Khartoum. The study group and control group were in the same range of age (5-30 years).

Gender and age were initially recorded in an appropriate form (Appendix).

The appropriate adult patients and parents of children were instructed about the daily use of royal jelly, and were asked to observe and report any changes that may occur. The follow up was maintained every 15 days for a period of 3 months.

3.2 Methods:

3.2.1 Dosage and administration of royal jelly:

Royal jelly was used as powder, in pure form. It was weighed in 200 mg, partions kept in sterile plastic containers, at room temperature. Royal jelly was administered under the tongue in a daily dose taken before breakfast, and that for a period of three months.

3.2.2 Blood and urine collection:

Initially 3 ml of Blood and some urine were collected from every participant to be tested for glucose level. After starting administration of royal jelly blood and urine samples were also collected every 15 days and subsequently tested for glucose level.For insulin autoantibodies(IAA) blood was allowed to clot at room temperature(about 2-3 hrs). The serum was then separated by centrifugation at 1000 rpm for 20 minutes.1 ml of serum obtained from every subject was kept at -20˚C.

3.2.2.1 Method for measurement of glucose level in urine:

Glucose level in uncenrifuged urine was measured using urine strips; the test depends on change in colour, due to glucose –specific glucose oxidase / peroxidase reaction.

The test strip was immersed into urine for a approximately 2 seconds, so that the reagent areas were covered.

Results for semiquantitation were read exactly at 60 sec. after immersion.

The reagent areas on the strip were compared with the corresponding colour chart on the container.

3.2.2.2 Measurement of glucose level in blood Plasma on fasting and two hours post prandial (Varely, 1980):

Venous blood samples were collected in vials containing antiglycolytic/anticoagulant mixture (fluoride/EDTA).The samples were centrifuged, plasma collected, and then glucose level was measured by the auto analyzer oxidase method, which depends on colour change.

In this method glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide produced was reacted in the presence of peroxidase with 4- aminoantipyrine and p- hydoxybenzene

sulphate to form a dye (a quinonimine).The intensity of the dye (colour) produced was measured spectrophotometrically at 520 nm.

Glucose concentration was calculated according to the following equation:

Glucose concentration in mg/dl =

$$\frac{\text{Sample-blank}}{\text{Standard-blank}} \times \text{Concentration of standard in mg/dl.}$$

3.2.2.3 Measurement of insulin autoantibodies (IAA) by the enzyme- linked immunosorbent assay (ELISA):

The isletest IAA kit (Biomerica, IN., 92663 California-U.S.A) was used for the invitro detection of circulating human IgG autoantibodies specific against human insulin, according toVardi**,** *et al*.**,** 1988.

3.2.2.3.1 Principle of the test (Engvall *et al*., 1971):

The assay depends on the capacity of antigens to bind through hydrophobic interaction to an inert carrier surface, which forms an immune adsorbent for the attachment of specific antibody. Thus when serum samples are added the amount of bound specific antibody can be measured by

causing the antigen/antibody complex to react with an enzyme labelled antiglobulin conjugate. The substrate for the enzyme is then added and the colour developed is measured spectrophotometrically.The intensity of the colour is directly proportional to the concentration of the specific antibody in the serum sample.

3.2.2.3.2 Protocol:

Sera of IDDM patients and healthy control subjects were tested for the presence of specific IgG to human insulin.

Microwell strips coated with human insulin were firmly snapped into a 96 well microtitre plate.

100 µl of diluted test sera (10 µl of sera in 1.0 ml of buffer) were dispensed in duplicates into appropriate wells according to specific indexing system and the wells were labelled in a schematric diagram. 100µl of positive and negative control sera were added also in duplicate to the appropriate wells. 2 wells were left empty at this point. The Plate was then covered (to prevent contamination) and incubated at 2-8°C overnight (12-16 hrs.).Next morning the plate contents were decanted and the wells were thoroughly washed with washing buffer (3x5) and blotted dry. Then 100 µl of IAA-anti human IgG enzyme (peroxidase) conjugate were added to appropriate microwells.The plates were covered and let stand at room temperature for about 1 hour

then the Contents were decanted and the washing repeated. 100 µl of diluted substrate solution containing colour developer were added to all microwells including the 2 wells that were left empty, which will act as the Blank. The plate was incubated in the dark at room temperature for 30 minutes. Then 50µl of stopping solution (1N NaoH) were added into each micro well. The optical density of each well including the blank was recorded at 405 nm in a spectrophotometer using ELISA reader.

3.2.2.3.3 Calculation of data:

The spectrophotometer readings were recorded in absorbance units (optical density O.D).

The average reading of duplicate samples or control (negative/positive) was calculated:

Average reading = (1^{st} O.D +2^{nd} O.D) ÷ 2

The average O.D of the –ve control is $\bar{N}$.

The average O.D of the +ve control is $\bar{P}$.

The average O.D of the sample data is $\bar{S}$.

The cut off point (X) of each one = N X 2.5.

According to the kit used (Isletest IAA) the negative control should not read more than 0.600.The average of positive control should not read more than the cut off point X(the cut off point is the lowest limit of positivity).

Therefore the result of a patient sample is either positive or negative. It is positive when the average of O.D is > X, and it is negative when the average of O.D is < X.

3.3 Statistical analysis:

Data were subjected to statistical analysis using SPSS computer program (Statistical package for social sciences).

ANOVA (Analysis of variance), T-test, and chi-square test.

CHAPTER FOUR

RESULTS

Mean age of population studied was 12.6±5 years (5-30 years). Males constituted 40.6%; females constituted 48.95%; children were 51.05% and adults were 43.75%.

4.1 Results of the first study:

Glucose level on fasting and 2 hours postprandial was measured in blood and urine, every 15 days, in case of the study sample (group B) and the control (group A)

4.1.1 Blood glucose level for group A and group B:

4.1.1.1 FASTING BLOOD GLUCOSE LEVEL:

Results in Table 1 show that, the mean level of fasting plasma glucose in group **B(**study) after administration of royal jelly for a period of 3 months was 216.83±63.200 mg/dl.Whereas the mean value for the control group **A(**control**)** was 276.94±62.30mg/dl.The difference is highly significant (P<0.00).

Also the difference was highly significant between adults and children of both groups (A and B) (Table 2).

In table 3 the mean level of fasting plasma glucose for males of group A was 195.13± 40.1mg/dl, compared to 229.45± 69.32 mg/dl for males of group B, and we have a significant difference when we compared the results of females from group A with females of group B, (P = 0.05).

TABLE (1) Mean *fasting* plasma glucose level after daily dose of royal jelly for 3 months in *group (B)* compared to *control (group A)*

	N (86)	Mean mg/dl±SD	P-value
GroupA(control)	30	276.94 ±62.30	0.00* ** Sig.
Group B(study)	56	216.83± 63.20	

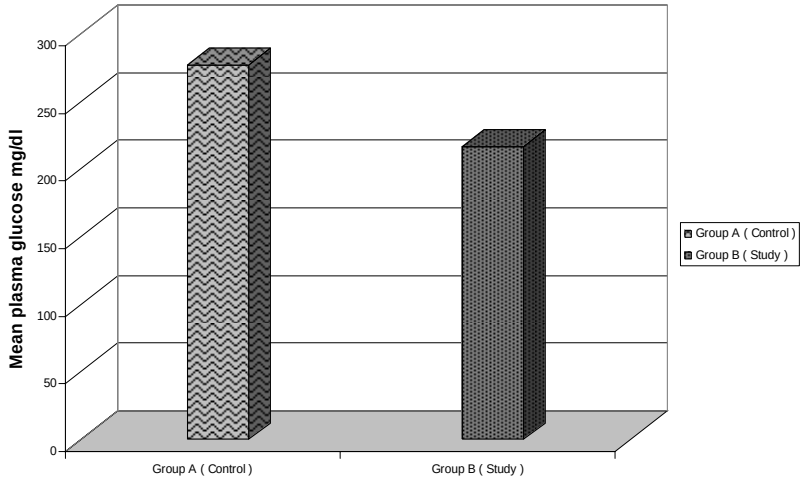
TABLE (2) Mean *fasting* plasma glucose level after daily dose of royal jelly for 3 months in *group B* (study) compared to *group A* (control) according to *age.*

	N	Mean mg/dl ±SD	P-value
Adult(groupA)	11	299.9±54.96	0.00* * * (Sig.)
Adult(group B)	23	238.1±59.5	
Child(group A)	19	263.61±63.8	0.00* * * (Sig.)
Child(group B)	33	201.98±62.3	

