GOVERNMENT DEGREE COLLEGE FOR WOMEN, KARIMNAGAR, TELANGANA						
1.3.2 Percentage of students undertaking project work/field work/internship (Data for the latest completed academic year)						
Name of the department	**Nature of the work**	**Title of the project work/field work/ internships**	**Programme Name**	**Programme Code**	**List of students undertaking project work/field work/internship (Upload excel file)**	**Link**
			B.Sc(Life Science)		Juveria Khanam	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Nasera Butool	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Arutla Ramya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Donta Susmitha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Applied nutrition	Project work	General literature on diabetes	B.Sc(Life Science)		Esampally Saisri	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Golle Supriya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Jadi Aishwarya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Janjarla Rasagna	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Konkata Srilekha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Applied nutrition	Project work	Capparis fascicolariscrude extracts: potent source of	B.Sc(Life Science)		Lavudya Prathyusha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Rabia Amber	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Rabiya Khatoon	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Sankasala Ishwarya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf

			B.Sc(Life Science)		Sanober Khanam	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Applied nutrition	Project work	Methods of protein purification	B.Sc(Life Science)		Shimaila Maheen	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Uzma Siddiqua	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Edipelly Lavanya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Talari Sriya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Ravula Priyanka	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf

GOVERNMENT DEGREE COLLEGE OF WOMEN

KARIMNAGAR – 505001

APPLIED NUTRITION

STUDENT STUDY PROJECT

(2021-22)

TOPIC

LYCOPENE EXTRACTION FROM TOMATO AND TOMATO PRODUCTS

SUPERVISOR

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CERTIFICATE

Certified that this study is a bonafide Student Study Project done by the following B.Sc. students of Government Degree College of women, Karimnagar District under the Supervision of A. CHANDRA SHEKHAR, Asst. Professor of Biochemistry.

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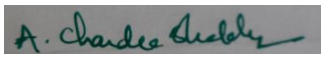

Supervisor

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INTRODUCTION:

Benefits are one of the specific issues that will greatly influence the food industry in the next few years. There has been a growing interest in functional foods. These are obtained from Health traditional foods with an ingredient able to provide or promote a beneficial action for human health. These ingredients are plant extracts, antioxidants, polyunsaturated fatty acids and others (Herrero, Cifuentes & Ibanez, 2006; Shi, Le Maguer& Brayan, 2002). Among the different compounds with functional properties, antioxidants are the most widely studied. Tomato fruit and its products contain the potent antioxidant lycopene and are the predominant source of lycopene in the human diet (Shi, Qu, Kakuda, Yeung & Jiang, 2004; Giovannucci, 1999).

The chemical properties of lycopene derive from its structure and make it hydrophobic and soluble in organic solvents (hexane, chloroform, benzene, triglycerides). According to scientific literature, lycopene is assimilated from the product of tomatoes better when using it with oil. The lycopene extraction with oil is a replacement for toxic organic solvents (hexane, chloroform, benzene), and a more environmentally friendly and faster extraction procedure. The scientific literature offers no information about the solubility of lycopene (Shi, Le Maguer& Brayan, 2002; Borel, Grolier, Armand, Partier, Lafont, Lairon& Azais-Braesco,1996). This work attempted to prepare a lycopene solution and find an excellent and cheap source of lycopene.

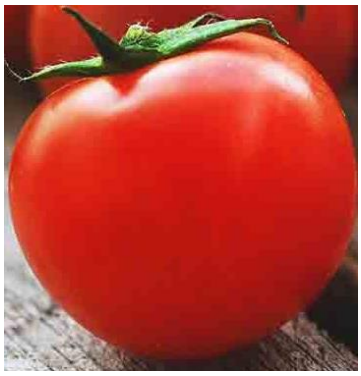
The lycopene content in tomato typically ranges from 70 to 130 mg/kg and depends on the variety, geographic location, technique of cultivation, climatic conditions and degree of ripeness of tomato fruits. The tomato extract described in this application is the ethyl acetate extract of ripe tomato fruits with lycopene content ranging from 150 to 250 mg/kg. The lycopene content of tomato extract ranges from 5% to 15%, depending on the nature of the fruit from which it was extracted, and the amount of tomato seed oil that is included in the extract

KEY WORDS :

Tomato, tomato products, pectinase, lycopene, DPPH, β-carotene, TPC

In the recent era, tomatoes and its products has gained immense attention owing to the presence of lycopene, a carotenoid pigment, which holds the ability to control numerous ailments like cancer and other degenerative illnesses by hindering the activity of free radicals (Nasir et al., 2014). On-going epidemiological studies reveal that regular intake of tomatoes has an inverse relationship with the danger of developing cancers at various anatomical sites including, the stomach, lung and prostate gland (Viuda-Martos et al., 2014). Antioxidant ability of lycopene to delocalize free radical species lies in the presence of conjugated carbon–carbon double bonds, which makes it quite beneficial for the human beings. Owing to this, its demand is quite high in food, feed, pharmaceutical and cosmetic industries. Tomatoes and its products (pulp and paste) are regarded as one of the richest sources of lycopene. The amount of lycopene in tomatoes varies between 90 to 190 lg/g fresh weight (Alda et al., 2009). Tomato paste is a thick concentrate made from the ripened tomatoes with the skin and seeds removed, while, tomato pulp is an unprocessed form, obtained by passing the fresh raw fully red tomatoes from the fine pulper machine removing the seeds (Rodriguez-Azua et al., 2014). Considerable amount of lycopene is present in the chromoplast of the plant tissues. In tomatoes, its biosynthesis carries on a rapid pace during the ripening process, in which thechloroplast transforms into chromoplast. Voluminous sheets of lycopene are present in the outer part of the pericarp while the inner jelly like part mainly contains beta-carotene (Choudhari and Ananthanarayan 2007; Kiokias, 2016). Owing to the beneficial effects of lycopene, there is a need of time to improve its extraction methods. In the past, simply organic solvents only such as hexane, ethyl acetate, benzene, ethyl ether, acetone, ethanol and petroleum ether etc were used to extract lycopene from its natural sources. Moreover, different combinations of solvents were used to achieve the same objective in more effective way (Periago et al., 2004). These solvents alone or in combinations were not effective in extracting maximum lycopene, as they do not have the power to dissolve the cell wall constituents (cellulose and pectin), mainly responsible for the binding of lycopene (Agarwal and Rao, 2000). The application of hydrolytic enzymes that degrade and assist in the release of lycopene in high amount is a widely reported method for its efficient extraction. As plant cell wall comprises mainly of cellulose and pectin, but the later mainly binds the lycopene, so pectinase are employed for this purpose.

They breakdown the respective bindings and release this important functional component which could be further extracted out by organic solvents (Zuorroa et al., 2011). The current study was planned for the extraction of lycopene from raw tomato and tomato products (paste and pulp) through solvent and enzyme aided extraction and its characterization through both the methods. Moreover, antioxidant potential of lycopene also determined while using different parameters. i.e. DPPH, TPC and β-carotene



MATERIALS AND METHODS :

Locally available bright red tomatoes (Solanumlycoperisicum. L)were procuredand stored at 2-4 °C. Fresh tomato products i.e. tomato paste and tomato pulp were prepared according to the prescribed methods.

Sample Preparation. The tomatoes were washed under running stream of water to remove all dirt, dust and foreign materials attached to their surface. De-heading and trimming of the tomatoes were carried out manually using a knife. These were processed into tomato paste and pulp. Tomato pulp was obtained by passing the fresh fully red tomatoes through the fine pulper machine and then the seeds and skin were separated following the protocols described by Dauthy (1995).

Tomato paste was prepared from ripened tomatoes by removing skin and seeds. It was cooked for several hours and reduced to a thick, red concentrate.

Extraction Method. Two methods were used for the extraction of lycopene from the raw tomatoes and its products. That included simple solvent and enzyme aided extraction methods.

Simple Solvent Extraction. Conventional method involved the simple application of organic solvents to the samples, for lycopene extraction .The fresh bright red fully mature tomatoes were grinded extensively, while the tomato paste and the pulp were properly homogenized to break the cell structure of these natural food materials for the efficient extraction of lycopene from them. About 20g of each sample was taken in the 250mL of the conical flask. Then these samples were extracted overnight in the orbital shaker with the solvent mixture of 200mL of hexane and acetone in the ratio of 75:25 respectively at room temperature. The extract from each flask was filtered with Whatman No. 1 filter paper. The solvent from extract was separated at 50°C in a rotary vacuum evaporator leaving behind crude extract only. The crude extract of each sample was stored at 4°C until use.

Enzyme-aided Extraction. Enzyme-aided extraction is a contemporary technique to acquire beneficial components from natural food materials as followed by. All the samples (tomato and tomato products i.e. paste and pulp) were first treated with the enzyme pectinase to remove the bindings of pectin, to get the lycopene in the free state for the better extraction latterly by the solvent mixture of hexane and acetone (75:25). For this purpose, the samples were properly homogenized and grinded extensively according to the requirement. About 3 g of the grinded sample was taken in 250 mL of the conical flask. Around 20mL of 0.2M acetate buffer of pH 5 was added to sample in the flask. Main purpose for the addition of the buffer was to provide appropriate environment for enzyme, to work efficiently. After buffer addition, thousand international units of the pectinase were added in the flask and incubated at 60°C for 20 minutes. After the completion of incubation process, filtration of the contents was carried out. Filtrate obtained was subjected to solvent extraction using 60mL hexane and acetone in the ratio of 75:25 respectively at room temperature. Vortex the contents for proper mixing of filtrate and solvent. Then the extraction was carried out in separating funnel for 15-20 minutes. Upper phase was non-polar in nature comprising lycopene and pooled together while lower aqueous phase was discarded. The crude extract of each sample was stored at 4°C until use.

Lycopene extract from tomato is produced from a tomato variety with high lycopene content, within the range of 150 to 250 mg/kg. This particular variety is not generally marketed for direct consumption, but is used primarily in the production of this lycopene extract. The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethyl acetate. The final product is obtained after solvent removal by evaporation under vacuum at 40-60°C.

