CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.7588515

Available online at: http://www.iajps.com *Review Article*

PHARMACOLOGICAL EVALUATION OF *EUPHORBIA HIRTA* L. FOR DIABETES AND DIABETIC DYSLIPIDEMIA

Shailendra Kumar Gupta *, Sailesh kumar Ghatuary, Satkar Prasad, Saurav Jain
RKDF School of Pharmaceutical Science, Bhabha University, Bhopal

Article Received: November 2022 **Accepted:** December 2022 **Published:** January 2023

Abstract:

The present research study was entitled focused on the importance of crude plant extract as promising treatments. In continuation, the active component responsible for medicinal properties from this plant were isolated and assessed for its mechanism by α-glucosidase and DPP IV inhibitory activity. In-vivo toxicity studies and pharmacological activity of extract and isolated compounds were carried out and compared with standard drugs; sitagliptin and miglitol.

Experimental work performed in this thesis consists of four parts. The first part dealt with phytochemical evaluation and isolation of active constituents. In second part, the in-vitro enzyme inhibitory activity was carried out and third part involves toxicity assessments of the plant and isolated compounds. Final part dealt with in-vivo efficacy of extract and potent isolated compounds in HFD fed and low STZ induced type 2 DM model.

Qualitative phytochemical analysis have shown the presence of flavonoids, tannins, phenolic compound, triterpenoids, glycosides and absence of alkaloids, saponins, steroids and anthraquinone glycosides. Total phenolic content was almost double to the total flavonoid contents. Both these contents were found higher in the methanol extract than aqueous and hydroalcoholic extract.

Conclusively, isolated compounds hirtacoumaroflavonoside (HCF) and hirtaflavonoside- A (HFA) and extract as such could be a potential agent for treating diabetes and diabetic dyslipidemia. In addition, they have showed in-vitro enzyme inhibition. Moreover they demonstrated better safety profile. These compounds can be further taken up for the detailed pharmacokinetic (PK) study, pharmacokinetic (PK) and pharmacodynamic (PD) correlation and toxicological studies. If aforementioned experiments provide promising results, preclinical to clinical translation of isolated compounds hirtacoumaroflavonoside (HCF) and hirtaflavonoside-A (HFA) and extract in diabetes is possible.

***Key words**: Glibenclamide, Antihyperlipidemic, Euphorbia hirta, Streptozotocin.*

Corresponding author:

Shailendra Kumar Gupta,

RKDF School of Pharmaceutical Science,
Bhabha University, Bhopal

QR code

*Please cite this article in press **Shailendra Kumar Gupta** et al, **Pharmacological evaluation of euphorbia hirta l. For diabetes and diabetic dyslipidemia**, Indo Am. J. P. Sci, 2023; 10 (01).*

INTRODUCTION:

Diabetes mellitus (DM) is a global health 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 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 β-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 β- cell defect, which makes Asian Indians more susceptible to diabetes and early coronary artery disease (Deepa *et al.*, 2006; Enas *et al.*, 2007; Unnikrishnan *et al.*, 2016).

It is a general belief that medicinal plants are "natural" and are hence harmless inherently. However, the consumption of plants could produce toxic effects which may be attributed to several factors including inherent toxicity (such as hepatotoxicity, heamatotoxicity or renal toxicity etc.) of main constituents and contaminants (heavy metals, pesticides, microorganisms, radioactivity, toxic organic solvents etc.) (Bateman *et al.*, 1998; Youns *et al.*, 2010). The patients and general public along with the animal welfare are mainly interested in fast access to effective and safe drugs. Based on the long- term use by humans one might expect herbal plants used in traditional medicine to have low toxicity. Nevertheless, the surveys have demonstrated that many herbal plants used in traditional medicine exhibited adverse effects (Ertekin *et al.*, 2005; Koduru *et al.*, 2006).

Hence, it is emphasized that the traditional use of plant, no guarantees the safety of the plant. This increases concern regarding the potential toxic effects resultant from the short-term and long-term use of herbal plants. The data of the toxicity studies should be obtained in order to increase the confidence in the safety of plants to humans, mainly for use in the drug discovery (Ukwuani *et al.*, 2012). Therefore, a assessment of herb toxicity is a serious issue. Organization for Economic Co-operation and Development has provided standard guidelines and methods for various toxicology studies such as single dose acute toxicity study, determines median lethal dose (LD_{50}) (OECD, 2001) and subacute and chronic toxicity studies are repeated dose toxicity studies for 28 days and 90 days which gives no observable adverse-effect level (NOAEL) of the toxicant (OECD,1995).