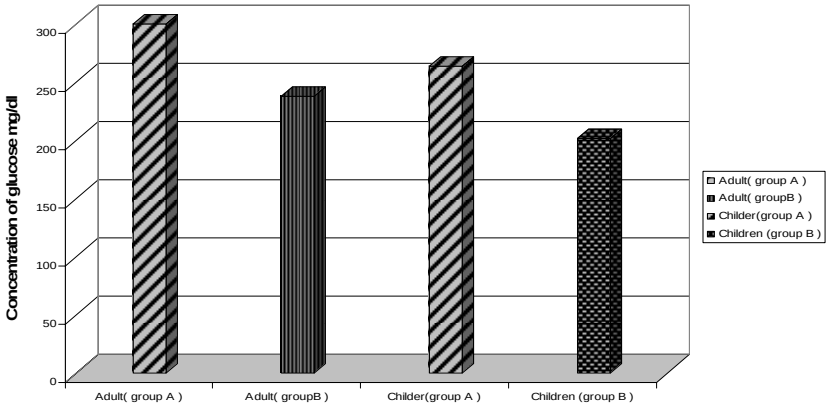
TABLE (3) Mean *fasting* plasma glucose level after daily dose of royal jelly for 3 months in *group B* as compared to *group A* according to *gender.*

	n	Mean mg/dl ±SD	P-value
Male(groupA)	16	195.13±40.1	0.04* * (sig.)
Male(group B)	26	229.45±69.32	
Female(group A)	14	279.1±56.1	0.05* * (Sig.)
Female(group B)	30	205.9±56.3	

Fig(***1***) Mean **fasting plasma** glucose level after daily dose of royal jelly for 3 months in **group B** (study) compared to **group A** (control)



Fig(***2***) Mean **fasting plasma** glucose level after daily dose of royal jelly for 3 months in **group B** (study) compared to **group A** (control) according to **age**



Fig(***3***) Mean **fasting plasma** glucose level after daily dose of royal jelly for 3 months in **group B** (study) compared to **group A** (control) according to **gender**

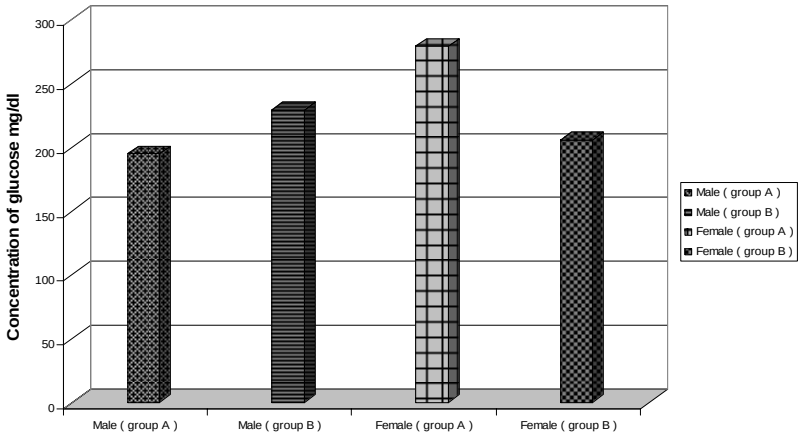


Table 4 shows mean fasting plasma glucose level for children patients in group B was201.98± 62.30mg/dl compared to 238.12± 59.45mg/dl for adult (P< 0.05).The difference were significant.

Results (Table 5, 6) also show that there were no gender or age related difference in fasting blood glucose level among group B patients. Mean fasting blood glucose level for female 205.89± 56.26mg/dl, and for male was 229.45± 69.32mg/dl (P> 0.05). Similarly the mean level for female children (204.04± 61.35mg/dl), and the mean level for male children (199.200± 65.85mg/dl), the different was not significant (P>0.05). Table 7 shows the mean fasting of blood glucose level for female adult was 209.078± 48.85 mg/dl, and for adult male was264.75±57.36 mg/dl. Significant difference was detected (P<0.05).

4.1.2 Postprandial blood glucose level:

Results showed in table (8) there is a highly significant difference between **group** B and group A**.** Table 9 shows mean level of 262.56± 68.23 mg/dl for children and 282.98± 59.98 mg/dl for adults, with no significant difference. No significant difference was observed between mean postprandial values for males and females (Table 10).

Within groups as shown in Table 11, 12 mean plasma glucose level 2 hours postprandial was265.45 ±70.40 mg/dl

for female children and 259.25± 68.15 mg/dl for male children .Mean value for adult female was 255.40 ±57.60 mg/dl, and 315.16 ±64.46 mg/dl for adult males, significant difference was observed ($P<0.05$).

TABLE (4) overall *fasting* plasma glucose level in *group B* patients after daily dose of royal jelly for 3 months according to *age.*

Age	n (56)	Mean mg/dl±SD	P-value
Child (5-21years)	33	201.98±62.30	0.0* * Sig.
Adult (13-30years)	23	238.1±59.45	

* *= significant.

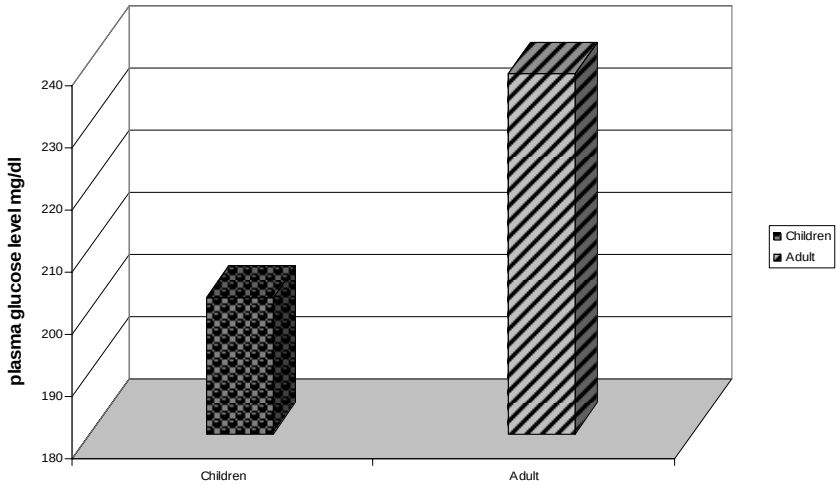
TABLE (5) overall *fasting* plasma glucose level in *group B* patients after daily dose of royal jelly for 3 months according to *gender .*

Gender	n (56)	Mean mg/dl±SD	P-value
FEMALE	30	205.89±56.26	0.1* (N.sig).
MALE	26	229.45±69.32	

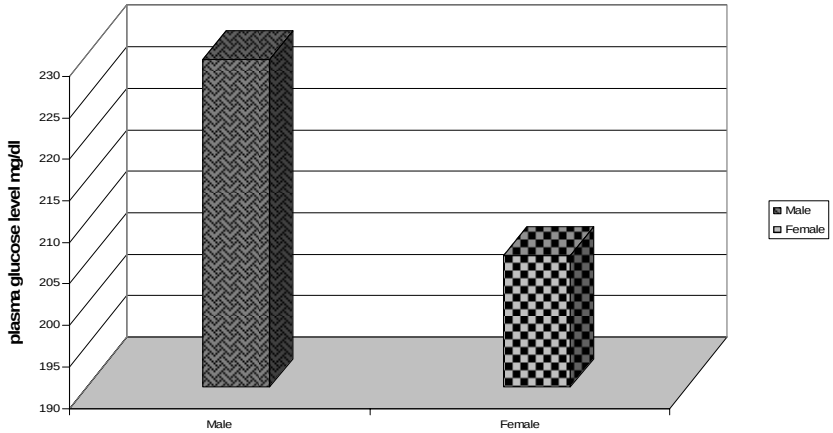
TABLE (6). *Comparison of* Mean *fasting* plasma glucose level in *group B children* according to *gender.*

Gender	n (33)	Mean mg/dl±SD	P-value
FEMALE	19	204.04±61.35	0.76* (N.sig).
MALE	14	199.20±65.85	

Fig(***4***) Mean **fasting plasma** glucose level in **group B** patients after daily dose of royal jelly for 3 months accordind to **age**



Fig(***5***) Mean **fasting plasma** glucose level in **group B** patients after daily dose of royal jelly for 3 months accordind to **gender**



Fig(***6***) Mean **fasting plasma** glucose level **group B** children acoording to **gender**