Table 1. Chemical composition of lycopene extract from tomato

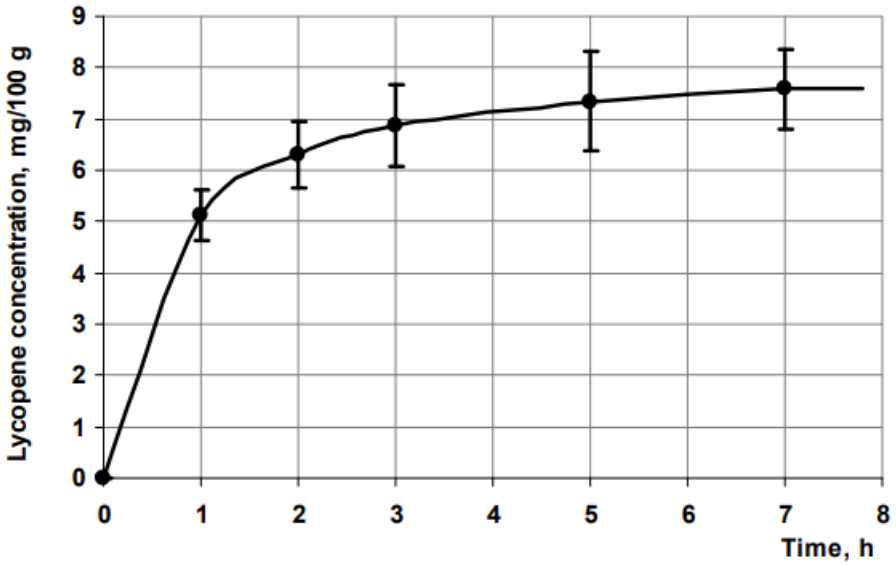
Compound	Content [%]	
	Min	Max.
Unsaponifiable matter	13.4	31.4
Lycopene	4.9	15
Phytoene	0.5	1.1
Phytofluene	0.4	0.9
β-Carotene	0.1	0.5
Tocopherols	1.0	3.0
Sterols	1.5	2.5
Others (i.e. waxes)	5.0	8.4
Fatty acids and acylglycerols	69	74
of which*		
Myristic acid (14:0)	0.5	0.6
Palmitic acid (16:0)	22.5	23.0
Stearic acid (18:0)	5.1	5.4
Oleic acid (18:1)	12.4	13.5
Linoleic acid (18:2)	46.7	48.7
Linolenic acid (18:3)	8.8	10.9
Arachidic acid (20:0)	0.9	1.1
Behenic acid (22:0)	0.5	—
Free fatty acids	5	
Water	0.5	0.9

Compound	Content [%]	
	Min	Max.
Water and soluble matter	2.7	4.8
Lactic acid	0.5	0.7
Other organic acids		0.1
Others	2.2	4.0
Total Phosphorus	0.4	0.5
Organic phosphorus	0.3	0.5
Phospholipids (estimated from phosphorus determined by ICP)	8.9	14
Nitrogen	0.16	0.31
Ash	0.7	0.8

RESULTS AND DISCUSSION

Extraction Methods. The effect of using different extraction methods on lycopene contents from raw tomato and its products is well mentioned in Figure 1. It can be elucidated that the simple solvent extraction method was not significant throughout the process of extraction whereas the enzyme (pectinase) aided extraction showed maximum results for lycopene recovery from all the products. By the solvent extraction method the maximum extraction rate was observed in tomato paste that was 21.3mg/100g whereas raw tomatoes and pulp followed by it that was 2.78 and 2.65mg/100g. On the other hand, enzyme-aided method had remarkable effect on the lycopene extraction rate in tomato paste that was 38.3mg/100g whereas pulp had least content of 3.71mg/100g. On an average basis, it was obvious from the results that tomato paste proved high in lycopene content by 29.8mg/100g, owing to the fact that thermal processing (cooking) and mechanical texture disruption (homogenization) of raw tomatoes are convenient ways to enhance bioavailability by breaking down sturdy cell wall structure (pectin), by disrupting chromoplast membranes and cellular integrity, thereby improving the accessibility of lycopene at the initial stage, which eventually helped in the better extraction of lycopene

The solubility of lycopene in fresh tomatoes and oleoresin from tomatoes containing 10% of lycopene was compared. It is known that lycopene is assimilated from the product of tomatoes better when using it with oil, but scientific literature offers no information about solubility of lycopene .It was determined that dissolution of oleoresin makes it possible to obtain oily lycopene solutions of a few times higher concentrations. However, the results showed that lycopene from tomato oleoresin concentration in oil ranged between 7-8 mg/100 g . The preparation of such solutions is not simple because oleoresin is prepared from tomato pulp by extraction with organic solvents. The use of such solvents would preferably be eliminated. It has been determined that it is possible to obtain oily lycopene solutions of non-inferior quality by direct extraction from tomato pulp with oils. Data from this study suggest that the solubility of lycopene from fresh tomatoes in oil at 20 °C is 7.5 mg/100 g.



During the processing of tomatoes into pastes or sauces, a large proportion of lycopene is removed by discarding peels. Peels are particularly rich in carotenoids . The best possibility to refine lycopene is to use tomato peels left after the production of juices. It has been established that in the peels of overripe tomatoes β-carotine amounts to 5.65 mg/100 g and lycopene amounts to 65.15 g/100 g. Lycopene concentration in tomato pulp without peels is only 4.224 mg/100 g and there is 7-15 times more lycopene in tomatopeels than in tomato flesh

Conclusions

The tomato procesing waste has been proved to be an excellent and cheap source of carotenoids, because during the processing of tomatoes into pastes or sauces, a large proportion of lycopene is removed by discarding peels. It has been established that there is 7 to 15 times more lycopene in tomato peels than in tomato flesh (up to 65.150 mg/100 g of lycopene is found in peel). Data from this study suggest that lycopene solubility in oil at 20 °C ranged between 7–8 mg/100 g and that the vegetable oils with extracted lycopene could be used for the creation of functional food with natural lycopene

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GOVERNMENT DEGREE COLLEGE OF WOMEN

KARIMNAGAR – 505001

APPLIED NUTRITION

STUDENT STUDY PROJECT

(2021-22)

General Literature on Diabetes

SUPERVISOR

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Assistant professor of biochemistry

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CERTIFICATE

Certified that this study is a bonafide Student Jignasa Study Project done by the following B.Sc. (BZAN & MZAN) students of Government Degree College of women, Karimnagar District under the Supervision of A. CHANDRA SHEKHAR, Asst. Professor of Biochemistry.

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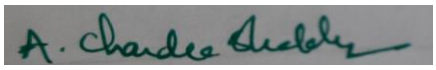
5	KONKATA SRILEKHA	B.Sc.



Supervisor

Diabetes mellitus

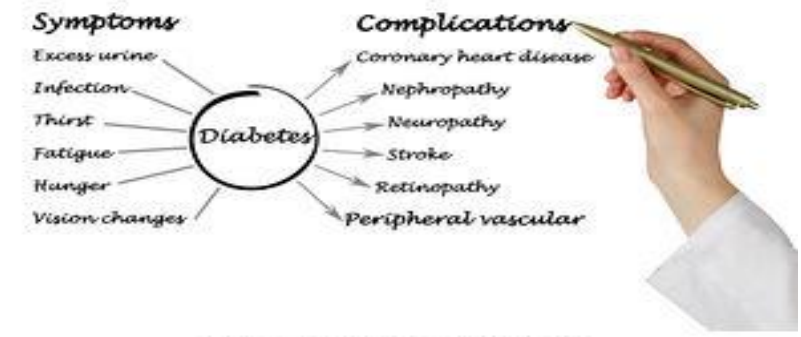
Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20) but may be the first symptom in those who have otherwise not received a diagnosis.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral artery disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplantation. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle atrophy and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function. Being diabetic, especially when on insulin, increases the risk of falls in older people.

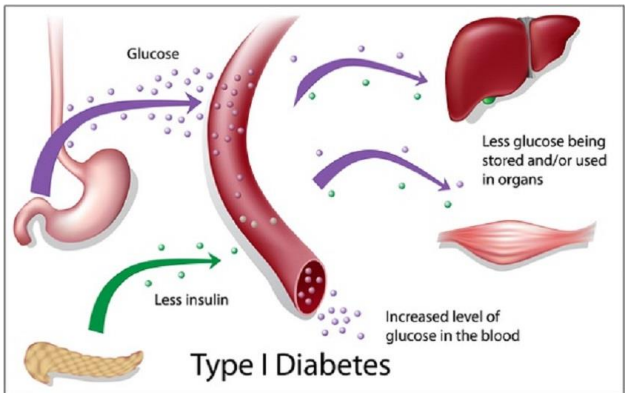


Comparison of type 1 and 2 diabetes

Feature	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body size	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in identical twins	50%	90%
Prevalence	~10%	~90%

Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes, and "other specific types". The "other specific types" are a collection of a few dozen individual causes. Diabetes is a more variable disease than once thought and people may have combinations of forms. The term "diabetes", without qualification, usually refers to diabetes mellitus

Type 1



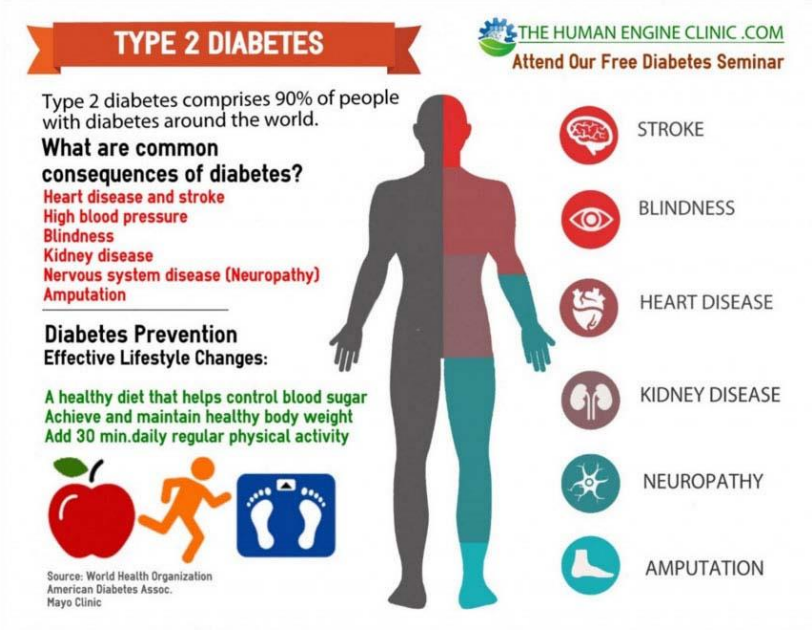
Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

"Brittle" diabetes, also known as unstable diabetes or labile diabetes, is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. Still, type 1 diabetes can be accompanied by irregular and unpredictable high blood sugar levels, frequently with ketosis, and sometimes with serious low blood sugar levels. Other complications include an impaired counter regulatory response to low blood sugar, infection, gastro paresis (which leads to erratic absorption of dietary carbohydrates), and endocrine pathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes.

Autoimmune attack in type 1 diabetes.

Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. Several viruses have been implicated, but to date there is no stringent evidence to support this hypothesis in humans.Among dietary factors, data suggest that gliadin (a protein present in gluten) may play a role in the development of type 1 diabetes, but the mechanism is not fully understood.

Type 2



Mechanism of insulin resistance in type 2 diabetes mellitus.

Type 2 DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion.[8] The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is the most common type of diabetes mellitus.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, high blood sugar can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the liver's glucose production.

Type 2 DM is primarily due to lifestyle factors and genetics.A number of lifestyle factors are known to be important to the development of type 2 DM, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Even those who are not obese often have a high waist–hip ratio.

Dietary factors also influence the risk of developing type 2 DM. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. The type of fats in the diet is also important, with saturated fat and trans fats increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk Eating lots of white rice also may increase the risk of diabetes.A lack of physical activity is believed to cause 7% of cases.

Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases, insulin may be required.

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

Maturity onset diabetes of the young (MODY)

Maturity onset diabetes of the young (MODY) is an autosomal dominant inherited form of diabetes, due to one of several single-gene mutations causing defects in insulin production. It is significantly less common than the three main types. The name of this disease refers to early hypotheses as to its nature. Being due to a defective gene, this disease varies in age at presentation and in severity according to the specific gene defect; thus there are at least 13 subtypes of MODY. People with MODY often can control it without using insulin.

Other types

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than cause.

Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The ICD-10 (1992) diagnostic entity, *malnutrition-related diabetes mellitus* (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

"Type 3 diabetes" has been suggested as a term for Alzheimer's disease as the underlying processes may involve insulin resistance by the brain.