MATERIALS AND METHOD:

Materials

Chemicals and reagents

Acarbose- Medley Pharmaceuticals Ltd, India
All analytical grade solvents- Merck, Darmstadt, Germany
Casein-Himedia Laboratories, Mumbai, India
Cholesterol- Himedia Laboratories, Mumbai, India
dl-Methionine-Himedia Laboratories, Mumbai, India
H-gly-pro-*para*-nitroanilide(GP-*p*NA) Enzo life Sciences, USA Lard Local market, Kharibaoli, Delhi, India
p-nitrophenyl-α-D-glucopyranoside (PNPG)Sigma-aldrich, St. Louis, US Silica gel, 230-400 mesh Merck, Darmstadt, Germany Sitagliptin-Ranbaxy Research Laboratory, India. Sodium carboxy methyl

cellulse-Himedia Laboratories, Mumbai, India Streptozotocin (STZ)-Sigma-aldrich, St. Louis, USA TLC silica gel 60 F_{254} plates-Merck, Darmstadt, Germany
Tris buffer-SD fine chemicals, India
Vitamin and mineral mix-Sarabhai chemicals, Baroda, India Yeast powder-Himedia laboratories, Mumbai, India α-glucosidase (EC 3.2.1.20) Sigma-aldrich, St. Louis, USA

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_{1C}) kit Asritha Diagnostic, Hyderabad, India
HDL cholesterol kit Reckon Diagnostics Pvt. Ltd., Baroda, India
Rat GLP-1 ELISA assay kit BioVendor, Japan
Rat Insulin ELISA assay kit Alpco Diagnostics, Salem, USA

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 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 ºC in 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 re- suspended 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, flavonoids, saponins, steroids, carbohydrates, glycosides, tannins, phenolic compounds, protein, amino acids and triterpenoids (Harborne, 1973; Kokate *et al.*, 2005).

Quantitative phytochemical screening

Evaluation of total Phenolic content (TPC)

Total phenolic contents of three extracts of *E. hirta* were determined by the Folin- Ciocalteu method (Ainsworth and Gillespie, 2007). Briefly, 1 ml either of three extracts (0.5, 1, 2 and 4 mg/ml concentration in methanol) or standard gallic acid (25, 50, 100,
200 and 400 µg/ml concentration in methanol) was mixed with 5 ml of Folin-Ciocalteu reagent (mixture of phosphomolybdate and phosphotungstate) and 4 ml of 1 M Na_2CO_3.

After 15 minutes the absorbance was measured at 765 nm against the blank in spectrophotometer. The results were expressed as mg gallic acid equivalents (GAE)/g of dry extract.

Evaluation of total flavonoid content (TFC):

Similarly flavonoid contents of three extract of *E. hirta* were determined by the aluminium chloride assay (Chang *et al.*, 2002). Briefly, 500 µL either of three extracts (0.5, 1, 2 and 4 mg/ml concentration in methanol) or standard quercetin (6.25, 12.5, 25, 50 and 100 µg/ml concentration in methanol) was mixed with 1.5 ml of methanol, 0.1 ml $AlCl_3$ (100 µg/ml concentration), 0.1 ml, CH_3COONa (1 M) and 2.8 ml distilled water. After 30 min absorbance was taken at 415 nm against the blank in spectrophotometer. The results were expressed as mg quercetin equivalents (QE)/g of dry extract. *Isolation of phytoconstituents by medium pressure liquid chromatography (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 g (18% yields) dark brown residue under reduced pressure. The residue was suspended in distilled water (1 L) and sequentially fractionated with *n*-hexane (3× 1 L) and ethyl acetate (3× 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. 8) to get uniform particle size.

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 Table 5.1.1. 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.