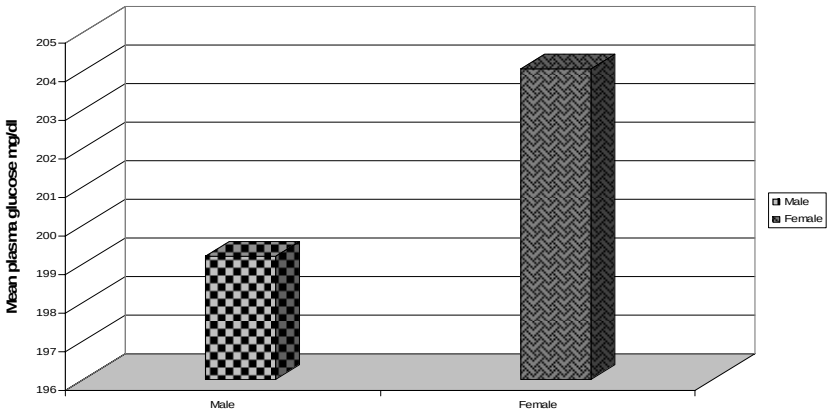


TABLE (7) comparison of Mean *fasting* plasma glucose level in *adults of group B* patients according to *gender*

Gender.	N (23)	Mean mg/dl±SD	P-value
FEMALE	11	209.078±48.85	0.02** (Sig.)
MALE	12	264.75±57.36	

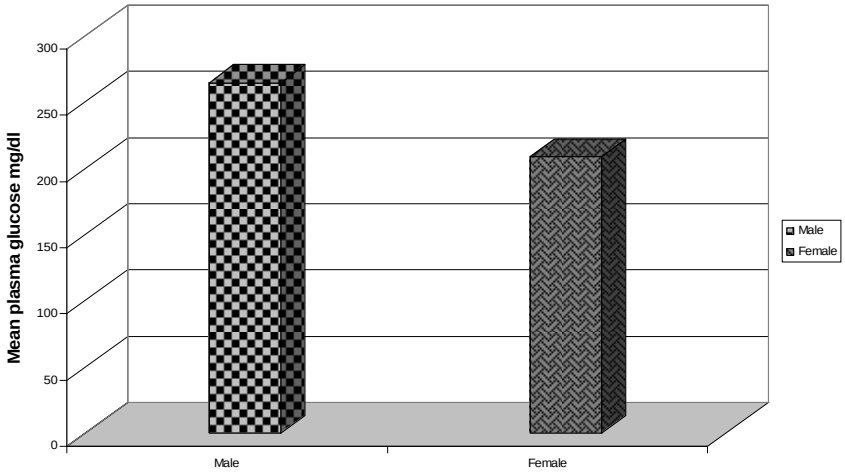
TABLE (8) Mean *Postprandial* plasma glucose level after daily dose of royal jelly for 3 months in *group (B)* compared to *control (group A)*

	N(86)	Mean mg/dl±SD	P-value
Group(A)	30	287.13 ± 53.1	0.000*** (Sig.)
Group(B)	56	272.0 ± 64.8	

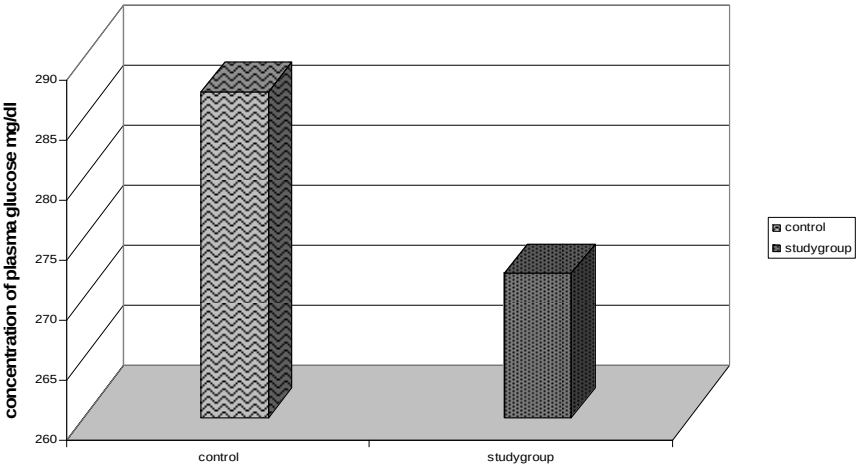
TABLE (9). *Postprandial* plasma glucose level in *group B* patients according *age.*

Age	n (56)	Mean mg/dl ±SD	P-value
CHILD(5-12)	30	262.56 ±68.23	0.37* (N.sig.)
ADULT(13-30)	26	282.98±59.98	

Fig(***7***) Mean **fasting plasma** glucose level in adults of **group B** patients according to **gender**



Fig(***8***) Mean **postprandial plasma** glucose level in **group (B)** patients after daily dose of royal jelly for 3 months compared to **control (group A)**



Fig(***9***) Mean **postprandial plasma** glucose level in **group B** patients according to **age**

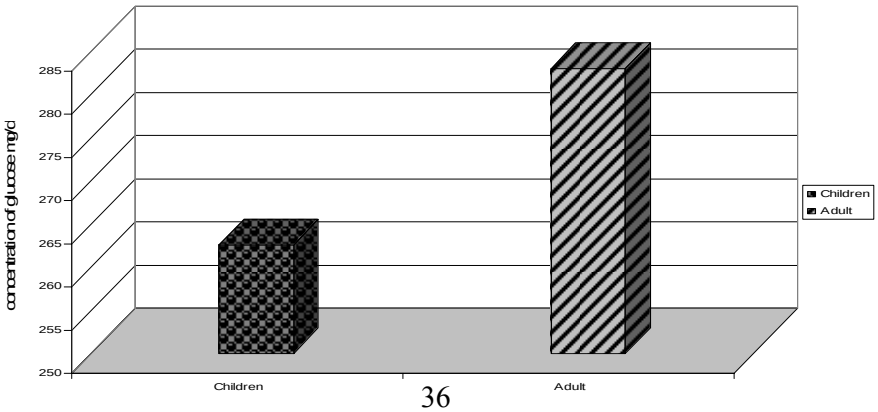


TABLE (10). *Postprandial* plasma glucose level in *group B* patients according to *gender.*

Gender	n (56)	Mean mg/dl±SD	P-value
FEMALE	30	260. 76±63.85	0.64* (N.sig.)
MALE	26	285.05±64.60	

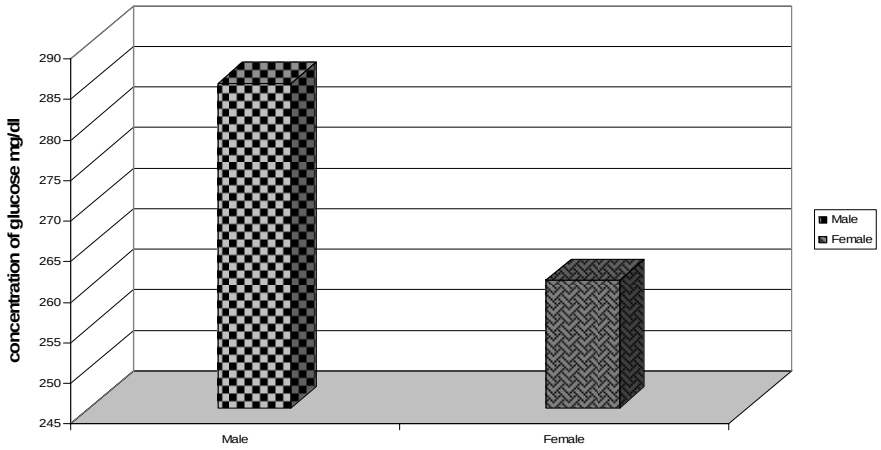
TABLE (11). *Postprandial* plasma glucose level in *group B children* according to *gender.*

Gender	n (30)	Mean mg/dl ±SD	P-value
FEMALE	16	265.45±70.4	0.75* (N.sig.)
MALE	14	259.25±68.15	

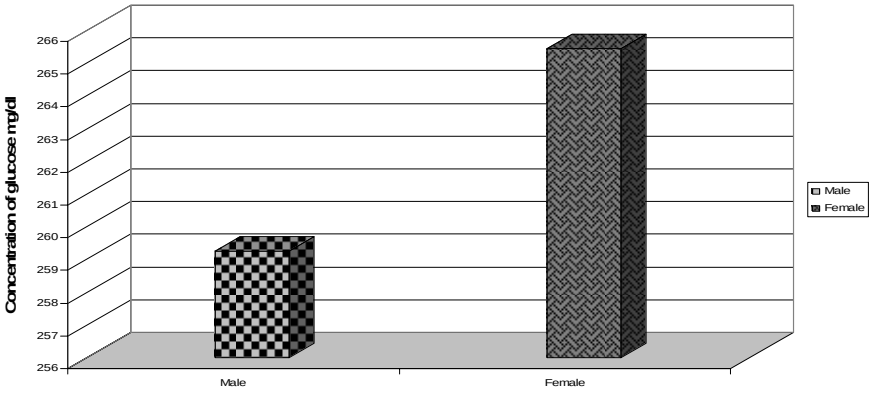
TABLE (12). Comparison of mean *postprandial* plasma glucose in group *B adults* according to *gender.*

