International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

Pharmacological evaluation of Euphoria hirta l. for Diabetes and diabetic Dyslipidemia

1 Sachi Sharma, 2 Manoj Kumar

Submitted: 02-01-2023 Accepted: 10-01-2023

I. INTRODUCTION

Diabetes mellitus (DM) is a global problem one of four priority non communicable diseases (NCDs) targeted for action by world leaders {WHO, 2016}. Together, the number of cases and the prevalence of diabetes has been rapidly increasing over the past few decades (WHO, 2016). The sudden increase in the incidence and prevalence of diabetes may be due to changes in the human behavior, lifestyle and environment Diabetes mellitus (DM) is a global health problem, one of four priority non communicable diseases along with genetic susceptibility (Laakso, 2010). According to the recent version of the International Diabetes Federation (IDF) atlas, there were 415 million (8.8%) of adult diabetic people around the world in 2015 and the number is expected to reach 642 million (10.4%) cases by the year 2040 (IDF, 2015). Approximately 5 million adult people died from diabetes in 2015, among them, more than 80% were from low-and middle-income countries (IDF, 2015; WHO, 2016). In addition to these human costs, the financial Cost for diabetes in the world was $673-1197 billion in 2015 and by 2040, it will be to exceed $802-1,452 billion (IDF, 2015). India had about 69.2 million diabetic patients in 2015 and expected to reach 123.5 million in 2040 (IDF, 2015).

Type 2 DM is a common metabolic disorder resulting from defect in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by polydipsia, glycosuria and polyuria (Lawrence et al., 2009). Defects in insulin action and or secretion leads to insulin resistance followed by Beta-cells of pancreas dysfunction. Most severe adverse outcomes of diabetes are vascular complications; both at the macrovascular level (i.e. coronary artery disease, peripheral vascular disease or cerebrovascular disease) and microvascular level (i.e. neuropathy, nephropathy or retinopathy) (UKPDS, 1998). The associated complication increased socioeconomic and medical burden of the disease, which impose global public health problem.

There are certain unique biochemical and clinical abnormalities in, Asian Indian Phenotype" refers to abdominal adiposity i.e., higher waist-hip ratio and higher waist circumference despite of lower body mass index, high visceral fats, increased insulin resistance, high prevalence of atherogenic dyslipidaemia, lower adiponectin and early Bcell defect, which makes Asian Indians more susceptible to diabetes and early coronary artery disease (Deepa et al., 2006; Enas et ai., 2007; Unnikrishnan et al., 2016)

The term "diabesity" has been coined by Sims and colleagues in the 1970s, to describe the strong relationship between obesity and type 2 DM (Sims et al., 1973; Haslam, 2010). A large number of clinical evidence attests to link obesity and an elevated risk for development of type 2 DM (Colditz et al., 1995; Field et al., 2001; Wannamethee et al., 2005; Narayan et al., 2007). The ,Metabolic Syndrome" is the pathological conditions where excessive body fats accumulated mainly in visceral parts, a group of sign and symptoms are impaired glucose tolerance, insulin resistance, type 2 DM, visceral obesity, dyslipidaemia, hypertension, and other co-morbidities such as pro-inflammatory and prothrombotic state and non-alcoholic fatty liver disease (Capurso and Capurso, 2012). Obesity was mosty common with the individuals who diagnosed type 2 DM (Ramarao and Kaul, 1999). In obese individual, the body weight is increased due to consumption of high energy diet such as fats, which is deposited in various body parts and decreased level of energy expenditure. The presence of altered lipid profile due to excess of fat intake could lead to increased fatty acid availability and oxidation which is mediated by insulin. Further, insulin-mediated reduction in hepatic glucose output and reduces the glucose uptake and or utilization in skeletal muscle leading to compensatory hyperinsulinemia occurs, which is a common feature of insulin resistance in obesity (Belfiore and lannello, 1998).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

Insulin, a peptide hormone produced by Beta-cells of pancreas, is the chief hormone of carbohydrate and fats homeostasis; it stimulates glucose absorption from blood to skeletal muscle and fat tissue, liver and muscle glycogen synthesis, and adipocytes fat deposition. Apart from carbohydrate and fat metabolism, insulin has some other important actions such as the increase protein synthesis, decrease protein catabolism, cell growth and survival, and anti-inflammatory effects (Saltiel and Kahn, 2001)

Glycogen is the primary storage form of glucose in the liver and skeletal muscles, serves as a reservoir for the glucose needs by the body (Baron et al., 1988). In the liver, insulin is responsible for conversion of glucose to glycogen; it stimulates glycogen synthase and inhibiting glycogen phosphorylase enzymes resulting increases intracellular glycogen synthesis (Stalmans et al., 1987). Diabesity is characterised by increased blood glucose levels, which result from glycogenolysis and gluconeogenesis in the liver and decreased glucose uptake by skeletal muscles leads to hyperglycaemia. The liver and muscles glycogen contents are reduced in individuals with type 2 DM (Shuiman et al., 1990; Magnusson et al., 1992)

II. REVIEW LITERATURE

Diabetes Mellitus

Diabetes mellitus (DM), a common metabolic disorder resulting from defect in insulin secretion or action or both, is described by hyperglycemia often accompanied by polydipsia, glycosuria and polyuria (Lawrence et al., 2009). Mainly, there are three types of diabetes mellitus.