Table 1. Qualitative chemical analysis of three extracts of *E. hirta*

Plant Constituents	Aqueous	Hydroalcoholic	Methanolic
Alkaloids	-	-	+
Anthraquinone Glycosides	-	-	-
Fats and Oils	-	+	+
Flavonoids			
Proteins	+	+	+
Saponin Glycosides			
	-	+	+
	-	-	-
Steroids & Terpenoids	-	-	+
Sugars	+	+	+
Tannins & Phenolics	+	+	+

+ = present, - = absent

Table1. Total phenolic and flavonoid contents of *E. hirta* extracts

Extract	Total phenolic content (mg gallic acid equivalent /g)	Flavonoids content (mg quercetin equivalent /g)
Aqueous (AEH)	45.29±1.78	21.08±1.69
Hydroalcoholic (HEH)	64.21±1.55	38.70±1.01
Methanolic (MEH)	118.04±1.94	53.35±2.02

Each value is mean ± SEM (n=3 in triplicates)

Several studies revealed that antidiabetic effect of polyphenols are due to not only the antioxidant effect, but also, via α-glucosidase inhibition (Xiao *et al.*, 2013), β-pancreatic cells protection (Zaklos-Szyda *et al.*, 2015), increasing insulin secretion (Li *et al.*, 2006) and enhancing insulin sensitivity (Ghorbani *et al.*, 2014) etc.

The antidiabetic and dyslipidemic capacity of flavonoids have been utilized in Indian and Chinese medical systems from centuries in the form of crude plant extracts. Meta- analyses and epidemiological studies advocated an inverse relationship between the flavonoid-rich diets consumption and development of many ageing-associated diseases including diabetes, osteoporosis, cancers and neurodegenerative disorders (Graf *et al.*, 2005). Several *in-vitro* and *in-vivo* studies also support a beneficial effect of flavonoids on glucose and lipids homeostasis (Hanhineva *et al.*, 2010). The present study showed presence of polyphenols and flavonoids in the *E. hirta* extracts which could be responsible for its usefulness in diabetes and dyslipidemia.

Isolation and structural elucidation of the phytoconstituents from methanolic extract of E. hirta

The medium pressure liquid chromatography on silica gel of the ethyl acetate fraction of methanolic extract with successive elution of *n*-hexane, *n*-hexane-ethyl acetate mixtures (75:25, 50:50, 25:75, *v/v*) and finally with ethyl acetate, resulted in the isolation of five pure compounds (***1-5***) . The physical characteristic of isolated compounds displayed in Table 2

Table 2. List of isolated compounds from ethyl acetate fraction of methanolic extract of *E. hirta*

Compounds	Column fractions	Eluant	%Yield* (w/w)	Code
1	22-31	*n*-hexane-ethyl acetate (75:25 *v/v*)	0.155	Que
2	35-39	*n*-hexane-ethyl acetate (75:25 *v/v*)	0.019	DMQ
3	42-57	*n*-hexane-ethyl acetate (50:50 *v/v*)	0.030	HCF
4	83-93	Ethyl acetate (100%)	0.010	HFB
5	65-71	*n*-hexane-ethyl acetate (75:25 *v/v*)	0.098	HFA

*Calculated on weight basis of dry powder of *E. hirta* in extract

Table 3. Physical characteristic of compound isolated from ethyl acetate fraction of methanolic extract of *E. hirta*

Isolated Compounds	Physical form	Color	Melting point	R_f Value (Mobile phase)
1 (Que)	Amorphous powder	Yellow	175-177 °C	0.67 (*n*-Hex-EtOAc, 75:25 *v/v*)
2 (DMQ)	Amorphous powder	Light yellow	160-162 °C	0.59 (*n*-Hex-EtOAc, 75:25 *v/v*)
3 (HCF)	Amorphous powder	Yellow	233-234 °C	0.78 (*n*-Hex-EtOAc, 50:50 *v/v*)
4 (HFB)	Amorphous flakes	Reddish brown	220-221°C	0.49 (*n*-Hex-EtOAc, 25:75 *v/v*)
5 (HFA)	Amorphous flakes	Yellowish green	198-200 °C	0.72 (*n*-Hex-EtOAc, 25:75 *v/v*)