Gender	N (26)	Mean mg/dl ±SD	P-value
FEMALE	14	255.40±57.60	0.00* * * (Sig.)
MALE	12	315.16±64.46	

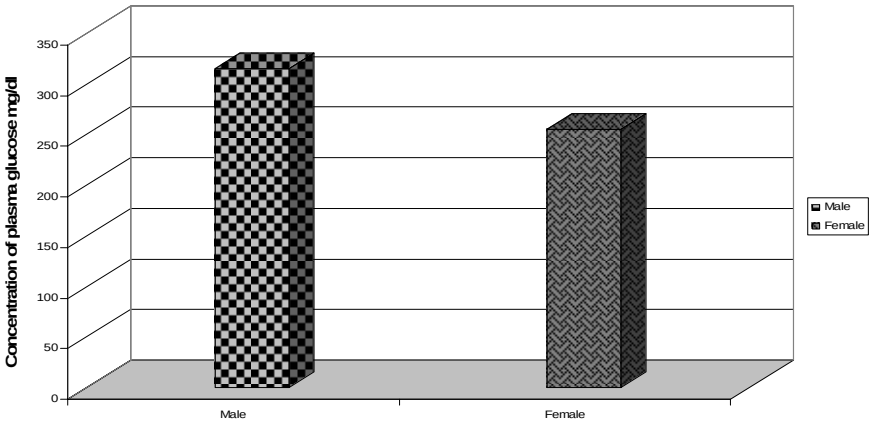
Fig(***10***) Mean **postprandial plasma** glucose level in **group B** patients according to **gender**



Fig(***11***)Mean **postprandial plasma** glucose level in **group B children** according to **gender.**



Fig(***12***)Mean **postprandial plasma** glucose level in **group B adults** according to **gender**.



4.2 Fasting urine glucose level for group A and B:

There was highly significant difference of royal jelly on urine glucose level in group B compared to group A which did not receive any royal jelly(P = 0.001).Table 13.

4.2.1 Post prandial urine glucose level:

Postprandial urine glucose level appears in table (14) showed no significant difference between patients of group B (royal jelly) compared to group A (no royal jelly).

4.3 Insulin autoantibodies (IAA):

This investigation involved 20 patients with good metabolic insulin control (B1) of the diabetes, and 36 patients with poor metabolic insulin control of the disease (B2), this latter acted as a control group.

Insulin autoantibodies (IAA) for type 1diabetes mellitus were measured by the enzyme linked immunosorbent assay (ELISA).

For the study group the minimum age for IAA positive subjects was 5 years, and the maximum age was 30 years, with mean age of 12.6 ± 5 years. The lowest level of IAA (O.D) was 0.353 for 6 years female patient, and 3.32 the highest level of I AA (O.D) for 13 years old, with mean level of IAA of 1.4±0.85 (O.D).

Table (15) shows that patients with good metabolic insulin control of diabetes showed lower levels of blood IAA as compared to patients with poor metabolic control of the disease. The difference was very significant ($p = 0.00$).

However, data showed, between patients within group **B1**which were under good metabolic insulin control had lower levels of blood IAA (Table 16 and 17) .Also there was no significant difference between patients with group B2, which were under poor metabolic control of insulin treatment, as related to age and gender. Table (18), (19).

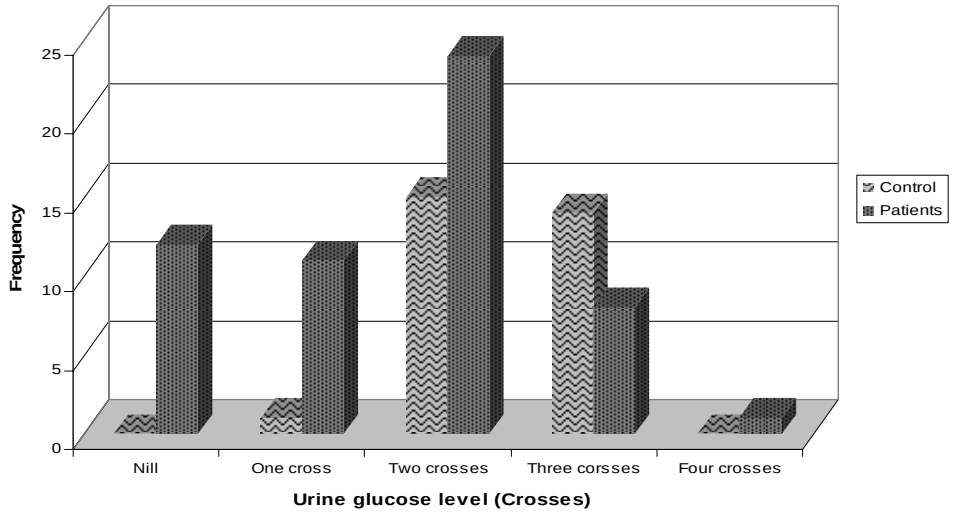
TABLE (13) Fasting *urine* glucose level in the study *group (B)* /3 months after daily administration of royal jelly compared to *control group (A).*

Glucose level (crosses)	Control group (A) (n=30)		Study group(B) (n=56)	
	Prevalence of crosses %		Prevalence of crosses %	
Nil	-	-	(12/56)	21.4%
+	(1/30)	3.3%	(11/56)	19.6%
++	(15/30)	50%	(24/56)	42.85%
+++	(14/30)	46.7%	(8/56)	14.3%
++++	-		(1/56)	1.8%
P-value =0.001				

TABLE (14). The effect of royal jelly on *postprandial urine* glucose level in *group B* patients compared to ***control (A)***.

Glucose level (crosses)	Control group (A) (n=30)		Study group(B) (n=56)	
	Prevalence of crosses%		Prevalence of crosses%	
Nil	-	-	(5/56)	9 %
+	-	-	(9/56)	16.1%
++	(16/30)	53.3%	(15/56)	26.8 %
+++	(11/30)	36.6 %	(22/56)	39.3 %
++++	(3/30)	10 %	(5/56)	9%
P-value =0.1				

Fig(*13*)Fasting Urine glucose level in study group B compared to control (group A)



Fig(*14*) The effect of royal jelly on post prandial glucose level in study group compared to control

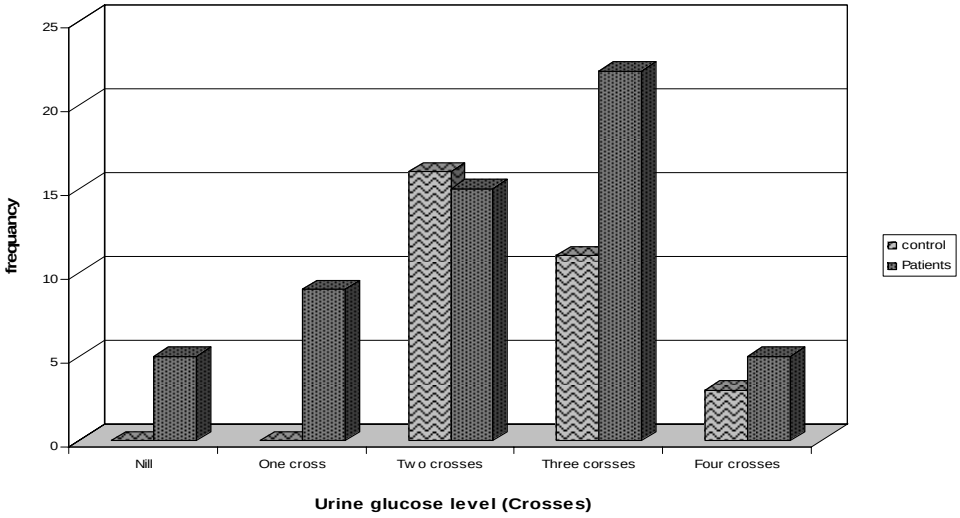


TABLE (15) Mean level of *serum IAA* of group *B1* compared to group *B2.*

Group	Mean optical density(O.D) ± SD	P- value
B1Good metabolic insulin control (n=20)	0.7± 0.2	0.000* * * (Sig.)
B2Poor metabolic insulin control (n=36)	1.8± 0.7	

TABLE (16) Mean level of *serum IAA* in group *B1* (good metabolic insulin control) as related to *age.*

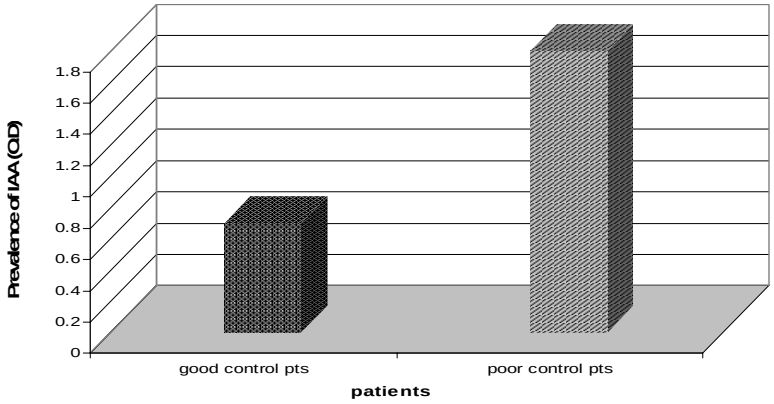
GROUP B1	Mean optical density (O.D) ±SD	P-value
Child(n=11)	0.6 ± 0.2	0.1* (N.sig.)
Adult(n=9)	0.76 ± 0.2	

IAA = Insulin autoantibodies.