The following is a comprehensive list of other causes of diabetes:

1. **Genetic defects of β-cell function**
    1. Maturity onset diabetes of the young
    2. Mitochondrial DNA mutations
2.
3. **Genetic defects in insulin processing or insulin action**
    1. Defects in proinsulin conversion
    2. Insulin gene mutations
    3. Insulin receptor mutations
4.
5. **Exocrine pancreatic defects**
    1. Chronic pancreatitis
    2. Pancreatectomy
    3. Pancreatic neoplasia
    4. Cystic fibrosis
    5. Hemochromatosis
    6. Fibrocalculous pancreatopathy

1. **Endocrinopathies**
    1. Growth hormone excess (acromegaly)
    2. Cushing syndrome
    3. Hyperthyroidism
    4. Pheochromocytoma
    5. Glucagonoma
2. **Infections**
    1. Cytomegalovirus infection
    2. Coxsackievirus B
3. **Drugs**
    1. Glucocorticoids
    2. Thyroid hormone

3. β-adrenergic agonists
4. Statins·

Pathophysiology

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, adipose tissue and muscle, except smooth muscle, in which insulin acts via the IGF-1. Therefore, deficiency of insulin or the insensitivity of its receptors play a central role in all forms of diabetes mellitus.

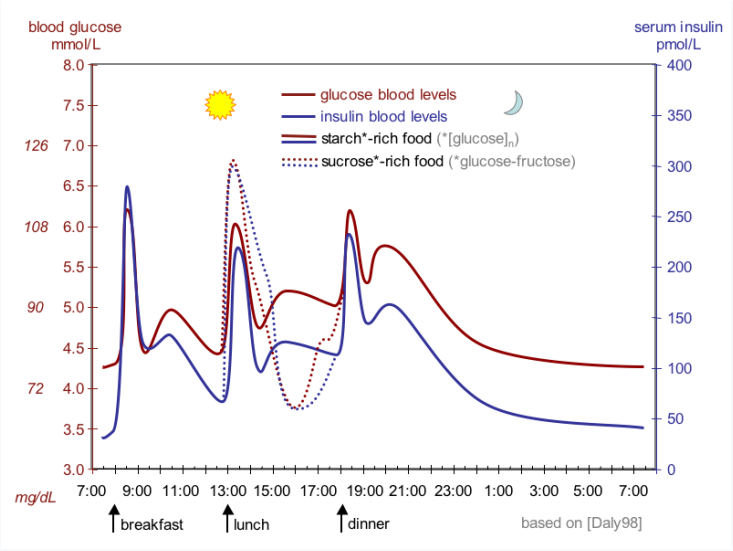
The body obtains glucose from three main sources: the intestinal absorption of food; the breakdown of glycogen, the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen.

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin.

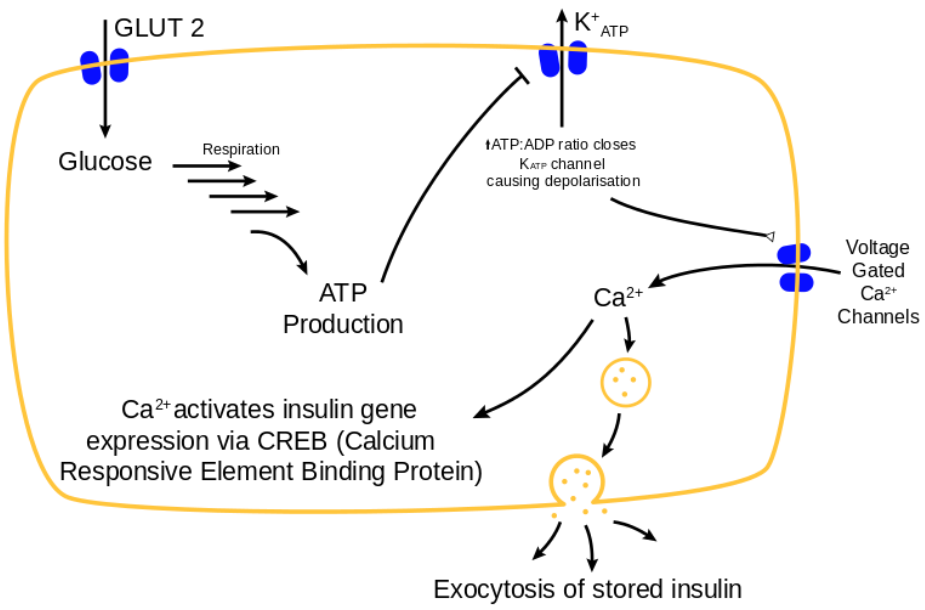
If the amount of insulin available is insufficient, or if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis.

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia).

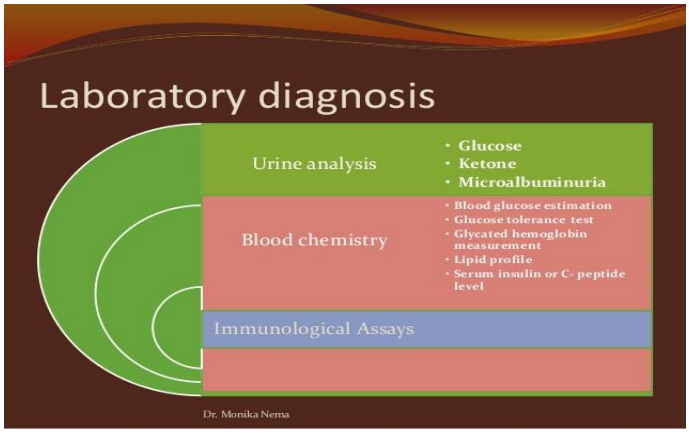
The fluctuation of blood sugar (red) and the sugar-lowering hormone insulin (blue) in humans during the course of a day with three meals. One of the effects of a sugar-rich vs a starch-rich meal is highlighted.



Mechanism of insulin release in normal pancreatic beta cells. Insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose.



Diagnosis



WHO diabetes diagnostic criteria

Condition	2 hour glucose	Fasting glucose	HbA_{1c}	
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	mmol/mol	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥6.1(≥110) &<7.0(<126)	42-46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42-46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

Diabetes mellitus is characterized by recurrent or persistent high blood sugar, and is diagnosed by demonstrating any one of the following:

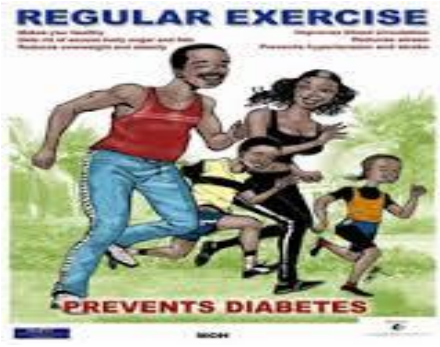
1. Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
2. Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
3. Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
4. Glycated hemoglobin (HbA_{1C}) ≥ 48 mmol/mol (≥ 6.5 DCCT %)

A positive result, in the absence of unequivocal high blood sugar, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test.According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

Per the World Health Organization people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose. people with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease.The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl).

Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any causes

Prevention



There is no known preventive measure for type 1 diabetes. Type 2 diabetes – which accounts for 85–90% of all cases – can often be prevented or delayed by maintaining a normal body weight, engaging in physical activity, and consuming a healthy diet. Higher levels of physical activity (more than 90 minutes per day) reduce the risk of diabetes by 28%. Dietary changes known to be effective in helping to prevent diabetes include maintaining a diet rich in whole grains and fiber, and choosing good fats, such as the polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help prevent diabetes. Tobacco smoking is also associated with an increased risk of diabetes and its complications, so smoking cessation can be an important preventive measure as well.

The relationship between type 2 diabetes and the main modifiable risk factors (excess weight, unhealthy diet, physical inactivity and tobacco use) is similar in all regions of the world. There is growing evidence that the underlying determinants of diabetes are a reflection of the major forces driving social, economic and cultural change: globalization, urbanization, population aging, and the general health policy environment.

Management

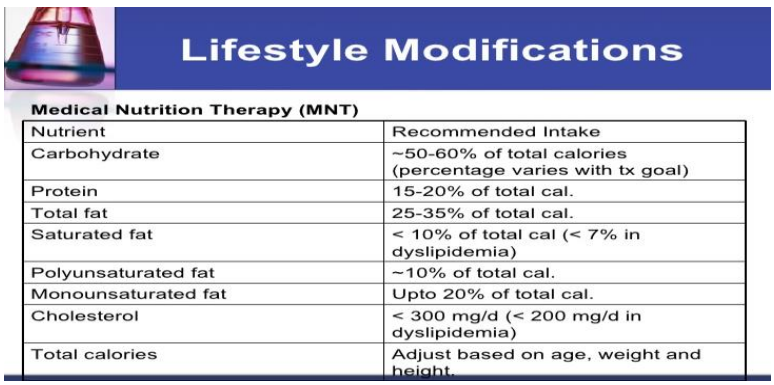
Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels. The goal of treatment is an HbA_{1C} level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise.Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however.

Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

There is no single dietary pattern that is best for all people with diabetes. For overweight people with type 2 diabetes, any diet that the person will adhere to and achieve weight loss on is effective.

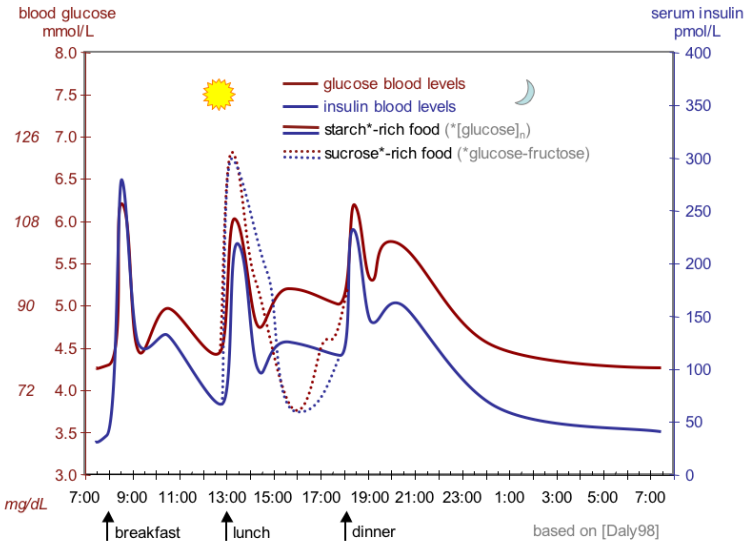


Medications

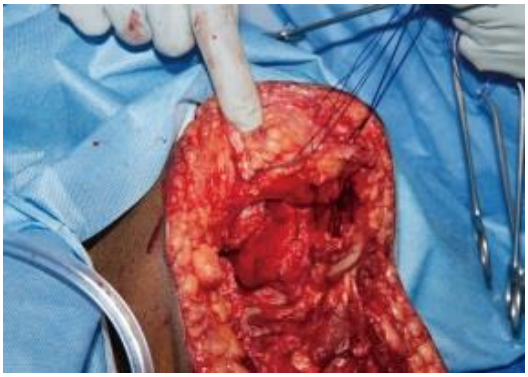
Medications used to treat diabetes do so by lowering blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available by mouth, such as metformin, while others are only available by injection such as GLP-1 agonists. Type 1 diabetes can only be treated with insulin, typically with a combination of regular and NPH insulin, or synthetic insulin analogs.

Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality.[4] It works by decreasing the liver's production of glucoseSeveral other groups of drugs, mostly given by mouth, may also decrease blood sugar in type II DM. These include agents that increase insulin release, agents that decrease absorption of sugar from the intestines, and agents that make the body more sensitive to insulin. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications. Doses of insulin are then increased to effect.