Type 1 diabetes mellitus

Type 1 DM, formerly known as, juvenile onset "insulin-dependent or ,immunemediated" diabetes and affects about 5% of the diabetes and the mainstream are children and youth (CDC, 2011). It is autoimmune disease in which body's defence (immune) system promote an erroneous attack and abolishes the Beta-cells of the pancreas which produce the insulin hormone. There is very low insulin of insulin or deficiency in type 1 DM. Sign and symptoms are excessive-thirst (polydipsia), excretion of urine (polyuria), hunger (polyphagia), weight loss, fatigue and vision changes, they may occur suddenly. If patients with type 1 DM left untreated, they results in death as a result of muscle and other tissues are starved for glucose in spite of high glucose levels in the bloodstream. Therefore, they require insulin for lifelong.

Type 2 diabetes mellitus

Type 2 DM, formerly known as, non-insulin-dependent"or, adult-onset is the most common form, and accounting for approximately 90-95% of diagnosed diabetes cases (CDC, 2011). It is strongly associated with obesity, more than 80% of adults with type 2 DM are obese or overweight (CDC, 2004), and obesity can cause insulin resistance and which lead to elevated glucose levels in the blood. Additional risk factors are a family history of diabetes, physical inactivity, and a history of diabetes during pregnancy (gestational diabetes).

Type 2 DM is characterized by defects in insulin action and or secretion leads to insulin resistance followed by Beta-cells of pancreas dysfunction. Sign and symptoms are similar to type 1 DM, but are often less severe. As a result, usually the disease may be diagnosed several years after onset, once complications have already aroused in type 2 diabetes. It is often diagnosed from associated complications or incidentally through an abnormal blood or urine glucose test. There are also an increasing number of patients who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not higher as in diabetes (CDC, 2011). They are at high risk of developing type 2 DM. Fortunately, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) spearheaded the Diabetes Prevention Program (DPP) clinical trial, with support by many components of the Centers for Disease Control and Prevention (CDC), the Indian Health Service (IHS) as well as the National Institutes of Health (NIH). They reported that people with pre-diabetes can intensely decrease the risk of developing type 2 DM with improvements in lifestyle modification or with drug treatment.

Gestational diabetes mellitus

Gestational diabetes is characterized by high blood glucose levels (glucose intolerance) during pregnancy and which affects 7-18% of the pregnancies (ADA, 2004). Symptoms are similar to type 2 DM and are associated with complications in the period immediately before and after birth. Gestational diabetes is most common among women with obesity or family history of diabetes. It is often diagnosed through prenatal screening, rather than reported symptoms. After the pregnancy, gestational diabetes usually disappears

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

but gestational diabetes women and her offspring are at higher risk of developing type 2 DM in later life. Approximately 35-60% of women who had gestational diabetes go on to develop type 2 DM in next 10-20 years (CDC, 2011), Gestational diabetes mellitus Gestational diabetes is characterized by high blood glucose levels (glucose intolerance) during pregnancy and which affects 7-18% of the pregnancies (ADA, 2004). Symptoms are similar to type 2 DM and are associated with complications in the period immediately before and after birth. Gestational diabetes is most common among women with obesity or family history of diabetes. It is often diagnosed through prenatal screening, rather than reported symptoms. After the pregnancy, gestational diabetes usually disappears but gestational diabetes women and her offspring are at higher risk of developing type 2 DM in later life. Approximately 35-60% of women who had gestational diabetes go on to develop type 2 DM in next 10-20 years (CDC, 2011).