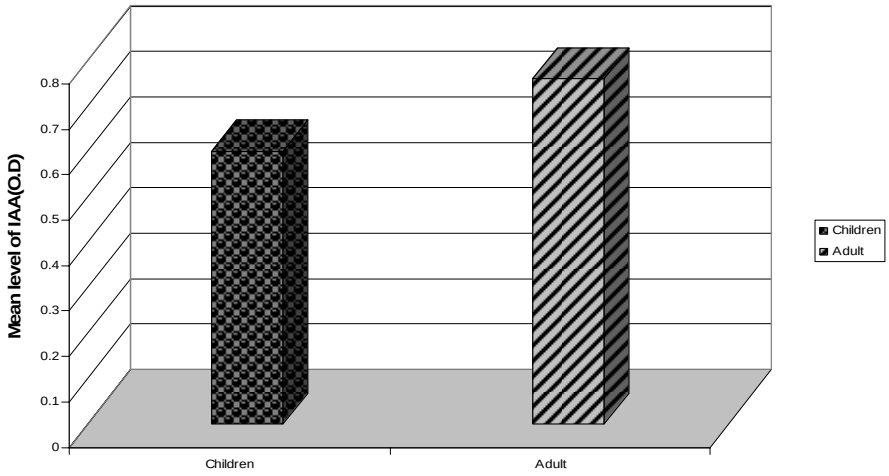
TABLE (17) Mean level of *serum IAA* in group *B1* (good metabolic insulin control) according to *gender.*

Gender	*Mean optical density (O.D) ±SD*	P-value
Male (n=5)	0.6 ± 0.25	0.6* (N.sig.)
Female (n=15)	0.7± 0.2	

Fig(***15***) Mean level of **serum IAA** of group **B1** compared to group **B2**



Fig(***16***) Mean level of **serum IAA** in group **B1** (good metabolic insulin control) as related to **age**



Fig(***17***) Mean level of **serum IAA** in group **B1**(good metabolic isulin control) as related to **gender**

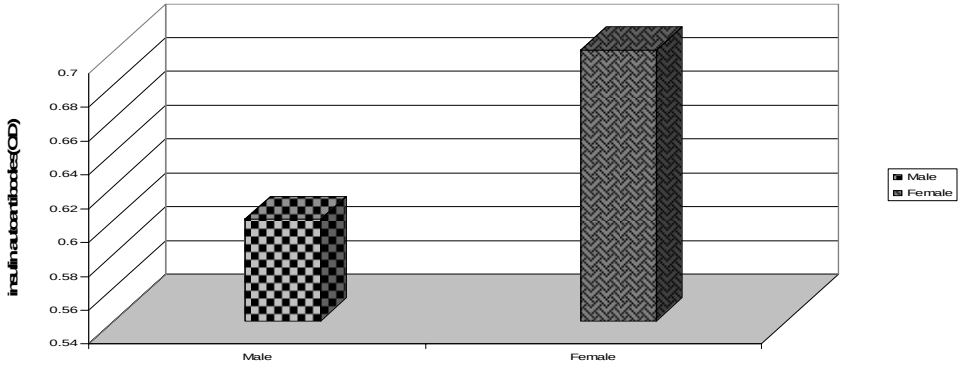


TABLE (18) Mean level of *serum IAA* in a group *B2* (poor metabolic insulin control) according to *age*

Age	*Mean optical density (O.D) ±SD*	P-value
Child (n=15) (5-12 years)	1.8 ± 0.75	0.8* (N.sig.)
Adult (n=21) (13-30 years)	1.7 ± 0.7	

TABLE (19) Mean level of *serum IAA* in group *B2* (poor metabolic insulin control) according to *gender.*

Gender	Mean optical density (O.D) ±SD	P-value
Male (n=5)	1.4 ± 0.4	0.5* (N.sig.)
Female (n=31)	1.6 ± 0.7	

Fig (***18***) Mean level of **serum IAA** in **group B2** (poor metabolic insulin control) as releated to **age**

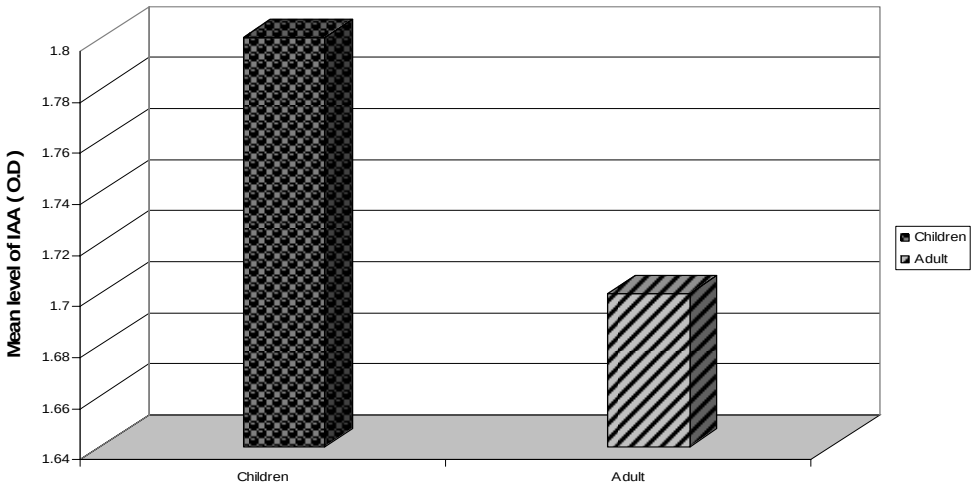
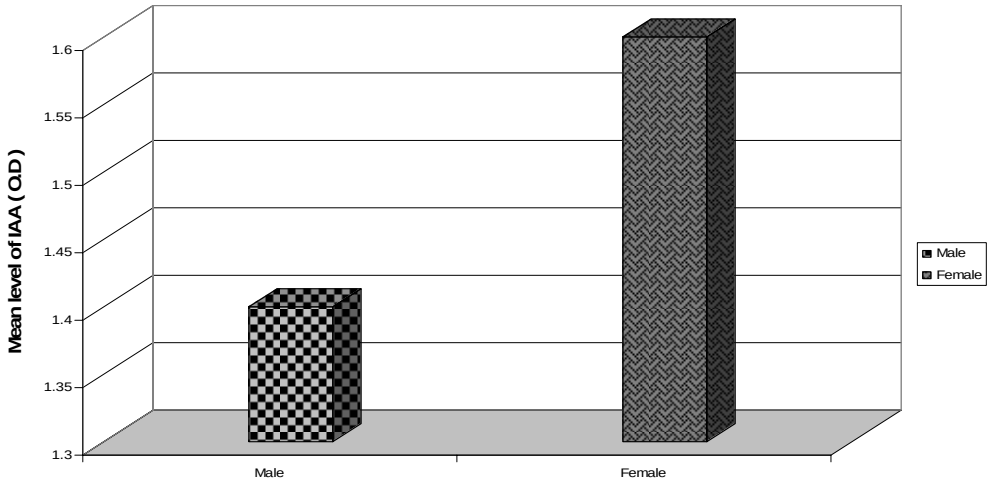


Fig (***19***) Mean level of **serum IAA** in **group B2** (poor metabolic insulin control) as related to **gender**



CHAPTER FIVE
DISCUSSION

Insulin dependant diabetes mellitus (IDDM) or type 1 diabetes mellitus is an autoimmune disease in which the beta-cells of the pancreas are destroyed by immunological mechanisms .This results in insulin deficiency which has to be corrected by administration of daily exogenous insulin.