Since cardiovascular disease is a serious complication associated with diabetes, some have recommended blood pressure levels below 130/80 mmHg. However, evidence supports less than or equal to somewhere between 140/90 mmHg to 160/100 mmHg; the only additional benefit found for blood pressure targets beneath this range was an isolated decrease in stroke risk, and this was accompanied by an increased risk of other serious adverse events. A 2016 review found potential harm to treating lower than 140 mmHg. Among medications that lower blood pressure, angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications angiotensin receptor blockers (ARBs) do not.Aspirin is also recommended for people with cardiovascular problems, however routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes.

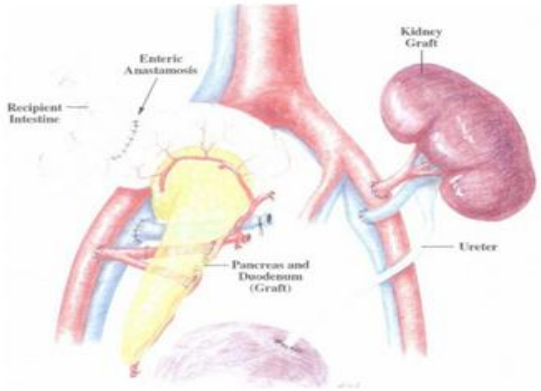


Surgery



Weight loss surgery in those with obesity and type two diabetes is often an effective measure. Many are able to maintain normal blood sugar levels with little or no medications following surgery and long-term mortality is decreased. There is, however, a short-term mortality risk of less than 1% from the surgery. The body mass index cutoffs for when surgery is appropriate are not yet clear it is recommended that this option be considered in those who are unable to get both their weight and blood sugar under control.

A pancreas transplant is occasionally considered for people with type 1 diabetes who have severe complications of their disease, including end stage kidney disease requiring kidney transplantation.



Support

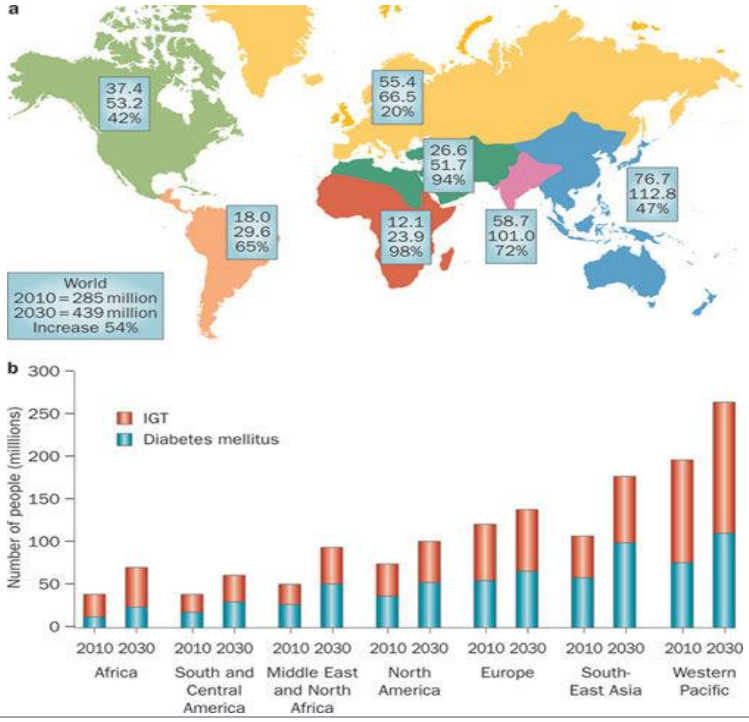
In countries using a general practitioner system, such as the United Kingdom, care may take place mainly outside hospitals, with hospital-based specialist care used only in case of complications, difficult blood sugar control, or research projects. In other circumstances, general practitioners and specialists share care in a team approach. Home telehealthsupport can be an effective management technique.

Epidemiology

As of 2016, 422 million people have diabetes worldwide, up from an estimated 382 million people in 2013and from 108 million in 1980.Accounting for the shifting age structure of the global population, the prevalence of diabetes is 8.5% among adults, nearly double the rate of 4.7% in 1980.Type 2 makes up about 90% of the cases. Some data indicate rates are roughly equal in women and men, but male excess in diabetes has been found in many populations with higher type 2 incidence, possibly due to sex-related differences in insulin sensitivity, consequences of obesity and regional body fat deposition, and other contributing factors such as high blood pressure, tobacco smoking, and alcohol intake.

The World Health Organization (WHO) estimates that diabetes mellitus resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death. However another 2.2 million deaths worldwide were attributable to high blood glucose and the increased risks of cardiovascular disease and other associated complications (e.g. kidney failure), which often lead to premature death and are often listed as the underlying cause on death certificates rather than diabetes. For example, in 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths worldwide,[16] using modeling to estimate the total number of deaths that could be directly or indirectly attributed to diabetes.

Diabetes mellitus occurs throughout the world but is more common (especially type 2) in more developed countries. The greatest increase in rates has however been seen in low- and middle-income countries,where more than 80% of diabetic deaths occur.The fastest prevalence increase is expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030. The increase in rates in developing countries follows the trend of urbanization and lifestyle changes, including increasingly sedentary lifestyles, less physically demanding work and the global nutrition transition, marked by increased intake of foods that are high energy-dense but nutrient-poor (often high in sugar and saturated fats, sometimes referred to as the "Western-style" diet).



History

Diabetes was one of the first diseases described, with an Egyptian manuscript from c. 1500 BCE mentioning "too great emptying of the urine". The Ebers papyrus includes a recommendation for a drink to be taken in such cases. The first described cases are believed to be of type 1 diabetes Indian physicians around the same time identified the disease and classified it as *madhumeha* or "honey urine", noting the urine would attract ants.

The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis.The disease was considered rare during the time of the Roman empire, with Galen commenting he had only seen two cases during his career. This is possibly due to the diet and lifestyle of the ancients, or because the clinical symptoms were observed during the advanced stage of the disease. Galen named the disease "diarrhea of the urine" (diarrhea urinosa).

The earliest surviving work with a detailed reference to diabetes is that of Aretaeus of Cappadocia (2nd or early 3rd century CE). He described the symptoms and the course of the disease, which he attributed to the moisture and coldness, reflecting the beliefs of the "Pneumatic School". He hypothesized a correlation of diabetes with other diseases, and he discussed differential diagnosis from the snakebite which also provokes excessive thirst. His work remained unknown in the West until 1552, when the first Latin edition was published in Venice.

Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400–500 CE with type 1 associated with youth and type 2 with being overweight.The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination. Effective treatment was not developed until the early part of the 20th century, when Canadians Frederick Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922.This was followed by the development of the long-acting insulin NPH in the 1940s.

Society and culture

The 1989 "St. Vincent Declaration" was the result of international efforts to improve the care accorded to those with diabetes. Doing so is important not only in terms of quality of life and life expectancy but also economically – expenses due to diabetes have been shown to be a major drain on health – and productivity-related resources for healthcare systems and governments.

Several countries established more and less successful national diabetes programmes to improve treatment of the disease.

People with diabetes who have neuropathic symptoms such as numbness or tingling in feet or hands are twice as likely to be unemployed as those without the symptoms.

In 2010, diabetes-related emergency room (ER) visit rates in the United States were higher among people from the lowest income communities (526 per 10,000 population) than from the highest income communities (236 per 10,000 population). Approximately 9.4% of diabetes-related ER visits were for the uninsured.

Other animals

In animals, diabetes is most commonly encountered in dogs and cats. Middle-aged animals are most commonly affected. Female dogs are twice as likely to be affected as males, while according to some sources, male cats are also more prone than females. In both species, all breeds may be affected, but some small dog breeds are particularly likely to develop diabetes, such as Miniature Poodles.

Feline diabetes mellitus is strikingly similar to human type 2 diabetes. The Burmese breed, along with the Russian Blue, Abyssinian, and Norwegian Forest cat breeds, showed an increased risk of DM, while several breeds showed a lower risk. There is an association between overweight and an increased risk of feline diabetes.

The symptoms may relate to fluid loss and polyuria, but the course may also be insidious. Diabetic animals are more prone to infections. The long-term complications recognized in humans are much rarer in animals. The principles of treatment (weight loss, oral antidiabetics, subcutaneous insulin) and management of emergencies (e.g. ketoacidosis) are similar to those in humans.

Research

Inhalable insulin has been developed.The original products were withdrawn due to side effects. Afrezza, under development by the pharmaceuticals company MannKind Corporation, was approved by the FDA for general sale in June 2014. An advantage to inhaled insulin is that it may be more convenient and easy to use.

Transdermal insulin in the form of a cream has been developed and trials are being conducted on people with type 2 diabetes.

Objective To assess the relation between adherence to a Mediterranean diet and the incidence of diabetes among initially healthy participants.

Design Prospective cohort study with estimates of relative risk adjusted for sex, age, years of university education, total energy intake, body mass index, physical activity, sedentary habits, smoking, family history of diabetes, and personal history of hypertension.

Participants 06 50 Native Hyderabadi's without diabetes, 50 Hyderabad's with Diabetes

Main outcome measures Dietary habits assessed at baseline with a validated 100 item food frequency questionnaire and scored on a nine point index. New cases of diabetes confirmed through medical reports and an additional detailed questionnaire posted to those who self-reported a new diagnosis of diabetes by a doctor during follow-up. Confirmed cases of type 2 diabetes.

Results : Participants who adhered closely to a Mediterranean diet had a lower risk of diabetes. The incidence rate ratios adjusted for sex and age were 0.41 (95% confidence interval 0.19 to 0.87) for those with moderate adherence (score 3-6) and 0.17 (0.04 to 0.75) for those with the highest adherence (score 7-9) compared with those with low adherence (score <3). In the fully adjusted analyses the results were similar. A two point increase in the score was associated with a 35% relative reduction in the risk of diabetes (incidence rate ratio 0.65, 0.44 to 0.95), with a significant inverse linear trend (P=0.04) in the multivariate analysis.

Conclusion: Adherence to a Mediterranean diet is associated with a reduced risk of diabetes

Introduction

Diabetes mellitus is an increasingly important global public health problem that threatens to reach pandemic levels by 2030 .[1] [2] As some randomised trials have consistently shown, increased physical activity and weight loss are efficient approaches for the control and prevention of type 2 diabetes.[3] [4] Diet has also long been believed to be an important risk factor for diabetes. Many studies have shown that the Mediterranean food pattern has a role in prevention of cardiovascular disease.[5] [6] [7] [8] [9] The similarity of some risk factors and some empirical and mechanistic evidence suggest that the Mediterranean diet can also protect against diabetes. The major protective characteristics include a high intake of fibre, a high intake of vegetable fat, a low intake of **trans** fatty acids, and a moderate intake of alcohol.[4] [10] [11] Moreover, a particular feature of the diet is the abundant use of virgin olive oil for cooking, frying, spreading on bread, or dressing salads.[12] This leads to a high ratio of monounsaturated fatty acids to saturated fatty acids. This ratio can be used to score adherence to a Mediterranean diet[8] as the traditional diet. Despite having a relatively high total fat content, this food pattern is rich in monounsaturated fatty acids (from olive oil) and poor in saturated fatty acids. Diets rich in monounsaturated fatty acids improve lipid profiles and glycemic control in people with diabetes, suggesting that a high intake improves insulin sensitivity.[13] [14] [15] [16]Together these associations suggest the hypothesis that following an overall pattern of Mediterranean diet can protect against diabetes. In addition to having a long tradition of use without evidence of harm, a Mediterranean diet is highly palatable, and people are likely to comply with it.[17]

Few prospective studies have evaluated the specific role of the Mediterranean diet on the risk of developing diabetes in initially healthy Mediterranean populations. A prospective follow-up study recently reported an inverse association between adherence to the diet and the incidence of diabetes.[18] All members of that study, however, had survived a previous myocardial infarction and the tool to assess dietary habits had inherent limitations because it included only a short list of items, and the authors did not attempt to measure the entire diet.