Diabetes related complications

DM is a multifactorial disease, associated with a number of vascular complications; both at the microvascular level (i.e. neuropathy, nephropathy or retinopathy} and macrovascular level (ie. peripheral vascular disease, coronary artery disease or cerebrovascular disease) (UKPDS, 1998). The late diagnosis of disease leads to the development of diabetic complications, and cause the premature death in the diabetic patients (Somaratne et al., 2008)

Diabetic Neuropathy involves damaged to the neural fibres and its prevalence is approximately 8% in newly diagnosed diabetic patients and more than 50% in patients with long-standing diabetes. Pre- diabetic patients are also associated with increased risk of neuropathy. Diabetic foot ulcers develop in about 15% of all diabetic patients and leading to non-traumatic limb amputation (Edwards et al., 2008).Diabetic Retinopathy is a severe complication and the leads to blindness among worldwide. It is a progressive multifactorial disease of the retina, where the pathogenesis is extremely complex. Diabetes manifests as hyperglycaemia which leads to damaging retina! Cells including ganglion, pigment epithelial, and muller cells. According to WHO, the prevalence of retinopathy is expected to rise, and in 2030, the people at risk of blindness will be double with the rising rate of diabetes epidemic (Wild et al., 2004).

Insulin resistance

Insulin resistance can be defined as the inability of a known quantity of insulin (exogenous or endogenous) to increase glucose utilization and uptake as well as glucose production in an individual as much as it does in a normal people (Lebovitz, 2001). It is a relative term due to inherent variability among cells, tissues and individuals for the insulin sensitivity. it is affected by factors such as age, body weight, concomitant illness, medications and physical activities. Moreover, insulin sensitivity differs in individuals over time in groups or populations. Therefore, insulin resistance has considerable pathological significance in type 2 DM or glucose intolerance patients who have reduced responses of insulin and can be easily distinguished with normal glucose tolerance people.

The major insulin-receptive tissues are liver, skeletal muscle and adipose tissues. Hepatic insulin resistance generally mentions as decrease the ability of insulin to suppress glucose production via gluconeogenesis and glycogenolysis (Petersen et al., 2005). Skeletal muscle insulin resistance generally marked by a decrease in uptake and utilisation of glucose from the blood (Koves et al., 2008). Adipocyte insulin resistance leads to increase release of free fatty acids and rates of lipolysis into the blood circulation (Hotamisligil et al., 1995).

The causes for insulin resistance and metabolic syndrome include genetic abnormalities of insulin action cascade proteins, fetal malnutrition and increases visceral adiposity {Lebovitz, 2001}. The ‚Metabolic Syndrome" is the pathological conditions where excessive body fats accumulated mainly in visceral parts, a group of sign and symptoms are impaired glucose tolerance, insulin resistance, type 2 DM, visceral obesity, dyslipidaemia, hypertension, and other co-morbidities such as pro-inflammatory and prothrombotic state, polycystic ovarian syndrome and non-alcoholic fatty liver disease (Reaven, 1991; Capurso and Capurso, 2012).

Diabetes dyslipidaemia

Type 2 DM Is associated with a 2-4 fold escalation in risk of CHD and diabetic dyslipidemia is the significant cardiovascular risk factor for diabetic patients and which affects 10-73% of diabetic population (Turner et al., 1998; Saydah et al., 2004). The high degree of glycaemia is a strong risk factor for microvascular and macrovascular complications in the diabetic patients,

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

cardiovascular disease complications cause approximately 80% of deaths in diabetic patients (Farmer, 2008). Asian Indians have higher risk of CHD than whites (O'Keefe et al., 1999). Diabetic dyslipidemia commonly manifested as decreased high-density lipoprotein cholesterol (HDL-C) levels and elevated triglyceride (TG) levels and or augmented low density lipoprotein cholesterol (LDL-C).

Hepatic lipase increased catabolism of HDL-C and leads to decreased HDL-C in type 2 DM subjects (Ginsberg, 1991). The HDL production is decreased due to reduced VLDL catabolism and lipoprotein lipase activity (Golay et al., 1987). Genetic composition of HDL-C has been altered in type 2 DM such as an apo A1/A2 is increased (Biesbroeck et al., 1982). Further, free cholesterol to lecithin proportion in HDL-C have been altered in type 2 DM patients (Lane et al., 1991).

A majority of LDL-C has been recognized as a risk factor for CHD (Austin et al., 1988), and a majority of small dense and glycated LDL-C presents in type 2 DM patients (Selby et ai., 1993; Haffner, 1998). Increased oxidised LDL-C has been found in diabetic patients (Bowie et al., 1993) and it is more atherogenic than non-modified LDL-C (Witztum and Steinberg, 1991}. Cholesterol transport has been defected in type 2 DM (Fielding et al., 1984). Moreover, the relative insulin deficiency and insulin resistance are major culprit's in type 2 DM patients, since it has an important role in the lipid metabolism regulations. Further, some other factors such as hypertension, obesity and glucose tolerance also play role in the pathophysiology of lipid abnormalities in type 2 DM (Reaven, 1991; Verges, 2005)

Traditional herbal medicines:

Potent therapeutic agents for diabetes mellitus In traditional systems, plant and plant products are used for the cure of diseases (Nadkarni, 1976). Pharmaceutical research conducted over the past fifty years shows that natural products are a potential source of novel molecules for drug development (Oubre et al., 1997; Farnsworth, 2008). Natural products have long been a thriving source for discovery of new drugs due to their chemical multiplicity and capability to act on various biological targets. Nowadays, the search of new molecules has taken in distinctly different route where the knowledge of ethnomedicine is being used as escort to lead the chemist towards different sources and classes of molecules (Gurib- Fakim, 2006; Jameel et al., 2014}. The use of ethnomedicine to recognize the possible leads in drug discovery is gaining importance as more and more of botanicals are being studied extensively,

There are numerous examples of development of new drugs from the plant origin such as morphine was isolated from the poppy plant (Papaver somniferum) about 200 years ago. Further, some drugs developed from plant sources have undoubtedly transformed medicine, like antibiotics (e.g. penicillin, erythromycin, tetracycline), lipid control agents (e.g. lovastatin and analogues), antimalarials (e.g. quinine, artemisinin), antiparasitics (e.g. avermectin), immune-suppressants for organ transplants (e.g. rapamycins, cyclosporine), and anticancer drugs (e.g. paclitaxel, irinotecan) (Harvey, 2008). In this context, various evidence has been published that a wide array of plant-derived active phytoconstituents, representing several classes of chemical moieties, demonstrate activity consistent for the treatment of diabetes mellitus (lvorra et al., 1989: Marles and Farnsworth, 1995). Now a days natural products and their active constituents continued to enter clinical trials (Harvey, 2008; Dias et al., 2012). 34% of new medicines were approved by USFDA between 1981 to 2010 were natural products or its direct derivatives (Newman and Cragg, 2012).

Today, synthetic drugs and insulin are mainly used for treating diabetes. However, these drugs come with considerable side effects, such as hypoglycaemia, drug-resistance, weight gain etc. In contrast, hundreds of traditional folk medicines have demonstrated their potential for the treatment of diabetes with less tolerability and adverse effects. Thus, there is an increasing need to search for more natural agents for the diabetes treatment from the traditional medicine. The traditional Indian system of medicine has very long term history of usage in a number of diseases and disorders, but lacks recorded safety and efficacy data. Hence, there is a need to develop and screen scientifically a large number of plant extract and active constituent's libraries for the treatment of diabetes. This approach can surely be a driving force for the discovery from Indian medicinal plants and leads to fruitful results for mankind.

AIMS AND OBJECTIVES

The present study was planned with the following aims and objectives:

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

1) Extraction and isolation of phytoconstituents from E. hirta.
2) In-vitro evaluation of extracts as well as isolated phytoconstituents for their glucosidase and Di-Peptidyl Peptidase (DPP) - IV enzyme inhibition.
3) Toxicity assessment (Acute and Sub-acute} of E. hirta in Wistar rats.
4) Evaluation of methanolic extract and isolated phytoconstituents in HFD+STZ rat model for the diabetes mellitus and diabetic dyslipidemia

Plant Profile Euphorbia hirta

Worldwide there are about 2000 species of Euphorbia genus belonging to family Euphorbiaceae ranging from annual weeds to trees (Lind and Tallantire, 1971}. About 80 species can be found in India, which are habituated in the warm areas of the country; among them, 30 species are used as herbal medicines. One such plant is Euphorbia hirta L., commonly known as asthma weed, snake weed, Dudhani, Dudhi, Feiyangcao, or pillbearing spurge {Kirtikar and Basu, 1991; Chopra and Chopra, 2006)

Dried and Fresh specimens of whole plant of Euphorbia hirta L.

Taxonomy (USDA, 2009}
Kingdom: Plantae
Subkingdom: Viridiplantae
Infrakingdom: Streptophyta
Superdivision: Embryophyta
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Superorder: Rosanae
Order: Malpighiales
Family: Euphorbiaceae
Genus: Euphorbia

Synonyms
Euphorbia pilulifera Linn. Chamaesyce pilulifera Linn (Nadkarni, 1976)

Distributions

This herb is native to India but widely distributed in many parts of Africa, America, Asia, Australia and Pacific countries as tropical weed {Sood et al., 2005; USDA, 2009).