n-Hex = *n*-Hexane, EtOAc = Ethyl acetate

Spectral analysis of the isolated compounds

The medium pressure liquid chromatography on silica gel of the ethyl acetate fraction of methanolic extract resulted in the isolation of five compounds, namely 2-(3,4- Dihydroxyphenyl)-5,7- dihydroxy-3-[{(2*S*,3*R*,4*R*,5*R*,6*S*)- 3,4,5-trihydroxy-6-methyl-2-tetra hydropyranyl}oxy]-4-chromenone (***1*, quercitrin**) and 2-(3,4-Dihydroxyphenyl)- 5,7-dimethoxy-3-[{(2*S*,3*R*,4*R*,5*R*,6*S*)- 3,4,5-trihydroxy-6-methyl-2- tetra hydropyranyl} oxy]-4-chromenone (***2*, dimethoxy quercitrin**), 7-*O*-(*p*-coumaroyl)-5,7,4'-trihydroxy-6- (3,3-dimethyl allyl)-flavonol-3-*O*-β-D-glucopyranosyl-(2"→1'")-*O*-α-L-rhamno- pyranoside (***3*, hirtacoumaroflavonoside**, **HCF**), 7,3'-dihydroxy-5,4'-dimethoxy-6-(2,2- dimethylpyrano)-8-(3,3-dimethylallyl)-flavonol-3-*O*-β-D-glucopyranosyl -(2"→1'")-*O*- α-L-rhamnopyranoside (***4*, hirtaflavonoside-B**, **HFB**) and 5,7,2',4'-tetra hydroxyl -9,2'- (isobut-1-enyl pyrano)-flavone-7-*O*-α-*L*-rhamnopyranoside (***5*, hirtaflavonoside-A**, **HFA**). The isolated compounds were characterized on the basis of UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMBC and Mass spectral techniques. According to the Sci-finder, pubchem database and literature review the compound ***3***, ***4*** and ***5*** are new one and isolated for the first time.

In-vitro evaluation

To investigate the direct inhibitory effect of the extracts and isolated compounds on α- glucosidase and DPP IV enzyme, we had performed *in-vitro* α-glucosidase and DPP IV enzyme inhibition assays.

In-vitro α-glucosidase inhibitory activity

α-Glucosidase located at epithelium of the small intestine and its inhibition would delay the digestion as well as absorption of carbohydrates which consequently suppress the postprandial hyperglycemia (Krentz and Bailey, 2005). *In-vitro* α-glucosidase inhibition was measured for aqueous, hydroalcoholic and methanolic extracts of *E. hirta* at 62.5- 2000 µg/ml concentration. All the extracts exhibited a dose dependent inhibition of α-glucosidase as shown in Figure 5.2.1. The maximum α-glucosidase inhibition was achieved by MEH (IC_{50} 546.21 µg/ml) followed by acarbose (IC_{50} 60.12 µg/ml), HEH (IC_{50} 856.78 µg/ml) and AEH, respectively as illustrated in Table 3.

Table 4. Maximum α-glucosidase inhibition and IC_{50} values of extracts, isolated compounds of *E. hirta* and reference drugs

Inhibitors	Maximum inhibition (%)	IC_{50} (µg/ml)*
AEH	42.18±2.9	-
HEH	54.16±1.7	856.78
MEH	75.18±2.2	546.21
1	57.91±4.24	67.87
2	52.18±3.03	86.73
3	91.26±3.13	18.39
4	75.76±3.61	55.03
5	64.67±3.74	49.51
Acarbose	68.62±4.9	60.12
Miglitol	89.43±3.63	8.31

Each value is Mean ± S.E.M (*n*=3). At least five serially diluted solutions of each analyte were taken for calculation of the IC_{50} values. *IC_{50} values were calculated by linear regression and expressed as concentration required for inhibition of α-glucosidase activity by 50%. AEH-Aqueous, HEH- Hydroalcoholic and MEH-Methanolic extract of *E. hirta*, ***1***-Quercitrin, ***2*-** Dimethoxy quercitrin, ***3*-** Hirtacoumaroflavonoside, ***4***-Hirtaflavonoside B, ***5*-**Hirtaflavonoside A.

Toxicological evaluation:

Herbal medicine is used for mankind since 4500-1600 BC by Rigveda and Ayurveda for the treatment of various diseases (Nadkarni, 1976) and most of the medicinal plants have scientific evidence with respect to the biological activities. However, there is little evidence or information available regarding the possible toxicity that herbal plants may causes to the consumers (Chandrasekaran *et al.*, 2009). Therefore, in screening natural products for the pharmacological activity, evaluation and assessment of the toxicity behaviour of herbal product extract or compound are usually a first footstep. Unexpectedly, conflicting results has been reported in the literature regarding the toxicity of *E. hirta*. Yuet Ping *et al.* demonstrated the acute and subchronic toxicity studies and reported its good safety profile (Yuet Ping *et al.*, 2013). Similarly, safe use of *E. hirta* has been described by two authors in acute toxicity study (Lanhers *et al.*, 1991; Kumar *et al.*,2010).

