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Anti-diabetic, Antioxidant, and Toxicity Studies of *Grewia abutilifolia* Leaves Extracts

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ABSTRACT

Various species of the genus *Grewia* are reported as glucose lowering agent besides other diverse pharmacological activity in ethno medicinal as well as in scientific studies. The present study pursues investigation on the in-vitro anti-diabetic, antioxidant, and cytotoxicity studies of the aqueous-alcoholic and ethyl acetate extract of *Grewia abutilifolia* leaves. Free radical scavenging activities of the *G. abutilifolia* were assessed by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay. The toxicity of extracts was evaluated through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on HepG2 cells, while neurotoxic effects were evaluated by the functional observational battery. Anti-diabetic potential of the extracts was assessed via four *in-vitro* tests; α-glucosidase inhibitory activity, α-amylase activity, glucose uptake in skeletal muscle cells 6-NBDG (6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) and GLUT4 uptake activity. In the DPPH assay, extracts showed concentration-dependent antioxidant effects. Ethyl acetate extracts displayed more antioxidant activity compared to aqueous alcoholic extract. Plant extracts did not exhibit any significant cytotoxic effect on the tested cells. Also, the extracts did not inhibit cell proliferation at lower concentration and showed mild anti-proliferative effect at very higher concentration having IC50 values 16 mg/mL and 35.28 mg/mL for aqueous-alcoholic and ethyl acetate extract, respectively. The extracts showed moderate to good anti-diabetic potential based upon the results of the glucosidase, α-amylase activity, glucose uptake and GLUT4 assays. Based upon the outcome of the study, *G. abutilifolia* was found to possess good anti-diabetic and antioxidant potential without any significant toxic effects. Plant can be explored further for its pharmaceutical applications.

Keywords: Toxicity, Pharmaceutical, Hot Extraction, MTT assay, α-glucosidase,6-NBDG, GLUT4 uptake.

Introduction

A set of disarray symbolized by anomalous glucose homeostasis with raised glucose levels could be diagnosed as Diabetes mellitus (Rusu, V et al., 2017). Diabetes is the most common chronic disease discussed under WHO's "Preventing chronic diseases a vital investment" in 2005 and type 2 diabetes is the most common narration for 90% of all diabetes (WHO, 2005). Diabetes affects around 422 million people worldwide, the majority of whom live in low- and middle-income countries, and it is responsible for 1.6 million fatalities each year. Over the last few decades, both the number of people

diagnosed with diabetes and the prevalence of the disease have significantly increased. The probability of secondary metabolic disorders like atherosclerosis, RA (rheumatoid arthritis), neuropathy, retinopathy, renal disease, blindness, and neurodegenerative are significantly escalated in diabetic patients. The disease also puts a socio-economic burden on the sufferers and family with life-threatening episodes at the last stages (Abbas et al., 2019). Type 2 diabetes involves pathogenesis pre-dominantly at adipose tissue and in the liver with respect to pancreatic islet impairment (Vinuela et al., 2020). Uncontrolled diabetes is linked to the emergence of complications that can jeopardise one's health and quality of life. (Cannon et al., 2018).In type 2 diabetes, cells of the body were inactive to insulin although it is available in the sufficient quantity, condition is called insulin resistance, ultimately leads to the elevation in the level of blood glucose (Moini et al., 2019). Management of Type 2 diabetes carried out generally by controlling glycemic index, lifestyle changing, using oral hypoglycemic medications and sometimes also by using extra insulin (Khoo et al., 2017). Herbal therapy has a long history of use in the treatment and prevention of ailments, including diabetes, when compared to mainstream medicine (Choudhury et el 2018). Diabetes mellitus medication has associated side effects including as weight gain and CVS (cardiovascular system) problems, which impacted the world economy $1.3 trillion in 2015 (Rivera-Mancíaet al., 2018; Bommer et al., 2018).Prediabetic condition involve higher glucose level compared to normal, but not reached up to level to be declared as diabetes. Majority of patient remain in prediabetic phase before actual development of diabetes, which usually remain unnoticed. Person with prediabetic condition have significant higher risk to develop type 2 diabetes (Tuso et al., 2014).

Research finding revealed that elevated glucose level for prolonged time induces higher Reactive Oxygen Species (ROS) due to enhanced autoxidation of glucose and protein glycation (Lobo et al., 2010; Jones et al., 2008).Higher degree of Protein glycation contributes in development various complications such as arterial hypertension, atherosclerosis, retinopathy, nephropathy, and other cerebrovascular as well as cardiovascular diseases (Jakus et al., 2004; Deo et al., 2009). Enzymes like α-amylase and α-glucosidase play vital role in breakdown and absorption of glucose, respectively. Inhibition of both enzymes is found to be effective in decreasing the postprandial glucose level and this strategy is widely used in management of hyperglycemia condition among diabetes patients. Some drugs like miglitol, acarbose, and voglibose inhibits the activity of α-amylase and α-glucosidase (Safamansouri et al., 2014; Adisakwattana et al., 2012;Deo et al., 2016; Sudhir et al., 2002; Etxeberria et al., 2012).