Description and Morphology

It is a small, erect or ascending annual herb reaching up to 50 cm, with hairy stems. The leaves are opposite, oblong, elliptical or oblong-lanceolate, with a slightly toothed margin and blacker on the upper surface. The flowers are numerous, small and crowded together in dense cymes about 1 cm in diameter. The fruits are yellow, hairy, three-celled, overturned capsules 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds (Kirtikar and Basu, 2003). Traditional uses E. hirta has a long history of being used for a variety of medicinal uses worldwide as shown in table.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

Country	Traditional uses	References
India	In respiratory disease (such as ,asthma,bronchial infections, Bronchitis,coryza and cough) diabetes, gastro-intestinal disorders (such as ,dysentery,intestinal infections owwel complainnts and helminthic Infestation), skin diseases, and kidney stones	(Anonymous ,2005)
China	To cure fever, prevention of itching,detoxification ,promote diuresis, and Induces prolactin secretion and also used in bronchitis acute mastitis,pulmonary abscess,diarrhea,eczema and pyogenic infections	(Huang et al 2012)
African countries	Commonly for asthma,intestinal amoebiasis,constipation and as a . livestock fodder	(Ekpo and pretorious,2007)
Philippines	Used in asthma,emphysema,chronic bronchitis, pulmonary cardiac disesase, Angina pectoris	(Singh et al.,2011)

Phytochemical studies

The plant has been extensively investigated and a number of chemical constituents from the plant have previously been reported are shown in Phytochemical investigation of E. hirta revealed the presence of several flavonoids, tannins and related polyphenols triterpenes, phytosterols and essential oils.

Class	Phytochemical constituents	References
Flavonoids	Afzelin,euphoebianin,isoquercitrin, Isorhamnetin,kaempferol,leucocyanidol, Luteolin,myricitrin,quercitin,rhamnetin And quercitol	(Blane and De saqui-sannes 1972; Yoshida et al;1988; Aqil and Khan,1999; Subbiah,2007; Singh et al., 2011; Wu et al.,2012)
Tannins and Related Polyphenols	Dehydroellagitannins,euphorin A,B,C and E, terchebin and isoterchein,galloyl glucose; 1,3,4,6-tetra-o-galloyl-beta-D-glucose, 2,4,6-tri-o-galloyl-beta-D-glucose,ellagic Acid,geraniin,quinic acid esters;o-caffeoyl Quinic acid 3,4-di-o-galloyl quinic acid	(Yoshida et al;1988, Yoshida et al;1990)
Triterpenes	Beta-amyrin,24-methyl cycloartenol,beta-sitosterol Cycloartenol,euphorbol hexacozonate,1-hexacosanol, Tinatoxin,beta-amyrin acetate,13 dodecanoate 20 acetate 12-deoxy 4 beta hydroxylphorbol and ingenol tri acetate	(Baslas and agarwal, 1980;Martinez et al; 1999)

Clinical studies

E. hirta extract was found to be active against dysenteric epidemia in 1964 {Ridet and Chartol, 1964). Further clinical trials studies showed 83.34% of healing in patients with colopathies related to Entamoeba histolytica. In the treatment of intestinal amoebas its activity was comparable to pharmaceutical product Flagentyl (secnidazole) (Martin et al., 1964).

According to WHO, nearly 75-80% of world population still depends on herbal plants for the treatments. Active constituents from plant sources directly used as therapeutic agent and phytoconstituents are also served as lead molecule for the synthesis of various drugs. WHO noted that about 25% of modern medicines are descended from plants sources used traditionally and research on traditional medicinal herbal plant leads discovery of 75% of herbal drugs.

Materials

Chemicals and reagents

Acarbose	Medley Pharmaceuticals Ltd, India
All analytical grade	Merck, Darmstadt. Germany
Casein	Himedia Laboratories, Mumbai, India
Cholesterol	Himedia Laboratories, Mumbai, India
dl-Methionine	Himedia Laboratones, Mumbai, India
H-gly-pro-para-nitroanilide (GP-pNA)	Enzo life Sciences, USA

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

Lard	Local market, Kharibaoli, Delhi, India
p-nitrophenyl-a-D-glucopyranoside (PNPG)	Sigma-aldnch, St. Louis, USA
Silica gel, 230-400 mesh	Merck, Darmstadt, Germany
Ranbaxy	Research Laboratory, India.
sodium carboxy methyl cellulse	Himedia Laboratones, Mumbai, India
Streptozotocin (STZ)	Sigma-aldrich, St. Louis, USA
TLC silica gel 60 F254 plates	Merck, Darmstadt, Germany
Tris buffer	SD fine chemicals, India
Vitamin and mineral mix	Sarabhat chemicals, Baroda, India
Yeast powder	Himedia laboratories, Mumbai, India

Diagnostic kits

ALP, ALT and AST kit	Span diagnostics, Surat, India
BUN and Creatinine (CR) kit	Span diagnostics, Surat, India
Glucometer and test-strips	Accu-check, Roche, Germany
Glycohemoglobin (HbA,,) kit	Asritha Diagnostic, Hyderabad, India
HDL cholesterol kit	Reckon Diagnostics Pvt. Ltd., Baroda,India
Rat GLP-| ELISA assay kit	BioVendor, Japan
Rat Insulin ELISA assay kit	Alpco Diagnostics, Salem, USA