In contrast, elevated levels of AST, ALT, creatinine and urea after oral administration of

1.6 g/kg bw of *E. hirta* for 14 days were reported and 20% mortality found in 14 days dosing of 10 g/kg bw of *E. hirta* (Adedapo *et al.*, 2004; Adedapo *et al.*, 2006). Further, Wong *et al.* has showed dose dependent injuries to kidney and liver in Spargue-Dawley rats (Wong *et al.*, 2013). Hence, to confirm the safety profile of Indian species of *E. hirta*, the current study was undertaken to evaluate and focus on the acute and subacute toxicity in an animal model.

Acute toxicity study:

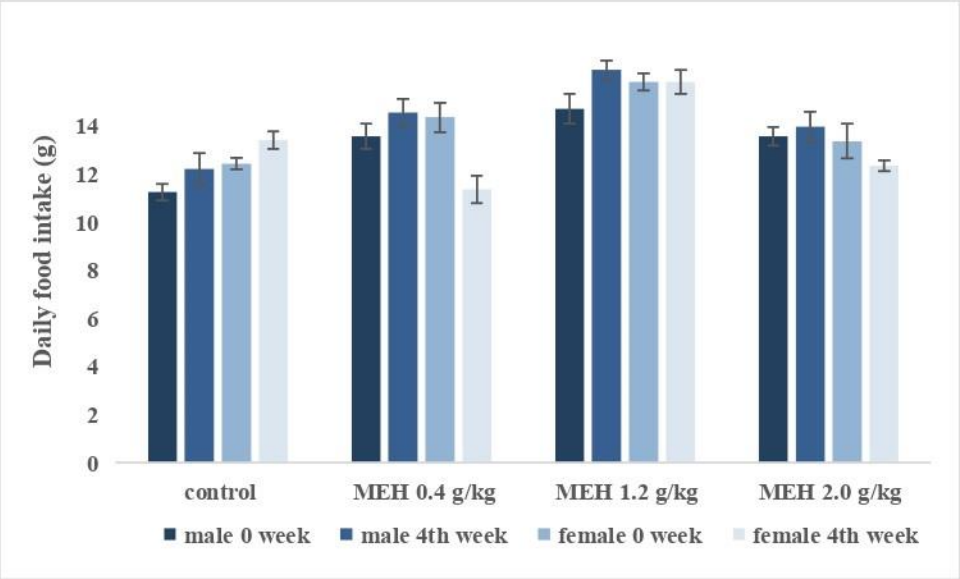
During the toxicity study of medicinal plants, LD_{50} determination is the first step to be conducted. The acute toxicity study may serve as classification and labelling of drug, mode of toxic action of a substance and help to determine a dose of a new compound (Yuet Ping *et al.*, 2013). In the present study, the acute toxicity of MEH and isolated compounds (***1-5***) were evaluated in Wistar rats. Doses used of MEH and isolated compounds ***1-5*** for the study were 1.0 to 5.0 g/kg and 5, 50 and 300 mg/kg of bw, respectively. All the tested doses showed no obnoxious physiological and behavioral changes. No ophthalmological abnormalities were recorded in any of the treatment groups. No significant changes in food and water intake as well in body weight were observed when compared with control group rats. Since there was no mortality and sign of toxicity even after 14 days, substances that present LD_{50} higher than 5.0 g/kg via oral route can be assumed practically safe (Kennedy *et al.*, 1986). Therefore, it may be suggested that acute toxicity of the methanolic extract of *E. hirta* is practically null via oral route and similar results have showed in studies done in mice (Lanhers *et al.*, 1991; Rajeh *et al.*, 2010) and isolated compounds are safe up to a single dose up to 300 mg/kg body weight.

Subacute toxicity study

Subacute toxicity studies assess the detrimental effects of repeated exposure of plant extracts over a portion of the average life span of experimental animals, such as rodents. Precisely, they provide evidence for organ toxicity and are planned to identify no observable adverse effect level (NOAEL) and to determine suitable dose regimens for long-term studies (Andersen and Krewski, 2009).