The immunological attack occurs on the beta cells several years (prediabetic asymptonali period) before the clinical onset of IDDM (Ziegler,et.al.,1989).Identification and classification of diabetes has been improved in recent years by autoantibody testing.Several autoantibodies have been implicated in this immunological attack, however the insulin autoantibody is the first islet autoantibody to develop during progression to IDDM (Kimpimaki,et,al.,2001),genetically predisposed individuals (with strong family history)should be periodically screened for this specific autoantibody; although positivity alone cannot be taken as an index for the disease nevertheles can be useful as an aid .It has been demonstrated that by the time clinical signs of IDDM appear, 80% of the islet are destroyed(Bach,1994).In this study the IAA investigation involved two groups: patients of group B1,were under good

metabolic insulin control whereas patients of group B2 were under poor metabolic insulin control.

Both groups received a daily dose of royal jelly for a period of 3 months besides a daily dose of insulin(2 injections).All patients were free of diabetic complications. Results showed that IAA present in both groups, however the mean level of IAA in group B2 sera was significantly higher than that in group B1 sera. This means that in the group under poor insulin metabolism (B2) royal jelly could not potentate or substantiates exogenous insulin. Another implication is that this high IAA response may partially be directed against the parley metabolized exogenous insulin and not wholly directed against the endogenous target. Therefore the role of royal jelly on IDDM cannot carefully estimated by measuring IAA level in blood. Therefore if insulin-specific antigen – specific immunosuppressors are concurrently administrated with royal jelly, then the association between poor glycaemia control and the high plasma level of IAA can be more accurately assessed also line predictive value of IAA for the appearance of IDDM in the Sudanese can be acunalely determined .And above all the effect of royal jelly on hypoglycemia as reflected in the plasma level of IAA can be acuately evaluated.

In both groups B1, B2 no age-related statistical difference in IAA plasma level was observed. However Sepe (1996) reported age related prevalence and that age is inversely correlated with age whereby he detected up to 100% prevalence in the age group less than 5 years compared to 40% in the age above 12 years and that IAA in almost absent in adult onset of IDDM .

In this study 45% of group B1 and 56% of group B2 were adults (Table 16, 18).This observation may early onset or may agree with Bach (1994) who started that IAA can appear at any age.

Although not observed in this study sex-related difference in IAA levels was reported by Dotta and DiMario (1996) where females attained higher values.

By the time clinical signs of IDDM appear 80% of the beta cells are already destroyed.

The effect of royal jelly on type 1 diabetes mellitus was assented by measuring glucose level in blood and urine on fasting and postprandial and that forte nightly over a period of 3 months.

A statistically significant difference was observed with respect to this parameter when mean level of group B patients (the study group) was compared with mean level in the

control (group A) on fasting and postprandial (Table 1, 8).The level values observed in the study group (B),Table 1 may be attributed to an effect of royal jelly. This effect may at least partially be attributed to an insulin –like peptide detected in royal jelly by Kramer, etal. (1982).

To study this effect on group (B) the data was broken down there after. When this positive effect was considered as related to age a significant difference in fasting glucose level was observed between adults and children of group (B) with a lower mean for children (Table 4).

The effect of royal jelly on children may be due to the concentration relative to body mass, as the dose was constant for all participants. However no gender- related difference among group B patients detected (Table5) or among group B children (Table 6) or among group B adults (Table 7).

To confirm the effect of royal jelly comparison between children and adults (Table 2) of the 2 groups, the result revealed a quite significant difference, similarly comparison between males and females of the 2 groups (table 3) indicated significant difference.

Surprisingly no age related difference was observed among patients in group B ,i.e. mean postprandial plasma glucose level for children was not significant for adults

(Table 9).Similarly no gender related difference in this parameter was detected(Table 10)among children (Table 11)or adults of group B(Table 12).

Although fasting plasma glucose level was significantly lower in the group which received royal jelly, surprisingly postprandial values were not significantly difference between those of the control group.

Fasting urine glucose level showed considerable reduction in the study group B compared to group A(Table 13),however there was no significant difference as for postprandial values compared to control (Table 14)

The mechanism underlying the reduction in plasma glucose level associated with intake of royal jelly is still not understood, however Bonomi (1983) suggested that the action of such compounds could be on endocrine glands, or they may become part of an enzyme system or may directly affecting intermediate metabolism.

Other studies (Elarafy, 2003) showed that when royal jelly is added to the diet, most people notice an increased hormonal activity in terms of an improved sense of well-being and more energy. The regulation of royal jelly due to a big mount of vitamins in its contents (Aeppler, 1992).

Between the study group, they were noted, when they given royal jelly, they gained weight after first month from starting using royal jelly.

The components of royal jelly, particularly the more unusual ones of those with known biological activity or present in greater quality have been tasted.

In other words, it is still not known how royal jelly works nor what is responsible for its amazing effects.However,researchers showed serve to emphasize the complexity and interdependence of different therapies and factors such as who is taking what, when and how much.

In all the popular and scientific literature on the composition of royal jelly, there is a fraction of royal jelly described as "other as yet unknown". This phrase not only emphasizes the incomplete state of analytical knowledge about the product, but also the lack of understanding of the biological activities.

These activities have not been proven definitely, nor have they been attributed to any of the known components.

An early explanation (Johansson and Johansson`, 1958) claming high vitamin content as a contributory factor can be refuted on the grounds that the same effects should then be an achievable with vitamin supplements or a glass of milk,

which contains amount of vitamins similar to the usual dose of royal jelly.

Some day science will figure out what these unidentifiable elements (Three percentages its constituents remain an undermined faction) are and some researcher will try to manufacture them (Saenz, 1984).

Activities or medicine may have synergistic effects which can not be explained by a list of compounds and their individual effects. Tests of such a hypothesis in clinical and scientific trails are needed.

There is continuing research over world-wide that has resulted in hard evidences showing royal jelly can make substantial contribution to man.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Results of this study indicated that the daily dose of royal jelly had a substantial effect on IDDM patients (children, adult, males, and females) with a more pronounced effect- on children.

In another part of this study results showed that this effect is only obtained under good metabolic insulin control. This implies that royal jelly cannot be used as a medicine but could be used as a partentiator.

Measurement of autoantibodies against islet –cells in cases of new- onset diabetes mellitus can help differentiate type 1 from type 2 diabetes.

Insulin autoantibodies (IAA) one of the most important of these autoantibodies.

The level of these autoantibodies is very high in the patients before diagnosed (untreated), and its decrease by the follow up treatment of insulin injection.

The result of this study, showed a significant difference of the IAA level between the patients under a good metabolic control of insulin and with a poor metabolic control of insulin treatment.

We believe that, the insulin control of the disease, cross active is also by many other environmental factors, are important factors one is the education of the patients and their relatives.

Information on autoantibodies in Sudan is still fragmentary. Studying these autoantibodies not only for early diagnoses of type 1 diabetes mellitus, but also for relatives (especially first degree of relatives).

It is recommended to study the other autoantibodies for type 1 diabetes mellitus to know the situation of the disease in Sudan, also to include them in primary investigations of children less than 10 years to maximize sensitivity.

Once therapies are developed that can prevent type 1 diabetes when applied in the prediabetic phases of β-cell autoimmunity.

Development of new and more sensitive technologies could cause significant advances by discovering novel auto-antibody specificities, which may have a higher predictive value than the traditionally available markers.

Royal jelly and type 1 diabetes mellitus give a good result with studying group. Young patients showed good response for fasting blood glucose level, and 2 hours post prandial.

There is a significant difference between the control (group) and the patients (group B) after using royal jelly in their physical and characters.

Decreasing of glucose level in blood and urine due to royal jelly ,with some patients if we know that they have the same conditions has many suggestion: one of it .that some of the patients controlling their disease by following the physician orders, and royal jelly was an addition helper for them, and others were not.

Exercise was important for some of the patients who are using royal jelly continuous for three months, and other was not.

Royal jelly and all honey bee products were a good material for studying, but the problem is a high price especially for royal jelly, due to technique of extraction from the hive.

Using royal jelly with insulin- dependant diabetes mellitus can be possible in the future if the complete study and research on, how it's working inside blood, and decreasing the level of glucose in the body.

Therefore, we recommended, should be taken royal jelly with insulin treatment for better metabolic control.

CHAPTER SEVEN

REFERENCES

Aeppler, C.W.1992.Tremendous growth forceGleoning in bee culture, **50**: 69-73.

Alsukkari magazine.2001.Alsukkari international.483 Belchers Lane Birmingham.

Ammon, R.and Zoch, E.1957.Zur Biochemie des futtersoftes der Bienenkonigin. Arzneimitt. Forsch.**7:** 699 - 702.

Angunawela, I.I.1987.The role of autoimmune phenomena in the pathogenesis of cataracrct.Immunology 61: 363-68.

Bach, J.F. 1994. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocr Rev 15:516-42

Bakkeskov,S., Nielsen,JH.,Marner,B.,Bilde,T.,Ludvigasson,J.and Lernmark,A.1982.Auto-antibodies in newly diagnosed diabetic children immunoprecipitate specific human islet cell protein.Nature,298:167-69.