Equipment

Accurate-mass 6530 series	Agilent, USA
Cooling Centrifuge (C-24)	REMI Instrument Ltd., Mumbai india
Counter STKS Automated Cell Coulter,Hialeah, Florida Counter	
Electronic balance	Scientific Systems, New Dethi, India
ELISA reader (ELx 800ms_BIO-TEK Instruments, INC. Winooski, Vermont, Automated microplate reader)	USA
FT-IR Spectrophotometer	Shimadzu, Japan
Homogenizer	REMI Instrument Ltd., Mumbai India
Incubator	Wisro Instruments, Delhi, India
Laboratory Centrifuge	REMI Instrument Ltd., Mumbai india
Melting Point apparatus	Perfit, India
Micropipette	Eppendorf, Hamburg, Germany
Milipore filtration unit	Milli Q, Massachusetts, USA
NMR instrument	Bruker, Switzerland
Oven	Wisro Instruments, Delhi, India
pH- meter	Microprocessor pH system, Punjab, India
Refrigerated centrifuge	Sigma, USA
Rotary evaporator	Buchi, Switzerland
Spectrophotometer	Shimadzu, Japan

III. METHODOLOGY

Phytochemical Evaluation

Collection of plant material

Euphorbia hirta whole herb was collected from the Maktabah Jafariyah Knowledge and Research Academy garden, Gujarat, India, in the month of May, 2011. Collected sample was authenticated by Dr. H. B. Singh, Head, at Department of Raw Materials, Museum and Herbarium, NISCAIR, New Delhi, India. A voucher specimen (Ref. NISCAIR/RHMD/ Consult/2011-12/1785/85) was deposited at NISCAIR.

Preparation of aqueous, hydroalcoholic and methanolic extracts

The whole plant of E. hirta was cleaned and air dried. Dried sample was minced to a coarse powder using grinder. About 1 kg of powdered was extracted with water, 50% methanol in water and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

methanol using Soxhlet apparatus at 80°C for 18 h separately. The extracts were filtered through Wattman no, 1 filter paper and subsequently, the filtrate was evaporated under reduce pressure at 50 celsius a rotary evaporator. Dark brown residues were obtained (14.52, 17.59 and 18.0% w/w for aqueous, hydroalcoholic and methanolic extract}. The dry residues were stored at 4 °C, and at the time of use, were resuspended in distilled water.

Qualitative phytochemical screening

All the extracts were subjected to routine qualitative chemical analysis to establish the nature of phyto- constituents present like alkaloids, flavanoids, saponins, steroids, carbohydrates, glycosides, tannins, phenolic compounds, protein, amino acids and triterpenoids (Harborne, 1973; Kokate et al., 2005).

Isolation of phytoconstituents by medium pressure liquid Chromatogrphy (MPLC)

Extraction and fractionation

The air dried and coarsely powdered of E. hirta (5 kg) was extracted with methanol using Soxhlet apparatus at 80°C for 18 h. The methanolic extract was filtered through Wattman no. 1 filter paper and concentrated to get 899 p (18% yields) dark brown residues under reduced pressure. The residue was suspended in distilled water {1 L) and sequentially fractionated with n-hexane (3x 1 L) and ethyl acetate (3x 1 L) to furnish n-hexane fraction (118 g, 2.4% yields) and ethyl acetate fraction (137 g, 2.8% yields) fractions and remaining water soluble fraction (590 g, 11.8% yields).

Preparation of slurry

The ethyl acetate fraction (120 g), in china dish was gently heated continuously in water bath and methanol Was added gradually under constant stirring, till desired consistency is achieved. Subsequently weighed quantity of silica gel (230-400 mesh) was added slowly with continuous mixing with the help of a steel spatula until the whole methanolic solution of plant extract got adsorbed on it. Resultant slurry was subjected to air drying followed breaking of larger lumps by rubbing between hands and finally passed through a sieve (No. &) to get uniform particle size.

Homogeneity of the fractions

The fractions collected were imperiled to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (which have the same Rf values) were combined together and concentrated. They were then crystallized with suitable solvent system.

Melting point

Melting points were determined on Perfit melting apparatus using open capillary method; generally melting point was uncorrected.

UV spectroscopy

Ultraviolet spectrum was recorded for the Amax in MeOH (1 mg/ml) using quartz cuvettes in UV-visible double beam Spectrophotometer. Further UV spectra were analyzed in presence of various shift reagents such as NaOH, AICl3, NaOAc, NaOAc+H3B03 to differentiate various functional groups in flavonoids.