We evaluated the association between adherence to a Mediterranean diet and the incidence of diabetes using a full validated food frequency questionnaire to measure the entire diet.

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Dietary assessment

We assessed dietary habits at baseline using a semi-quantitative food frequency questionnaire with 136 items, previously validated in Spain.[21] In the validation study, the correlation coefficients between the questionnaire and diet records ranged from 0.45 for vitamin A to 0.91 for alcohol.[21] With regard to gross misclassification, on average 3% of participants classified in the highest or lowest fifth

of the distribution by food record were assigned to the lowest or highest fifth by the food frequency questionnaire.[21] The questionnaire was based on typical portion sizes and had nine options for the frequency of intake in the previous year for each food item (ranging from never or almost never to six or more times a day). We used the sum of the consumption of each of several food items to estimate the overall consumption of the food group to which each item belonged (vegetables, fruits, cereals, legumes, fish, meats, fast food, dairy products). We applied the specified portion sizes for each item and summed up across all foods of that group. Then we computed the mean for all the participants in each category of the three categories of the score to create an estimated daily intake.
Nutrient intake scores were computed with a specifically developed computer program. A trained dietitian updated the nutrient data bank using the latest available information included in food composition tables for Spain. The questionnaire included questions on use of fats and oils, cooking methods, and dietary supplements. For example, we calculated total consumption of olive oil (a major source of monounsaturated fat in this population) from the nutrient score of olive oil consumption, taking into account the amount and type of olive oil used for cooking or frying and the use of olive oil for salad dressing or as a spread on bread or other foods.

Adherence to the Mediterranean diet was appraised according to the score created by Trichopoulou et al,[22] which is extensively used because it is simple and has variants created to evaluate multiple diet-health relations.[23]Originally, this index included only eight components to define the Mediterranean diet[22]: high ratio of monounsaturated: saturated fatty acids, moderate intake of alcohol, high intake of legumes, high intake of grains, high intake of fruit and nuts, high intake of vegetables, low intake of meat and meat products, and moderate intake of milk and dairy products. The same authors subsequently added high intake of fish.[8] The index assigns a score of 0 or 1 according to the daily intake of each of the nine components. With the exception of alcohol, the sex specific medians of the sample were used as cut-off points and the unit of measurement was grams per day.[8 22 23 24] For each of the six protective components (fatty acid ratio, legumes, grains, fruits, vegetables, or fish) participants received one point if their intake was over the sample median. Participants received one point if the intake was below the median for the two non-protective components (dairy products or meat). For alcohol one point was scored if consumption was 10-50 g/day for men or 5-25 g/day for women. If participants met all the characteristics of the Mediterranean diet, their score was the highest (nine points), reflecting maximum adherence. If they met none of the characteristics the score was minimum (zero), reflecting no adherence at all.

Ascertainment of diabetes

Participants reported any medical diagnosis of diabetes at baseline and in each of the follow-up questionnaires. The baseline questionnaire also collected data on use of insulin or oral antidiabetic agents. Follow-up questionnaires recorded the date of any new diagnosis of diabetes (if applicable)

Participants were considered to have diabetes at baseline if they reported a medical diagnosis of diabetes or were receiving insulin or oral antidiabetic agents. Probable cases of new onset diabetes were defined as those participants who reported a diagnosis of diabetes made by a doctor in the follow-up questionnaire and did not have diabetes at baseline. We sent additional questionnaires to participants who reported probable new onset diabetes, requesting confirmation of the diagnosis, the type of diabetes, the date of the diagnosis, whether the diabetes was diagnosed during pregnancy, the highest fasting glucose value that they ever had, whether they had ever undergone an oral glucose tolerance test and its results, their current use of insulin or oral antidiabetic agents, and the incidence of some complications. We also asked them to send us the medical report detailing the diagnosis. A panel of physicians, blinded to the information about dietary habits, used the information provided by participants (additional questionnaires and medical reports) to classify the diagnosis as incident type 2 diabetes or not. The diagnosis criteria for type 2 diabetes were those of the American Diabetes Association (symptoms of diabetes plus casual plasma glucose concentration ≥11.1 mmol/l or fasting plasma glucose ≥7.0 mmol/l, or two hour post luunch glucose ≥11.1 mmol/l during an oral glucose tolerance test; in the absence of unequivocal hyperglycemia, confirmation by repeat testing on a different day is requested). Participants were classified as having confirmed incident type 2 diabetes only after undergoing necessary medical treatments .

Statistical analysis

To estimate incidence rate ratios and their 95% confidence intervals, we ran Poisson regression models with robust standard errors, controlling simultaneously for known risk factors for diabetes: sex, age, years of university education, total energy intake, body mass index, physical activity, hours sitting down a week, family history of diabetes, baseline hypertension, and smoking. For all analyses, we considered participants with the lowest level of adherence to the Mediterranean diet reference category.

When we designed the study, taking into account the planned age and sex distribution of cohort members and the available data about incidence of diabetes in Hyderabad from CDTA, we expected an overall rate of new onset type 2 diabetes of three cases for 1000 person years of follow-up. Thus, in the follow-up of 12 000 cohort members for an average 4.4 years we would have expected 158 new cases of type 2 diabetes and the statistical power would have been >90% for the comparison of the two extreme quarters, assuming a relative risk of 0.5 and a two tailed α error of 0.05. The statistical power for the trend test would be higher because both intermediate quarters would also be taken into account.

Because of the surprisingly low incidence of confirmed cases of diabetes, instead of using quarters, we grouped the exposure variable into only three categories. In addition to the reference category (lowest

adherence), we built two other categories: moderate adherence and high adherence. We did not use any imputation method for the missing data of participants lost to follow-up.

Results

During the follow-up period 50 Days, 25 participants initially free of diabetes (according to their baseline questionnaire) self-reported a new diagnosis of diabetes. After we excluded 10 women who developed gestational diabetes, six cases of impaired glucose tolerance mistakenly reported by participants as diabetes, three participants with prevalent type 2 diabetes at baseline who did not report it in the baseline questionnaire, one case of incident type 1 diabetes, and 21 participants who did not send us any additional medical report, we identified 33 cases of new onset confirmed type 2 diabetes among 100 Persons Investigated

Discussion

This large prospective study shows that a traditional Mediterranean food pattern is associated with a significant reduction in the risk of developing type 2 diabetes.

Interestingly, among participants with the highest adherence to the diet, there was a high prevalence of important risk factors for diabetes, such as older age, higher BMI, family history of diabetes, and personal history of hypertension and a higher proportion of ex-smokers. Therefore, we would have expected a higher incidence of diabetes among these participants. These higher risk participants with better adherence to the diet, however, had a lower risk of diabetes, suggesting that the diet might have a substantial potential for prevention. This finding is consistent with our previous report of an inverse association between a Mediterranean diet and the metabolic syndrome. The metabolic syndrome is closely associated with a generalized metabolic disorder of insulin resistance, which is one of the underlying causes of type 2 diabetes. Therefore a high biological plausibility exists to support the causality of our findings. In addition, a previous cohort study of survivors of myocardial infarction also reported that a higher adherence to a Mediterranean diet was associated with a reduction in the risk of type 2 diabetes, despite use of a relatively inaccurate tool for the dietary assessment. The inverse graded dose-response pattern and the significant inverse trend that we observed also support a causal relation.

Diet and disease

Experimental evidence suggesting that a diet similar to the Mediterranean diet and rich in plant based foods might protect against diabetes can also be found in the Indian diabetes prevention program me. That trial promoted a plant based diet (together with increases in physical activity) and found a significant reduction in the incidence of diabetes, although there are clear differences between the Mediterranean and Indian food patterns.

There is an analogy between coronary heart disease and diabetes because patients with type 2 diabetes and no coronary heart disease have a risk of coronary heart disease similar to patients without diabetes but with prior coronary heart disease. There is evidence that a Mediterranean diet protects against coronary heart disease, and the analogy between coronary heart disease and diabetes suggests that this diet might also prevent diabetes. It has been shown to provide protection against coronary mortality and the incidence of non-fatal myocardial infarction. In addition, both cohort studies and randomized trials[36] have found that adherence to a Mediterranean diet protects against mortality in patients who already have established coronary heart disease.

The potential mechanisms explaining the protective effect of a Mediterranean diet on diabetes have been reviewed elsewhere. Two trials have shown that virgin olive oil protects against insulin resistance and the metabolic syndrome. Indexes of insulin resistance were significantly improved among participants allocated to a Mediterranean diet rich in virgin olive oil. Olive oil is rich in monounsaturated fatty acids, and a diet rich in monounsaturated fatty acids is beneficial among those with diabetes and might lead to improved insulin sensitivity and better lipid profiles than diets rich in carbohydrate. People allocated to a Mediterranean diet secrete more glucagon-like peptide-1,a finding also seen in animals. The non-fat minor components of virgin olive oil also exert a beneficial effect on pro-inflammatory cytokines.

Apart from olive oil, adherence to an overall Mediterranean-type food pattern is related to lower plasma concentrations of inflammatory markers and markers of endothelial dysfunction. these biomarkers are predictive of the future occurrence of type 2 diabetes. In addition, a large cross sectional study nested in the nurses' health study found that increased adherence to a Mediterranean diet was associated with higher levels of adiponectin, and higher levels of adiponectin are associated with a reduced risk of diabetes.

Limitations

The number of new cases of diabetes was small, despite the follow-up of several thousand people for over four years. This small number of incident cases is a major drawback and can compromise the statistical power of our study. Nevertheless, our participants had high absolute levels of consumption of the typical food items of the Mediterranean diet, even among those participants classified as poorly compliant. For example, among those in the lowest category of adherence to the diet, the estimated mean daily absolute consumption (g/day) of olive oil (12), vegetables (308), fruits (176), cereals (77), and legumes (17) can be considered as healthy for the standards of nutritional epidemiological studies. This high consumption of plant based foods in our cohort could be because our participants were from a Mediterranean country and were highly educated and health conscious. The small number of new cases observed in this study should therefore not be surprising. If the Mediterranean diet is actually protecting against diabetes, we would expecta low incidence in a young cohort (mean age is 37.8 years) with these characteristics. The low number of observed cases precluded assessment of the

specific role of single dietary factors because we would have needed multiple adjustments for other dietary factors (in addition to the confounders already included in the model) and the statistical power to adjust for so many factors would have been low. The Mediterranean food pattern has the potential to minimise confounding by including nutritional confounders in the score and capturing effect modification among the nutritional variables.[5]

Diabetes might have been under-reported in our participants, despite their high educational level and easy access to medical care—half of them are health professionals. The proportions of participants aged over 65 years across increasing categories of adherence to the diet were 0.6% ,1.7% , and 3.5% . Therefore, older participants were more compliant with the diet. If selective under-reporting of diabetes was present among the older participants, this would provide an alternative non-causal explanation for our findings. We acknowledge this possibility, but the proportion of selective underdiagnoses of diabetes among older people is likely to be lower as they receive closer medical attention and routine medical care in Spain includes assessment of fasting glucose.