Bakkeskov,S.,Annstoot,H.,Christgau,S.,Reetz,A.,Solimena,M .,Coscalho,M.,Folli,F.,Richter-olesen,H.,and DeCamilli,P.1990.Identification of the 64 Kda autoantigen in insulin dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase

.Nature, **347**:151-56.

Bell GI, Pilkis SJ, Weber IT, Polonsky KS. 1996. Glucokinase mutation, insulin secretion, and diabetes mellitus. Annu Rev Physio. **58**:171-186.

BenFenati, L.Sabatini, A.G.and Nanetti, A.1986.Mineral composition of royal jelly.Apicolltura Italy, No.2:129-143.

Bingley, PJ Bonifacio.E. Williams, AJ., et.al.1997.Predication of IDDM in the General population: Strategies Based on Combinations of Autoantibody markers, Diabetes, 46(11):1701-10.

Blum, M. S., A. F. Novak, and S. Taber III. 1959. 10 hydroxy-2-decenoic, an antibiotic found in royal jelly. Science 130 (3373): 452-453.

Bonomi, A.1983.Acquisizioni in temadi composization chemical ediattivita biologica Della pappareale.Apitalia, **10** (15):7-13.

Bottaazzo, G., Florin-Christensen, A., Doniach, D. 1974. Islet cell antibodies in diabetes mellitus with auto immune polyendocrine deficiencies. Lancet III; II: 1279-83.

Chmidt's, H.W.DR.1956.Royal jelly in diet, prophylaxis, and therapy.

Christie, MR., Hollands, J.A.Brown, T.J., Michellsen, B.K.and Delvoitch, T.L.1993.Detection of pancreatic islet 64,000Mr.Autoantigens in IDD.Distinct from GAD.J.clin.Invest. **22:**240-48.

Christie,MR.,Genovese,S.,Cassidy,D.Bosi,E.,Brown,TJ.,Lai, M.,Bonifacio,E., Bottazzo,GF. 1994. Antibodies to islet 37 K antigen, but not to glutamic acid decarboxylase,discriminate rapid progression to IDDM in endocrine autoimmunity,diabetes.vol. **42**.pp.1254-1259.

Clark Pm. 1999. Assays for insulin, proinsulin (s) and C-peptide. Annuli biochem. **36** (PT5): 541-64

Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National diabetes data group.1979.Diabetes, **28**: 1039-57 .

DeAizpuria, Henry, J.*et.al*. 1992."Glutamic acid decarboxylase autoantibodies in preclinical insulin-dependent diabetes ", Proceedings of the national academy of science.vol. **89**, pp. 9841-45.

Derivici,A. and Petrescu,A. 1965. Experimental studies in vitro and in vivo on the virulicidal action of royal jelly.Lucr. stint.stat.cent.seri.Apic.**5 :** 135-143.

Destrem,H.1956.Expērimentation de la gelēe royale d'abeille

en partique gēriatriquel (134 cas). Rev.France.Geront 3.

Donadieu,Y.1980.La gelēe royale. Maloine,Paris.

Dotta, F.and DiMario, U.1996.Antigenic determinants in type 1 diabetes mellitus.APMIS, **102:796**-74.

Dozio, N.,Belloni,C.,Girardi, A.M.,Genovese, S.,Sudoyez, J.C., Bottazzo,G.F.,Pozza,G., and Bosi,E.1994.Heterogeneous IgG subclass distribution of islet cell antibodies.J.Autoimmun.,**7**: 45-53.

Eisenbarth, G.S., J.Connelly, and J.S.Soeldner.1987.The natural history of type diabetes .Diabet./Metab.Rev.,**3**:873-891.

Elamin, A.; Omer, M.I.A.; Hofander, Y.; Tuvemo, T.1989. Prevalence of insulin-dependent diabetes mellitus (IDDM) in school children in Khartoum, Sudan. Diabetes Care. **12:**430-2

Elamin, A.E.; Athh, A.; Ismail, B.; Turemo, T.1992.Clinical pattern of childhood type 1(IDDM) in the Sudan. Diabetologia, **35**: 645-8.

Elarafy, I.A.2003.Chemical characteristics of Honey bee products and their effect on diabetic patints.Ph.D.thesis collage of Agricultural studies, Sudan University of Science and Technology.

Elhabiby, M. 1998. Biochemical and immunological studies of diabetes mellitus in Sudanese patients. Ph.D. Thesis.16-20.

ElMahdi, E.M.A.; El Mahdi, A., and Daw Mukhtar, S.1989.Pattern of diabetes mellitus in the Sudan.Trop. Geog.Med. **41**:353-357.

Engvall, E.1971.Enzyme Linked Immunosorbent Assay (ELISA).J.Immunol .**109**: 129-135.

Felig, D.; Baxter, JD. ; Brodus, AE.and Frohman, L.1987.Endocrinology and metabolism.2nd Edition, 1105-1166.London, UK.

Fujii, A., Kobayashi,S. Ishihama,S. Yamamoto, H. and Tamura,T.1990.Augmentation of wound healing by royal jelly.Japan.J.Pharmacol., **53**(3):331-337.

Fujiwara, S. Journal of biological chemistry; July 5 1990. **265;** 11333-7.

Ganong, W.F.2003.Review of Medical Physiology.21st edition 336-358.

Gorsuch, AN., Spencer,KM., Lister,J., McNally,JM., Dean,BM., Bottazzo,GF., Cudworth, AG.1981.Evidence for a long prediabetic period in type 1 (insulin-dependent)diabetes mellitus. *Lancet* **2**: 1363-1365.Medline.

Guyton,A.C. and Hall,J.E.1996.Text book of medical physiology.9th edition.

Internet site,www.Royal-jelly-co-uk/diabetes/htm.2003.

Johansson, T.S.K., Johansson, M.P.1958.Royal jelly .Bee world **39:** 254-277-286.

KimPimaki,T.,Kupila,A.,Hamalainen,AM.,Kukko,M.,Kulmala,P.,Saola,K.,Simell,T.,Keskinen,P.,Iconen,J.,Simell,O., Knip,M.2001.The first sigs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1Diabtes predication and prevention study clin Endocrinal Metab.**86** :4782-4788.

Kramer, K.J., Childs, C.N. and spires, Redland Jacobs, R.M.!982. Purification of insulin-like peptidea from insects' haemolynph and royal jelly. Insects' biochemistry **12**(1): 91-98.

Kramer, K.J., Tager, H.S., Childs, C.N. and spiers, R.D.1977. Insulin like hypoglacemic and immunological activities in honey bee royal jelly. Journal of insect physiology .**23**(2): 293-295.

Krell,R.1996.Value –added products from beekeeping **124** : 204-206.

Laackso, M.; Pyorala, K.1985.Age of onset and type of diabetes .Diabetes care **8**:114-117.

Lavie, P. 1968. Propriētēs antibactēriennes et action physiologique des produits de la ruche et des abeilles-Invol.3 of: Chauvin, P.1-115.

Lendrum,R.,Walker,G.and Gamble,Dr.1975.Islet cell antibodies in juvenile diabetes mellitus of recent onset.Lancet,i:880-82.

Lercker, G., Coboni, M.F., Vecchi, M.A, Sabatini, A.G.and Nanetti, A.1992.Caratterzzazionedei principal costituentidella gelatine reate.Apicoltura **8**: 11-21.

Mahdi, S. 2000. Effect of bee honey on blood glucose level of Sudanese type 2 Diabetics.M.Sc.Thesis.Sudan University of science and technology.

Martinetti,R.and Caracristi,C.1956.Azione eccitometabolica della gelatina reale nell´uomo.Atti del 1 convegnohazionale per 10 studio dell`applicazione dei prodotti delle api nel campo- medico-biologica, Bologna, Italia,139-144.

McCance, DR.; Hanson,RL; Charles,MA.; Jacobsson,LT.; Pettitt,DJ.;Bennett,PH.; etal. 1995. Wich test for diagnosing diabetes? Diabetes care.**18**: 1042-4.

McCance,DR.;HansonRL.;PettittDJ.;BennettPH.;HaddenDR.;KnowlerWC.,1997.Diagnosing diabetes mellitus: do we need new criteria? Diabetologyia. **40:** 247-55.

Naserke, HE., Bonifacio, E., Ziegler, AG.1999.Immunoglobulin G insulin autoantibodies in BABYDIB offspring appear postnatally: sensitive early detection using a protein A/G-based radioinding assay. *J Clin Endocrinol Metab*. **84** 1239-1243 Abstract.