In-vitro enzyme inhibitory activity

In-vitro a-glucosidase inhibitory activity

The a-glucosidase inhibitory activity of three extracts and isolated compounds were measured as described with slight modifications (Dong et al., 2012). Briefly, a volume of 50 uL of sample solution and 50 uL of 0.1 M phosphate buffer (pH 6.8) containing alpha glucosidase solution (0.1 U/ml) was incubated in 96 well plates at 37celsius for 10 min. After pre-incubation, 50 ul of 2.5 mM p-nitrophenyl-a-D- glucopyranoside (PNPG} solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 OC for another 20 min. Then the reaction was stopped by adding 100 uL of 0.2 M Na2CO3 into each well, and absorbance reading (A) was recorded at 405 nm by micro-plate reader (BioTek, USA} and compared to a control which had 50 uL of buffer solution in place of the sample.

Toxicological evaluation

Animals

Wistar albino rats of either sex (150-200 g weight; 8-10 weeks old) were procured from animal house of, India. The animals were maintained in standard conditions (Temp. 25+2°C; RH 504+5%; 12:12 h dark/light cycle). Industrialized dry food pellet diet (Amrut rat feed, manufactured by Nav Maharashtra Chakan Oil Miils Ltd, Delhi) and water were available ad libitum. The experimental protocol was approved by Institutional Animai Ethics Committee of RGPV(CPCSEA registration No.173/ CPCSEA 28 OCT-2011; Protocol number: JH/IAEC/2011/744) and care was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

(CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Observations

Body weight of animals was weekly recorded and food consumption and water intake were daily monitored. Animals were observed for signs of abnormalities during whole treatment. Food consumption was assessed by subtracting the amount of food left in the cages from the initial food weight and expressed as grams per rat per day. Food efficiency was calculated as the ratio between body weight gain over the 28 days experimental period and cumulative food intake, and expressed as a percentage (Sangiao Alvarellos et al., 2009). Animals were observed twice daily for mortality and morbidity by individually handled and carefully examined once before treatment and consequently once a week to observe abnormal Behaviors and appearances, especially abnormalities of the appearance, condition, posture and position, behavior, and urinary and fecal excretions.

IV. RESULTS & DISCUSSION

Phytochemical evaluation

Extraction of E. hirta

Qualitative chemical analysis

Qualitative analysis was done for three extracts of E. hirta and results are tabulated in. Qualitative chemical tests indicated that the methanolic extract had diverse phytoconstituents such as flavonoids, tannins, phenolic compound, alkaloids, steroids, triterpenoids, fats, oils, sugars while as Saponins and anthraquinone glycosides were absent

A number of scientific studies have reported that the flavonoids, tannins, steroids, terpenoids and alkaloids have protective effects in various diseases (Choo et al., 2012; Gao et al., 2004; Kim et al., 2006). In recent time, a number of natural items traditional medicines and foods have been investigated and subjected to clinical trials for diabetes and diabetic dyslipidemia (Modak et al., 2007). Presence of major phytoconstituents in the methanolic extract makes it a potential candidate for further investigation.

V. SUMMARY AND CONCLUSIONS

Diabetes mellitus (DM), a common metabolic disorder resulting from defect in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by polydipsia, glycosuria and polyuria. DM, a global public health problem, is now emerging as an epidemic world over and has prevalence of 415 million in 2015 and the number is expected to reach 642 million by the year 2040. The mortality is about 5 million in 2015, more than 80% are in low-and middle-income countries and will be 7th leading cause of death by 2030. India has about 69.2 million diabetic patients in 2015 expected to reach 123.5 million in 2040. Diabetic dyslipidemia is the most significant cardiovascular risk factor for diabetic patients and an account for approximately 80% mortality in individuals with diabetes. Due to severe adverse effects from the synthetic drugs, there has been a shift in universal trend from synthetic to herbal medicine, which we can say Back to Nature'. Drug discovery from plants involves multidisciplinary approaches combining botanical, ethnobotanical and phytochemical techniques. India is one of the world"s 12 leading biodiversity center. It is endowed with more than 47,000 known species of plants. Medicinal plants have been known for eras and are highly esteemed all over the world as a rich source of medicine and being used for prophylactic and curative purposes of various ailments. Plants contain a large variety of secondary metabolites, such as fatty acids, terpenoids, phenylpropanoids, flavonoids, glycosides and alkaloids, which cumulatively account for approximately 200,000 compounds Euphorbia hirta is a small annual herb from the Euphorbiaceae family, widely used in the various traditional medicine systems for various ailments, including diabetes, respiratory diseases, gastro- intestinal disorders, kidney stone and skin diseases. Phytochemica! analysis of this medicinal plant revealed the presence of several flavonoids, tannins and related polyphenols, triterpenes and phytosterols and essential oils. However, E. hirta has not been investigated so far for its potential for type 2 DM and its complications, mechanism of action and the chemical entities responsible for its curative action.