All our participants are university graduates and the generalizability of our findings to other groups with less education should be assumed only on biological grounds but not at all on "representativeness" of the general population. Also, the building of the diet score was based on sample specific median cut-off points, and our participants had high absolute levels of consumption of favorable foods and low absolute intakes of detrimental foods. Therefore, it will be difficult to compare our results with those of non-Mediterranean countries where levels of consumption of favourable foods in the general population are much lower. This is an additional limitation for external validity, but we selected the score proposed by Trichopoulou et al for the sake of comparability with previous studies.

A potential caveat might be the quality of our nutritional assessment. Food frequency questionnaires are known to contain a certain degree of measurement error, which might affect results that depend on such questionnaires to assess diet and risk of chronic disease. Total energy intake was included as a covariate in the model to achieve the equivalent of an is caloric diet and to reduce measurement error in the score. Measurement error, however, would probably have introduced non-differential misclassification, and the implications for the results of this error would have been to bias the estimates towards the null.

A potential limitation, inherent to every observational design, is the possibility of residual confounding by unmeasured or unrecorded factors. Our major confounders, however, were sex and age. Additional adjustment for other factors made only negligible changes in the estimates, suggesting that residual confounding is unlikely.

Conclusion

Our prospective cohort study suggests that substantial protection against diabetes can be obtained with the traditional Mediterranean diet, rich in olive oil, vegetables, fruits, nuts, cereals, legumes, and fish but relatively low in meat and dairy products. The limited number of cases of diabetes and the possibility of under-reporting, however, requires that further larger cohorts and research

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GOVERNMENT DEGREE COLLEGE OF WOMEN

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APPLIED NUTRITION

STUDENT STUDY PROJECT

(2021-22)

TOPIC

CAPPARIS FASCICULARISCRUDE EXTRACTS: POTENT SOURCE OF NATURAL ANTIBACTERIAL ACTIVITY

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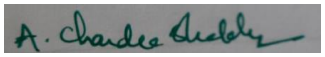

Supervisor

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1.0 INTRODUCTION

The herbal products usually symbolize safety in contrast to the synthetic drugs that are harmful to human beings and environment (Chin et al., 2006). Majority of the medicinal plants especially of Indian origin have been in continuous use for the treatment of different types of aliments. However, there are still several plants which have not been evaluated for their use as traditional medicine. Extraction and isolation of therapeutically valuable natural compounds is necessary to scientifically validate the newly found medicinal plants.

1.1 Description

A scrambling shrub or climber, usually with hooked spines on the stem.The most notable difference between these varieties is that var. *fascicularis* has indented leaf-tips whereas var has pointed leaf-tips. Produced on leafless side branchlets which resemble spikes or racemes. The fruit are spherical and 6-15 mm in diameter, ripening to purple-black. *Capparis* is a dominating genus of the family Capparidaceae. *Capparis* spp. are xerophytic, growing in a broad range of climatic conditions, such as dry deserts to cooler terrains of mountain either as shrubs, trees or creepers. The female flowers of some of the

Capparis species are used as vegetable and fruits are used in pickle production because of their high nutritive ingredients like proteins, carbohydrate, minerals and vitamins. Whole plant or parts are used for curing asthma, rheumatism, diabetes, paralysis, toothache, as antihelmintic, antiallergic, snakebite antidote, etc. Out of the many *Capparis* species, a few are of specific interest for curing particular ailments, like tuberculosis, cancer, rheumatism or diabetes, which still requires extensive study.

Simultaneously, it will be valuable to evaluate utility potential of flowers/fruits in cancer patients due to high titre of spermidin containing alkaloids, which are implicated in tumorogenesis.

The review highlights medicinal importance of the *Capparis* products and unnoticed threatened status in their respective niches for sustainable use and long lasting conservation. Being harsh terrain species, plant needs to be considered for strategic planning for greening deserts hilltops(4).

*Capparis fascicularis*Flowers of *Capparis fascicularis* var.

Capparis fascicularis var. *fascicularis*

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms

(unranked): Eudicots

(unranked): Rosids

Order: Brassicales

Family: Capparaceae

Genus: *Capparis*

Species: ***C. fascicularis***

Binomial name

Capparis fascicularis

2.0 MATERIALS AND METHODS

2.1 PREPARATION OF EXTRACTS

Leaves were washed with water, dried under shade, leaves were homogenized to coarse powder whereas, fruits was shade dried and homogenizes to coarse powder (100 grams) was used for extraction with methanol, water, acetone, ethanol, chlorofom and subjected for dryness under reduced pressure by rota vapor at 40-50 ^{0}C for 3 h. Further on the CAPPARIS FASCICULARIS leaf, , fruits extracts

2.2 QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

The extracts were subjected for the preliminary phytochemical analysis using standard methods described by Trease and Evans (1989) and Harborne (1998).

2.3 DETERMINATION OF TOTAL PHENOL CONTENT

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent by following described method with slight modifications [7].Separately, 1 ml of various alcoholic extracts of plant extract of different concentrations (50,100 and 150 µg/ml) and standard solution of tannic acid (10 µg/ml) was added separately to 100 ml volumetric flask separately, that contained about 60 ml distilled water and followed by the addition of 5 ml of Folin–Ciocalteu reagent. The content was mixed thoroughly and kept constant for about 10 min. To this, add 15 ml Na_2CO_3 (20 %) and make up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-visible spectrophotometer.

2.4 DETERMINATION OF FLAVONOID ASSAY

Flavonoid content was measured by aluminum chloride colorimetric assay with slight modification.[8] 1 ml of plant extract alcoholic extracts with different concentrations (50,100 and 150 µg/ml) and standard solution of catechin (10 µg/ml) was added separately to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture 0.3 ml of 5% $NaNO_2$ was added, followed by the addition of 0.3 ml of 10% $AlCl_3$ after 5 min. After incubation period of 6 min 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared

2.5 ANTIBACTERIAL ACTIVITY

The bacterial species selected were *Methycilin resistant Staphylococcus aureus*, *Bacillus subtillis*, *,Escherichia coli,klebcella* . All these strains were obtained from department of biochemistry GDC(W) Karimnagar.

2.5.1 PREPARATION OF BACTERIAL SUSPENSION

The bacterial strains were inoculated into sterilized nutritive broth and incubated at 35 ± 2°C for 24 h. The turbidity of the resulting suspensions are diluted with same nutritive broth to obtain a transmittance of 25% at 580 nm, this percentage was calculated spectrophotometrically using Bausch & Lomb spectrophotometer comparable to McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 × 108 CFU/ml (a stock standard from which a working standard was drawn with concentration of 1 × 108 CFU/ml).

The antibacterial activity of these extracts was carried out according to the method described by Raman with slight modifications [9]. Each selective medium was inoculated with the test organism suspended in nutritive broth. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts of CFL and CFF at various concentrations and corresponding wells with positive and negative control. The concentration of the

methanol,ethanol,aceton,water extracts employed at concentrations 50, 100 and 150 µg/ml simultaneously, Azythromycin (10 µg/ml) is used as positive control. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h. The inhibition zone diameter was measured in mm.

eagent blank at 510 nm.

2.6.1Potato Dextrose Agar (PDA) Medium (pH – 6.7)

Potato - 250g Dextrose - 15g Agar - 18g Distilled water - 1000ml

The potato tubers were peeled off and weighed for about 250g tubers were chopped in to small pieces in to the sterile conical flask. After boiling the supernatant were collected and dextrose (15g) with agar (18g) to dissolve the ingredients. The medium was mentioned and adjusted to 6.5pH. finally the medium was sterilized in pressure cooker for 20min.

3.0 RESULTS AND DISCUSSION

3.1 PHYTOCHEMICAL ANALYSIS OF THE PLANT EXTRACTS

Extracts of ***CAPPARIS FASCICULARIS*** extracts resulted in the presence of various types of chemicals. Fruit and leaves extracts were given positive results for all phyto chemicals tested. The results were shown in table 1.

Table-1: Phytochemical analysis of leaf ,stem, root and flower extract of different solutions , absent or present

	leaves					***fruit***				
	wateer	***aceton***	***chloroform***	***methanol***	***ethanol***	***water***	***aceton***	***chloroform***	***methanol***	***ethanol***
glycoside	+	+	+	+	+	+	+	+	+	+
coummarins	+	-	-	-	-	+	-	-	-	-
leucoanthocyanine	+	-	+	-	-	+	-	+	-	-
steriodes	+	+	-	+	+	+	+	+	+	+
terpenoides	+	+	+	+	+	-	+	+	+	+
saponins	+	-	-	-	-	+	-	-	-	+
Tannins (led acet)	+	+	-	+	+	+	-	-	+	+
flavonoide	+	-	-	-	-	+	-	-	-	+

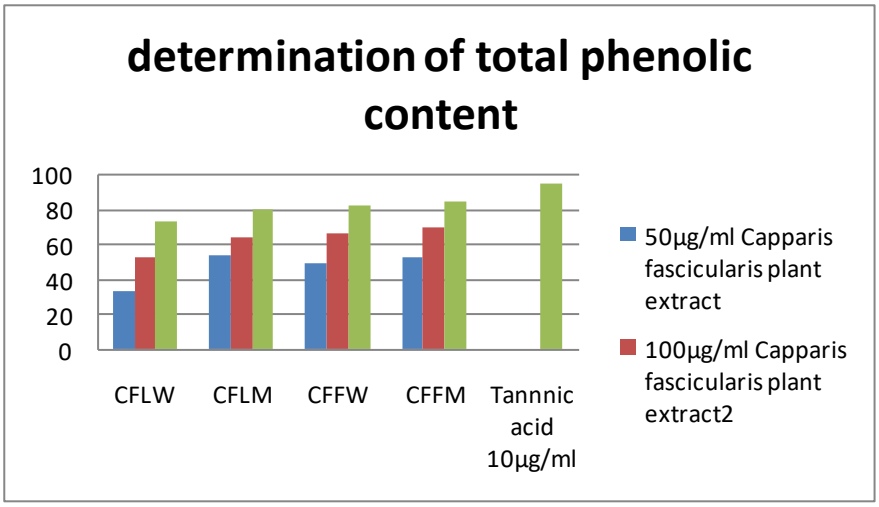
3.2 DETERMINATION OF TOTAL PHENOL CONTENT

Total phenolic content of ***plant water and methanol extract***revealed that all the plant extracts possessed concentration dependent increase in the amount of phenols. The percentage yield of phenolic content was found to be 33, 52, 73 and 53, 64, 79 at 50, 150, 250 µg/ml of plant extracts respectively. The highest yield was noticed with fruit and leaf extracts were comparable with reference standard tannic acid 61.2, 80.5, 94.1 at 10 µg/ml (Figure 2).