National Diabetes data group.1995.Diabetes.2ed.Bethesda, MD: National institutes of health, National institute of diabetes and digestive and kidney diseases.NIH publication no.95-1468.

Nye, M.J., Shuel, R.w., Dixon, S.E. 1973.Gluconic acid in the food of larval honey bees.J.Apic.Res.**12** (1):9-15.

Osman, S.A.and Ismail, S.A. 1977. Biochemical studies on Egyptian royal jelly.II.protein and amino acids. Bulletin. **28** (1): 305-321 of the faculty of Agriculture, Cairo University.

Palmer, JP., Asplin,CM.,Raghu,P.K.,Clemens,P.,Lyen,K.,etal.1986. Anti-insulin antibodies in insulin-dependant diabetes before insulin treatment-anew marker for autoimmune beta cell damage?Adolesc.Endocrinol.,15:111-116.

Palmer,JP.,Asplin,CM.,Clemons,P.,Lyen,K.,Tatpati,O.Raghu, PK.,Paquette,TL. 1983. Insulin antibodies in insulin-dependent diabetes before insulin treatment. Science **222**:1337-1339.

Peakman, M., and Vergani.D.1997.Basic and clinical immunology.ch.13 Endocrine autoimmune disease.P.183-184 .1st edition, churchil Livingstone, U.K.

Pickup, J., Williams, S.G.1998.Textbook of diabetes, Black well since 2nd edition.

Report of the expert committee on diagnosis and classification of diabetes mellitus.1997.Diabetes care.**20 (7):**1183-97.

Saenz, A.DR.1984. Biology, biochemistry, and the therapeutic effects of royal jelly in human pathology.Puplished by the Pasteur institute of Paris.

Sasaki, M., Tsuuta, T.and Asada, S.1987.Role of physical property of royal jelly in queen differentiation of honey bee. In chemistry and biology of social insects (edited by Eder, J., Rembold, H.).Murich, German federal republic, Verleg J.Papemy 306-307.

Schranz, D., Lernmark, A.1998.Immunology in diabetes: an update. Diabetes Metab. Rev.**14**: 3-29.

Sepe, V. 1996. “Islet related autoantigen and the pathogenesis of diabetes mellitus”. Molecular pathogenesis of diabetes mellitus, Leslie RDG.**22**:68-89.

Sera, J.andEscola, R.1991.Study of the microbiological quality and Bacteriostatic activity of queen food (Royal jelly), effect of organic acids.Deutsch lebensmittell-Rundschau 87(8):256-529.

Skrha, J.1998.Possible makers of diabetic vascular disease. Diabetes Reviews international**, 7;** 1: 12-4

Takenaka, T. 1984. Studies on protein and carboxylic acid in royal jelly. Bulletin of the faculty of Agriculture, Tamagawa University. No.**24:** 101-149.

Takenaka, T. 1984.Proteins and peptides in royal jelly. Nippon nogeikaku Kaishi 57 (12), 1203

Takenaka, T., Yatsunami, K.and Echigo, T.1986.Changes in quality of royal jelly during srorage.Nippon shokchin kogyo gakkaishi.**33** (1): 1-7.

Takenaka, T.1982.Chemical composition of royal jelly .Honey bee Science **3**(2): 69 - 74.

Tang, L.F.1995.Activated T cells in the epiretinal membrane of eyes of patients with proliterative diabetic retinopathy.Clinical.Exp.Opthalumal. **233**: 21-25.

Tang, S.1995.Activated T-Lymphocytes in the epiretinal membranes from eyes of patients with proliferative diabetic retinopathy.Graefes Arch.Clin.Exp.Opthalmol., **233:** 21-25.

Vardi,P.,Ziegler,A.,Mathenes,J.H.,Dib,A.,Keller,R.J.,Ricker, A.,Wolsdorf,J.L.,Herskowitz,R.D.,Rabizadeh,A.,Eisenb arth,G.S.and Soddner,J.S.1988.Concentration of insulin autoantibodies at onset of type 1 diabetes :inverse long-linear correlation with age .Diabetes Care ,11:736-39.

Varely,H.,Alan,H.G.and Mauice ,B.1980.Practical clinical biochemistry .Vol.1 General topics and commoner tests,5th edition,Williams Heinerman Medical Books Ltd.,London.

Verge, C.; Gianani, R.; Kawasaki, E. 1996. Predication of type 1 diabetes in first degree relatives using combination of insulin GA, and ICA512bdc/IA-2 autoantibodies,"Diabetes care, **45**(7):926-30.

Weaver, N., N.C.Johnston, R.Benjamin, and J.H.Law.1968.Novel fatty acids from the royal jelly of honey bees (*Apis melliferal*) lipids **3**(6):535-538.

Wetherill, C.M.1852.Chemical examination of the food of the queen bee.Rep.A cad.Nat.sci. philad.**6**:119-121.

WHO Expert committee on diabetes mellitus.1980.second

report. World health organization.Tech.Repser **646**: 1-80.

World Health Orgnization.1985.Diabetes mellitus .WHO technical reports.Series No.727, Geneva WHO.

World health orgnization.1994.Preventive of diabetes mellitus ,WHO technical report,series No.844,Geneva WHO.

World Health Orgnization.2002.www.WHO.org.Diabetes programme.Prevalence of diabetes worldwide.

Yatsunami, K. and Echigo, T.1985.Antibacterial activity of royal jelly. Bulletin of the faculty of Agriculture, Tamagawa University No.**25**, 13-22.

Ziegler,A.G.,Ziegler,R.,Varili,P.,Jackson,R.A.,Soeldner,J.S. and Eisenbrth,G.S.1989.Life table analysis of progression to diabetes of anti insulin autoantibodies – positive of individuals with type 1 diabetes.Diabetes,**38**:1320-325.

APPENDIX

1- Criteria for diagnosis:

Diabetes mellitus:

Elevation of plasma glucose (PG> 200mg/dl) and classic symptoms of diabetes, including, polydipsia, polyuria, and weight less, or fasting plasma glucose (FPG> 140mg/dl) and tow oral glucose tolerance tests (OGTTs) with the 2 hour PG 200mg/dl after 75 g OGTT.

2- Measurement of insulin autoantibodies (IAA) using ELISA kit:

2-1 Materials of IAA ELISA kit:

PLA IAA = IAA-Microwell strips (with the holder)

1. IAA-Anti-Human IgG Enzyme conjugate (conc.).
2. IAA-Sample diluent (concentration).
3. Islet conjugate diluent.
4. IAA-Positive control (human serum).
5. IAA-Negative control (human serum).
6. Islet substrate solution (PNPP).
7. Islet washing buffer (conc.).
8. Islet stopping solution (1 N NaOH).
9. ELISA Reader (spectrophotometer)

2-2Preparation of reagents:

- 5 ml of the islet conjugate diluent was transferred in to one bottle containing IAA-IgG enzyme conjugate (concentration).

- IAA-sample diluent buffer (25 ml) transferred in to 100 ml of distilled water.
- Islet wash solution transferred in to 480 ml of distilled water.

2-3 Serum sample preparation:

Accurately pipette 0.01 ml of serum sample in to 1.0 ml of the working sample diluent in an already labelled glass tube. Mix thoroughly.

3- Measurement of glucose level in the plasma:

	Sample	Standard	Blank
Test sample	0.01 ml	-	-
Standard	-	0.01 ml	-
Distilled water	-	-	0.01 ml
Reagent	1.0 ml	1.0 ml	1.0 ml

Read at 505 nm.

4- Vitamin content of royal jelly in µg per gram of fresh weight (Krell, 1996):

Vitamin	**Weight µg per gram**
B1 (Thiamine)	1.5 – 704
B2 (Riboflavin)	5.1- 2.5
Pantothenic acid	159-265
B6 (Pyridoxine)	1.0-48.0
Niacin (Nicotinic acid)	48.0-88.0
Folic acid	0.13-0.53
Biotin	1.1-19.8
Inositol	80.0-350.0

5- Some observations were not including in the study:

- In all study group of type 1 diabetes mellitus with out any complications, or family history of the disease.
- Using of royal jelly daily increased the weight (Fujii, et al., 1990) of the patients, after first month from using, by stimulating the metabolism.
- Most of the patients, talked about the changes of the attitudes of their children (becomes quieter and very sensive), which is gave us a good indicator on the action of royal jelly on all systems of the body, and secretion of the glands.
- Some of the patients decreased their insulin dose by physician, due to decreasing of glucose level in their blood after using royal jelly.

Patient´s Form.

Jaber-Abulez Diabetes center.

Serial No. ( )

Name……..……

Gender…….Telephone ….……

Age……Starting of royal jelly……/……/ 2004

Type of group:

B I	B 2

Date	Fasting plasma	urine	2 hrs.plasma	urine	Comments