Observations:

During the 28 days repeated oral dose study, MEH was administered to rats at 0.4, 1.2 and 2.0 g/kg bw. No mortality was observed till the endpoint of the experiment, and no clinical sign (such as piloerection, alteration in the locomotor activity) was observed during the experiment. Moreover, the change in body weight of the rats is not significant during the study period as of untreated control and treated groups and increased steadily from 194.24, 179.35, 180.57 and 201.35 g in initial week to 226.68, 206.83, 214.12 and 230.24 g at fourth week for male rats (Figure 5.3.1) and 200.26, 191.27, 183.89 and 203.46 g at initial week to 238.47, 219.62, 215.36 and 237.89 g at fourth week for female rats (Figure 5.3.1), respectively. Figure 5.3.2 showed daily food intake of control and treated group rats was not significantly affected. Food efficiency ratio of male 0.4 g/kg MEH treated rat (7.18) and in female 1.2 g/kg MEH treated rats (8.2) were lower than the other groups but statistically not significant to control group.



. Daily food intake of male and female rats treated orally with the methanolic extract of *E. hirta* by oral route for 28 consecutive days.

The values are expressed as Mean ± S.E.M (n=6). **MEH**-Methanolic extract of *E. hirta*

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. Phytochemical 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.

REFERENCES:

1. Sachi Saharma, Manoj Kumar, Pharmacological evaluation of Euphorbia hirta L. for diabetes and diabetic dyslipdemia vol. 8 Issue 1January 2023
2. Muhammad Zeeshan Ali, Malik Hassan, Mehmud, , Abdul Malik, Pharmacological

Evaluation of Euphorbia hirta, Fabonia Indica and Capparis Decidua and hyper tension through in-vivo and in –vitro- assays.
3. S. Kumar, R . Malhotra , D. Kumar, Antidiabetic and free radicles Scavenging potential of Euphorbia hirta flower extract.
4. Anup Kumar Mourya, Smriti Tripathi Zabir Ahmad, Ram Kumar Sahu, Antidiabetic and antihyperlipidemic effect of Euphorbia hirta in streptozotacin induced to diabetic rats.
5. Sunil Kumar , Rashmi, D. Kumar , Evaluation of antidiabetic activity of Euphorbia hoirta linn. In streptozotacin induced diabetic mice vol. 1(2), June, 2010
6. Vibha Rani, Anubhuti Gupta, Sanya Verma antidiabetic activity of Indian medicinal plant.
7. S. Subramanian, S. Bhubneswari, G. Prasath antidiabetic and antioxidant potentials of euphoria hirta leaves extract studied and streptozotocin induced experimental diabetes in rats . Issue:1st September 2011
8. Tahira Shamim, Mehmud Ahmed , Muhammad Muktar antihyperglycemic and hypolipidimic effects of mythanolic extract of euphorbia prostrator on alloxal induced diabetic rabbits. Issue August 2014
9. S. Pradeepa, S. Subramanian, B. Kaviyarasan Biochemical evaluation of antidiabetic properties of Pithecellobium dulce fruits studied in streptozotocin induced experimental diabetic rats.
10. S. Gupta, L. Azmi, P.K. Mohapatra, C.Rao Flavonoids from whole Plant of Euphorbia hirta and their Evaluation against Experimentally induced Gastroesophageal Reflux Disease in Rats ,Issue 2017
11. D. Katare, Effect of ethanolic extract of Euphorbia hirta on chronic diabetes mellitus and associated cardiorenal damage in rats, Issue 2018
12. Nasirzadeh Mr., Nourazar Ar Effect of Alcoholic Extract of Euphorbia cyparissias on Serum Glucose and Antioxidant Enzymes Level in Diabetic Male Rats , issue 2-014
13. Le Thi Bach , L. Dung, Nguyen Trong Tuan, N. Thanh THE PROTECTIVE EFFECT OF SOME EXTRACTS AND ISOLATED COMPOUNDS FROM EUPHORBIA HIRTA ON PANCREATIC β-CELLS MIN6 , Issue 2018
14. B. Bading Taika, M. Bouckandou, A. Souza, H.P. Bourobou Bourobou, L. MacKenzie and L. Lione, An overview of anti-diabetic plants used in Gabon: Pharmacology and Toxicology, Journal of Ethnopharmacology,
15. https://doi.org/10.1016/j.jep.2017.12.036