Table 2: Total phenols content ***of*** **CAPPARIS FASCICULARIS**

	water			Methanol		
	50	100	150	50	100	150
leafe	0.33	0.52	0.73	0.53	0.64	0.79
fruit	0.49	0.66	0.82	0.52	0.69	0.84

Figure 2: Determination of Total Phenol content at various concentrations of Chordia dichotoma *extract*



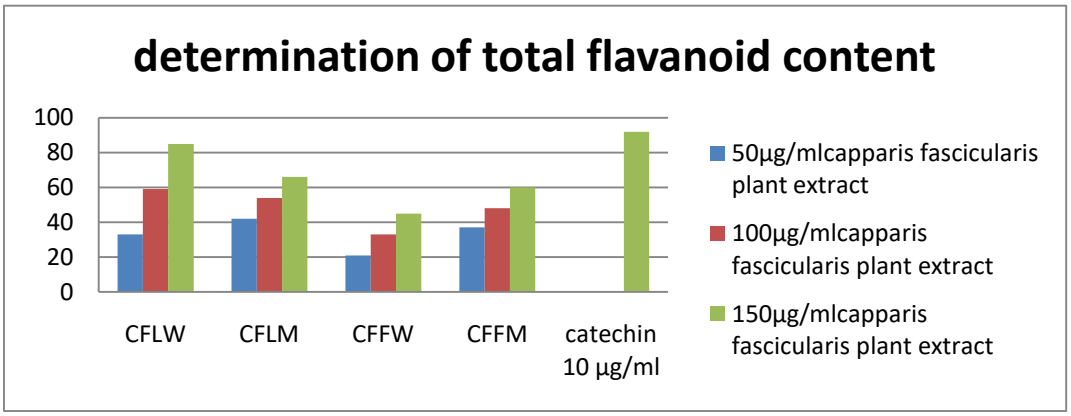
3.3 DETERMINATION OF TOTAL FLAVONOID CONTENT

The percentage yield of total flavonoid content is found to be 33, 59, 85 and 42, 54, 66and 21, 33, 45 and 37,48,60 at 50, 150, 250 µg/ml of plant extract respectively. The highest yield was noticed with stem bark and leaf extracts and are comparable with reference standard catechin 62.8, 75.4, 91.9 at 10 µg/ml respectively (Figure 3).

Table 3: Total flavonoid content ***of*** **CAPPARIS FASCICULARIS**

	water			methanol		
	50	**100**	**150**	**50**	**100**	**150**
Leafextrct	**0.33**	**0.59**	**0.85**	**0.42**	**0.54**	**0.66**
Fruit	**0.21**	**0.33**	**0.45**	**0.37**	**0.48**	**0.60**

Figure3: Determination of Total Flavanoid content at various concentrations of Chordia dichotoma extract



3.4 ANTIBACTERIAL ACTIVITY

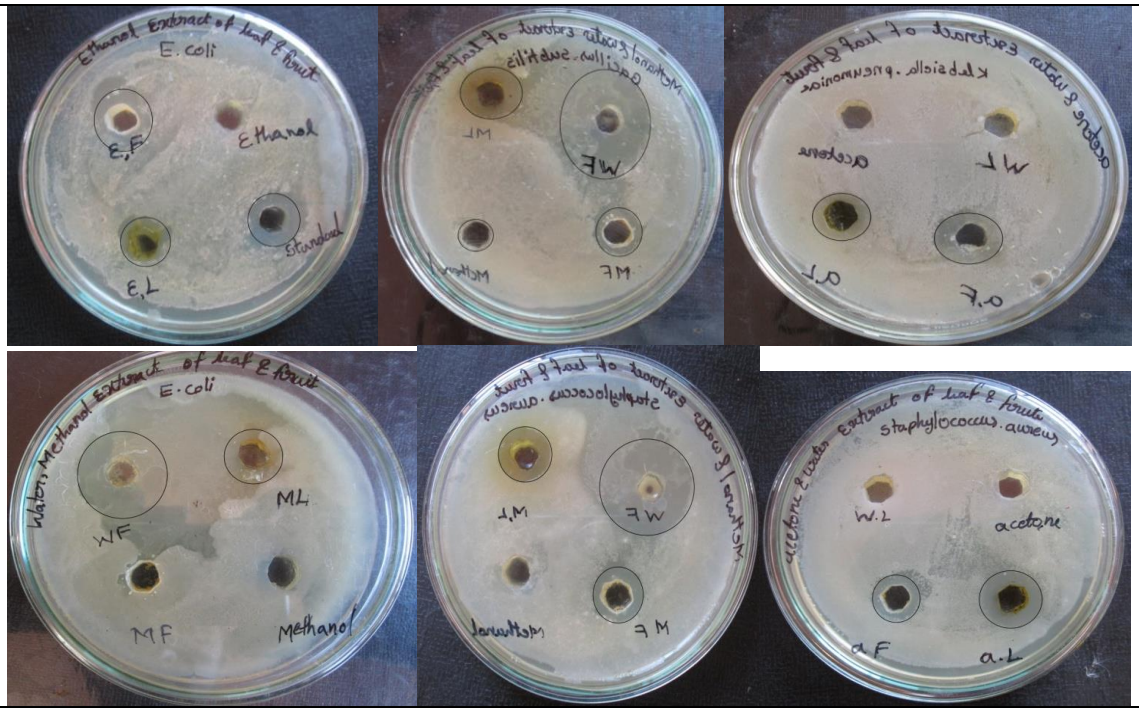
The *CAPPARIS FASCICULARIS* extracts of leaves and fruits showed good antibacterial activities. (Table 4). Fruit water exhibited significant activity againsBACILLUS.SUBTILISby producing inhibition zones 0.9mmat 50 µg/ml respectively. STAPHYLOCOCCUS.AUREUS,ESCHERICHIA.COLI were also more susceptible towards fruit water extract and exhibited 0.8 and 0.7 inhibition zones at 50,µg/ml respectively. Among the gram positive strains. The highest inhibition zone 0.9mm was produced by fruit extract against

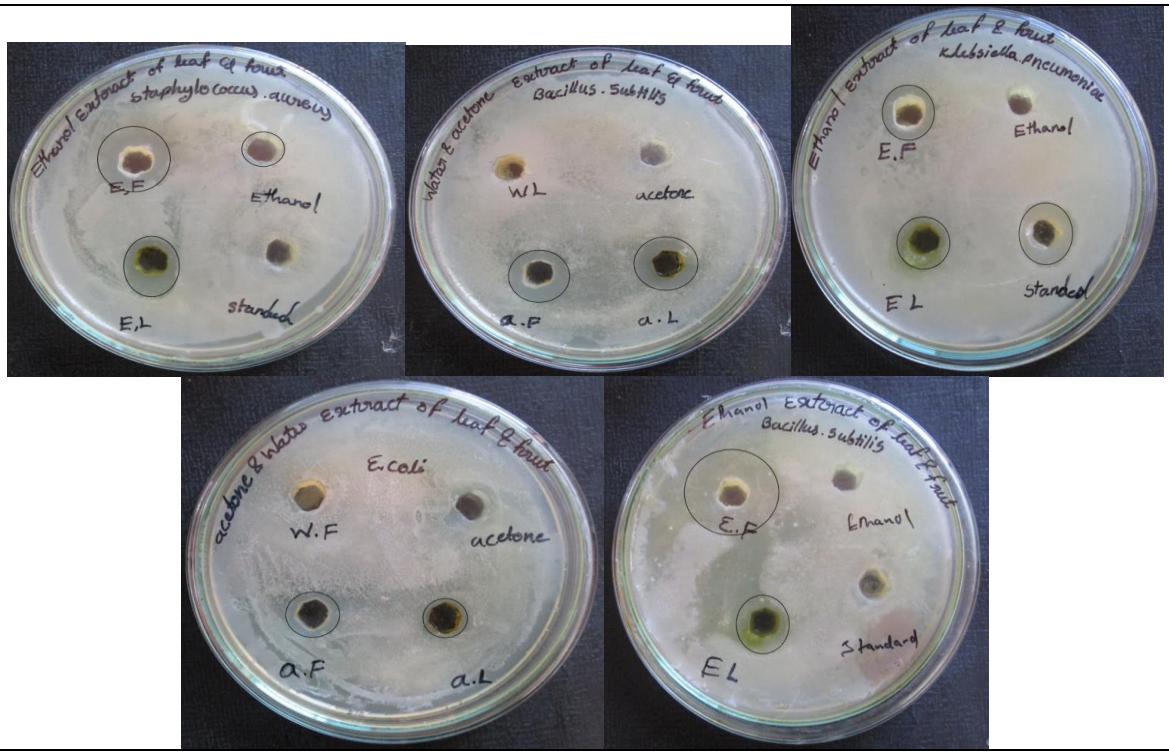
BACILLUS.SUBTILIS. Results of antibacterial activity of the extracts were compared with known standards.

		Fruit				leafe			
		water	aceton	methanol	ethanol	water	aceton	methanol	ethanol
Gram+Ve	BACILLUS.SUBTILIS	0.9mm	0.3mm	0.2mm	0.5mm	-	0.3mm	0.5mm	0.2mm
	STAPHYLOCOCCUS.AUREUS	0.8mm	0.2mm	0.3mm	0.3mm	-	0.4mm	0.3mm	0.2mm
Gram-Ve	KIEBSIELLA.PNUMONIAE	0.3mm	0.4mm	0.2mm	0.2mm	-	0.3mm	0.1mm	0.2mm
	ESCHERICHIA.COLI	0.7mm	0.2mm	-	0.2mm	0.3mm	0.1mm	0.1mm	0.2mm

(Table 4).

Table-4: Antimicrobial activity of leaf and fruit extract of **CAPPARIS FASCICULARIS**





4.0 CONCLUSION :

The extracts fo *CAPPARIS FASCICULARIS*possessed antibacterial activity. Therefore The plant which has been used as traditional medicine for the treatment of various human aliments.

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GOVERNMENT DEGREE COLLEGE OF WOMEN

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APPLIED NUTRITION

STUDENT STUDY PROJECT

(2021-22)

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METHODS OF PROTEIN PURIFICATION

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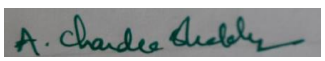


Supervisor

Protein purification:

Purity is defined by the general level of protein contaminants and also by the absence of contaminants of special interests such as microbes, toxins etc.

Protein purification is divided into ***five stages***:

1. Preparation of sources
2. Knowledge of protein properties
3. Development of an assay
4. Primary isolation
5. Final purification

I. Preparation of sources:

- ✓ The *raw materials* from which proteins can be isolated such as microbial culture or animals or plant sources should be selected.
- ✓ The amount of protein can be increased by increasing cultivation volume.

II. Knowledge of protein properties:

- ✓ Before employing any procedure, one should know about different properties of proteins such as- intracellular or extracellular occurrence, *denaturation temperature*, *pH range*, *ionic stability*, *molecular weight*, *charge*, *iso-electric point*, *binding partners* etc.

III. Development of an assay:

- ✓ An assay developed should be convenient, *easy*, *rapid*, and *precise* for purification.

IV. Primary isolation:

- ✓ This consists of separation of protein from other cellular components.

- ✓ For this propose there are different methods:

1. Concentration:

- ✓ Different methods can be employed for concentration of extracellular protein.
- ✓ *Ultrafiltration* is usually used to concentrate extracellular proteins from cell.
- ✓ Ultrafiltration is a membrane filtration in which hydrostatic pressure is applied which causes movement of solution across the semi-permeable membrane.
- ✓ Water and low molecular weight solute pass while other high molecular weight of molecules trapped in membrane.
- ✓ The protein molecules are adsorbed in the membrane surface.

2. Cell lysis: (For intracellular protein)

- ✓ The intracellular proteins are liberated by cell lysis. There are different methods for cell lysis.

a. Physical method:

- ➢ *Mechanical method:* Bead mill, *Homogenizer*, Microfluidizer, Sonicator etc.
- ➢ *Non-mechanical method:* Decompression, Osmotic shock, *Thermolysis*, Freeze thaw, Dessication, Cell bomb.

b. Chemical method:

- ➢ Antibiotics
- ➢ Detergents
- ➢ Chartrops
- ➢ Chelating agents

c. Enzymatic method:

- ➢ Autolysis
- ➢ Lytic enzyme
- ➢ Phage mediated lysis

After cell lysis, the cellular constituents are concentrated by ultrafiltration.