VI. CONCLUSIONS:

The present study was an attempt to evaluate the E. hirta for diabetes and diabetic dyslipidaemia. From the results of the study following conclusions were drawn;

1 Methanolic extract of E. hirta showed the abundance of total phenolic content and flavonoids than the aqueous and hydroalcoholic extract.

2In-vitro a-glucosidase enzyme inhibition study showed hirtacoumaroflavonoside (3, HCF) is potent inhibitor comparable to marketed drug miglitol. The maximum aglucosidase inhibition

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 300-309 www.ijprajournal.com ISSN: 2249-7781

was achieved in following order; HCF (3) > miglitol > HFB (4) > MEH > acarbose > HFA (5) >QUE (1) > HEH > DMQ (2) > AEH

3 Enzyme kinetic study revealed that, MEH, 1, 2, 4 and 5 inhibit a-glucosidase enzyme by mixed, non-competitive and uncompetitive manner and compound 3 by non-competitive manner

4 Based upon the outcomes of the toxicity studies, E. hirta was found to be safe after oral administration.

5 Single dose of isolated compounds on sucrose tolerance test indicated compound 1, 3 and 5 proved better suppression against hyperglycemia than acarbose in sucrose (2 g/kg bw) loaded normal rats

6 28 days treatment with MEH {at 200 and 400 mg/kg bw) and isolated compounds; HCF and HFA (at S and 10 mg/kg bw) demonstrated antidiabetic and anti dyslipidemic potentials in HFD+STZ induced type 2 DM rat model, and mechanism underlying for the same is inhibition of a-glucosidase and DPP IV enzyme apart from antioxidant activity.

REFERENCES

[1]. Abu-Sayeed, M., M.A. Ali, P.K. Bhattacharjee, A. Istam, G.R.M. Astag, M. Khan and S.Yeasmin, 2005. Biological evaluation of extracts and triterpenoids of euphorbia hirta. Pakistan Journal of Scientific and Industrial Research, 48(2): 122

[2]. Abubakar, E.M., 2009. Antibacteria! activity of crude extracts of euphorbia hirta against some bacteria associated with enteric infections. Journal of Medicinal Plants Research, 3(7): 498-505.

[3]. ADA, 2004. Gestational diabetes mellitus; american diabetes association. Diabetes Care, 27: S88.

[4]. ADA, 2016. American diabetes association; standards of medical care in diabetes: Summary of revisions. Diabetes Care, 39(Supplement 1): S 4-5.

[5]. WHO, 2016. Global report on diabetes. In: Executive Summary. World Health Organization, Geneva.

[6]. IDF, 2015. Idf diabetes atlas, seventh edition. International Diabetes Federation, Belgium.

[7]. Lawrence, J.M., E.J. Mayer-Davis, K. Reynolds, J. Beyer, D.J. Pettitt, R.B. D'Agostino, S.M. Marcovina, G. Imperatore and R.F. Hamman, 2009. Diabetes in hispanic american youth prevalence, incidence, demographics, and clinical characteristics: The search for diabetes in youth study. Diabetes Care, 32(Supplement 2): S123-S132.

[8]. UKPDS, 1998. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UK prospective diabetes study 38. BMJ: British Medical Journal: 703-713.

[9]. Deepa, R., S. Sandeep and V. Mohan, 2006. Abdominal obesity, visceral fat and type 2 diabetes-“asian indian phenotype”. In: Type 2 diabetes in south asians; epidemiology, risk factors and prevention. Jaypee Medical Publishers New Delhi: pp: 138-152.

[10]. CDC, 2004. Prevalence of overweight and obesity among adults with diagnosed diabetes- -United States, 1988-1994 and 1999-2002. Morbidity and Mortality Weekly Report, 53(45): 1066.

[11]. CDC, 2011. National diabetes fact sheet: National estimates and general information on diabetes and prediabetes in the United States. Department of Health and Human Services, Centers for Disease Control and Prevention.

[12]. Belfiore, F. and S. lannelio, 1998. Insulin resistance in obesity: Metabolic mechanisms and measurement methods. Molecular Genetics and Metabolism, 65{2): 121-128.

[13]. Capurso, C. and A. Capurso, 2012. From excess adiposity to insulin resistance: The role of free fatty acids. Vascular Pharmacology, 57(2): 91-97.

[14]. Field, A.E., E.H. Coakley, A. Must, J.L. Spadano, N. Laird, W.H. Dietz, E. Rimm and G.A. Colditz, 2001. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Archives of internal medicine, 161(13): 1581-1586.