3. Refolding:

- ✓ The first step in the refolding is the dissolution of the inclusion bodies (obtained from concentration) in a strong chaotropic solution of 6M urea, 2M thiourea.
- ✓ Chaotropic agents are denaturating agent.
- ✓ Chaotropic agents disrupt the intramolecular force between water molecules and allows protein and other macromolecule to dissolve easily.

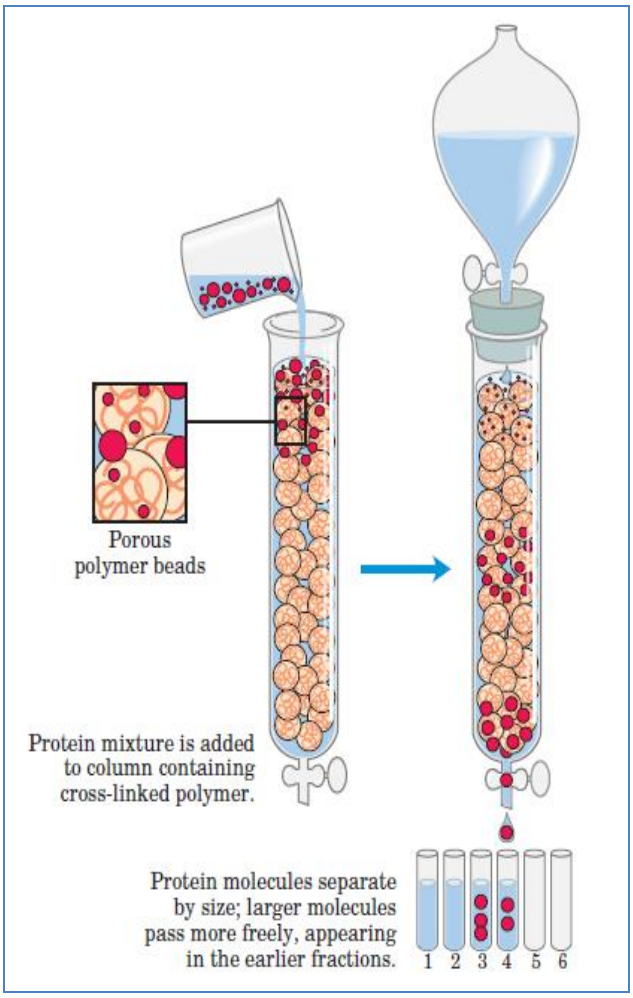
- ✓ The denaturated protein is then allowed to renature by removing the chaotropic agent by dilution, dialysis or by chromatographic separation.

V. Final purification:

- ✓ Chromatography is the usual method for obtaining pure protein.
- ✓ There are different types of chromatographic methods such as:

1. GEL FILTRATION (PERMEATION) CHROMATOGRAPHY

The gel filtration or gel permeation chromatography is used to separate the components in the mixture based on their sizes which is function of their respective molecular weights. In this technique, a column of gel beads or porous glass granules is allowed to attain equilibrium with a solvent suitable for the molecules to be separated. If the mixture of molecules of different size is placed on the top of such equilibrated column, the larger molecules cannot enter the small pores and pass through the interstitial spaces between the beads with little resistance. However, smaller molecules spend more time within the pores of the support medium, and hence move more slowly, than larger molecules. Therefore the small molecules are retarded. The degree of retardation of a molecule is proportional to the time it spends inside the gel pores, which is function of the molecule's size and pore diameter.



Gel Filtration Chromatography

***Exclusion limit*: -** The upper limit of molecular weight, beyond which molecules can not enter the gel bead pore and elute in the void volume of the column.

Types of gels:-

a) **Sephade**x – for proteins and most of the biomolecules.
b) **Polyacrylamide** – for smaller molecules
c) **Agarose** – for large molecules
d) **Styragel** – polystyrene gel for non-aqueous separations.

Applications: -

1. *Purification of biomolecules:* - Gel filtration is used for the purpose of purification of biological molecules such as proteins, enzymes, hormones antibodies, nucleic acids and even viruses also.
2. *Desalting:* - Another important application is the separation of large biological molecules from salts. By use of a column of Sephadex G-25, solutions of high molecular weight compounds may be desalted.
3. *Molecular weight determination:* - By this technique, the molecular weight of a solute can be calculated from the effluent volume. It is because, that the effluent volumes of globular proteins are largely determined by their molecular weight. It has been shown, that the effluent volume is approximately a linear function of the logarithm of the molecular weight.
4. *Solution concentration:* - Dilute solutions of macro molecules may be readily concentrated by utilizing the hygroscopic nature of the dry gel. Dry Sephadex G-25 absorbs water and other small molecules from the solution. This increases the macro molecule concentration without altering pH or ionic strength.

2. ION-EXCHANGE CHROMATOGRAPHY

Definition: **- Ion exchange chromatography** is a process that allows the separation of ions and polar molecules based on their affinity to the **ion exchanger**. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.

Principle: -

The separation of ionic molecules occurs by reversible exchange **of ions** between the ions present in the solution and those present in the **ion exchange resin**. Exchange of ions is the basic principle in this type of Chromatography. In this process **two types of exchangers** i.e., cationic and anionic exchangers can be used.

1. ***Cationic exchangers***: - Cationic exchangers possess negatively charged group, and these will attract positively charged cations. Cation exchange resins are employed to retain cations in the column. These exchangers are also called "**Acidic ion exchange**" materials, because their negative charges result from the ionization of acidic group.

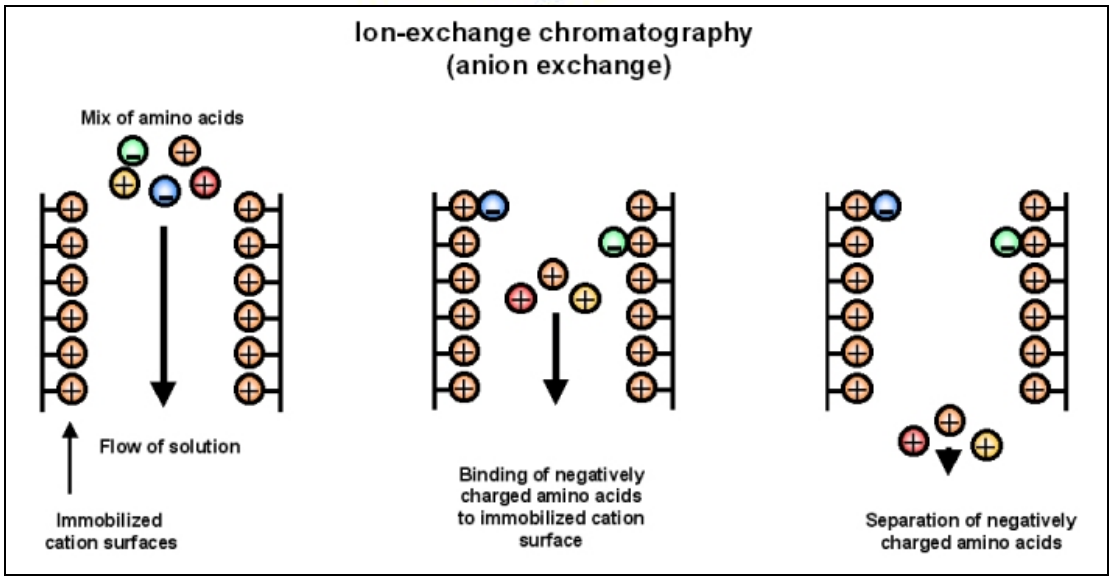
Using a cation exchange resin

2. ***Anionic exchangers***: - **Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called "Basic ion exchange" materials.** And anion exchange resins are used to keep anions into the column from the sample.

$$\text{Resin-OH}^- + \text{A}^- \longrightarrow \text{Resin-A}^- + \text{OH}^-$$

(in solution) (in eluting solution)

Anion Exchange Resin



Applications of Ion Exchange Chromatography:-

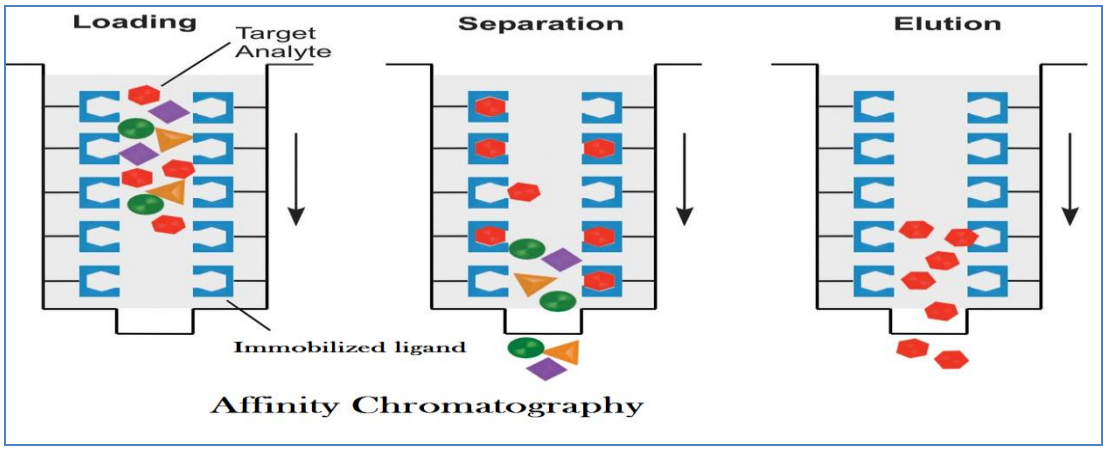
1. It is extremely used in the **analysis of amino acids**. The amino acid "Autoanalyzer" is based on in exchange principle.
2. To determine the **base composition of nucleic acids**. Chargaff used this technique for established the equivalence of Adenine and Thymine; Guanine and Cytosine.
3. This is most effective method for **water purification**. Complete deionization of water is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for **softening of drinking water**.
4. Proteins are also successfully separated by this technique.
5. It is also used for the **separation** of many **vitamins**, other **biological amines**, and **organic acids** and **bases**.

3. AFFINITY CHROMATOGRAPHY

The affinity chromatography is a type of liquid chromatography for the separation of sample components based on reversible "**biological interaction**" (molecular recognition) between a molecule and aspecific ligand bound to solid matrix in column.

e.g. enzyme with an inhibitor and antigen with an antibody.

Principle: **-** The stationary phase consists of a support medium (e.g. cellulose beads) on which the Ligand has been bound covalently. As the crude mixture of proteins is passed through the chromatography column, proteins which are specific for the ligand will bind to the stationary phase, while all other proteins will be eluted in the void volume of the column. Once the other proteins have all been eluted, the bound enzyme(s) can be eluted later. Theoretically affinity chromatography is capable of giving absolute purification in a single step.



Applications: -

1. ***Purification of macromolecules:*** **-** Affinity chromatography has been used to purify a large variety of macromolecules such as enzymes, antibodies, membrane receptors, nucleic acid and polysaccharides.
2. ***Purification of whole cells:*** **-** Whole cells have been purified using this technique. Cells separated by using this technique include fat cells, T and B lymphocytes, spleen cells, lymph nodes etc.

3. ***Metal chelate affinity chromatography: -*** Many proteins bind different metal ions for their functioning. To purify these proteins, the particular metal ion is immobilized on a gel matrix by chelation. When the mixture of proteins passed through the column, the specific proteins bind to the chelated metal ions.
4. ***Immobilized enzymes:*** - The enzymes are attached to gel beads in column. The suitable substrate is continuously passed through one end of the column and the product is removed from the other end.

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