Currently synthetic drugs used as enzyme inhibitors in diabetes management are related with several potential side effects. Plants-based drugs can be attractive and more safe alternative options of synthesized drugs for the management of hyperglycemia condition (Choudhury et al., 2012). Herbal products having high content of antioxidants and polyphenols are well established agents in reducing ROS and delaying in onset of diabetic problems. Advanced Glycation End Products (AGEs) and ROS formed due to chronic hyperglycemia significantly contribute in diabetic complications (Tan et al., 2018).In a study, Koch et al., 2016 evaluated the inhibitory effects of seven liquid nutritional supplements against α-amylase and α-glucosidase. Among the tested supplement, *Samoan noni* juice and chlorophyll extract exhibited the excellent inhibitory effects against α-amylase and α-glucosidase, respectively, even superior to the positive control acarbose (Koch et al., 2018).*Syzygiumpaniculatum* was evaluated for inhibition of α-glucosidase, α-amylase, and protein glycation. Methanolic extract of leaves displayed prominent α-amylase inhibitory activity with IC_{50} value of 14.29 µg/mL, while aqueous methanolic extract showed prominent inhibition (IC_{50}: 4.73 µg/mL) of α-glucosidase activity. Fruit extracts of *Syzygiumpaniculatum* inhibited formation of AGEs with IC_{50} value of 11.82 µg/mL (Kim et al., 2020).In an endeavor to diminish side effects and, at contemporaneous, to get hold on the nutritional and medicinal benefits of plants and their isolates, natural products have gained considerable attention worldwide. As per the plant list (2013), the *Grewia* genus has 673 species, in which few of its species are scientifically proven for anti-diabetic activity like *Grewia hirsuta, Grewia*

asiatica, Grewia optiva,Grewia nervosa and *Grewia mesomischa*(Natarajan et al., 2015; Khatune et al., 2016;Bari et al., 2019;Deo et al., 2016;Mongalo et al., 2018). *Grewia abutilifolia* is a shrub belongs to Tiliaceae family and it is known for its diverse medicinal values (Khasim et al., 2020). Ethnobotany studies divulge its various uses like a cooling agent, refreshing drink, anti-inflammatory, anti-rheumatism, demulcent and anti-diabetic (Quattrocchi et al., 2020).As time passed diverse medicinal effects of concerned genus are being established apart from its traditional use as a fruit and cooling drink (Mall et al) The sight of present study is to explore *Grewia abutilifolia* for innumerable biological activities, for instance, antioxidant activity, *in-vitro* and *in-vivo* toxicity study, and *in-vitro* anti-diabetic activity. In explore the anti-diabetic potential of the plant, various tests i.e. α-glucosidase inhibitory activity, α-amylase activity, glucose uptake in skeletal muscle cells (6-NBDG) and GLUT4 uptake activity were carried out.

Materials and Methods

Plant material

The *Grewia abutilifolia* leaves were collected during July-August of 2018 in and around Pune, Maharashtra, India. Leaves was washed properly under tap water and shade dried. Sample were authenticated in National Institute of Science Communication and Information Resources (voucher no Ref No: NISCAIR/RHMD 3290/91). Anhydrous plant material pulverized and screened through 100 mesh size.

Extraction Procedure

The dried powder was subjected to hot extraction. The extraction was accomplished with ethyl acetate and mixture of two solvents with different polarities i.e. aqueous ethanolic mixture (30:70 v/v ratio). In the first phase, extraction was performed in ethyl acetate (three cycles) using round bottom flask (RBF) fitted with condenser at 80 °C temperature for 24 hours. In the next phase, same sample was extracted with aqueous ethanolic mixture (three cycles) at 100 °C for 30 hours.

The extract was passed through Whatman filter paper (41) to accomplish filtration. Both extracts were concentrated with the help of a rotary vacuum evaporator. The samples were weighed and stored in a small bottle inside the fridge at (8 ºC). The percentage yield was determined with below formula:

Percentage Yield (%)= E/R×100

(E= Wt. of Extracted residue, R= weight of Raw material)

Cell culture

Rattus norvegicus's (Rat) L6 skeletal muscle cell and HepG-2 (human hepatocellular carcinoma cell) were procured from National Centre for Cell Science, Pune, India. Both cells were sustained in DMEM (Dulbecco's Modified Eagle Medium), adjunct with 10% FBS (Fetal Bovine Serum) and Sterptomycin100U/mL antibiotic followed a constant supply of 5% CO_2, maintaining temperature 37˚C. Cell was secured in 2% FBS and all experiments were accomplished succeeding approximately 90% conflux after 5 or 6 days.

Free Radical scavenging activity [DPPH((1,1-diphenyl-2-picrylhydrazyl) Test]

Antioxidant activity of the extracts were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as per the reported protocol. The DPPH assay is based upon blanching of DPPH's characteristic violet colour to reduced yellow form (Szerlauth et al., 2019; Adebiyi et al., 2019). 3500 µL quantity of 60 µM DPPH (methanolic) was mixed with 100 µL extract of plant having volume of 100 µL in various concentrations i.e 100, 80, 60, 40, 20, 10µG. All plant extracts were made-up in DMSO to attain the sample solutions. In the presence of antioxidant, violet colour of DPPH changes to yellow, which is an indication of reaction progress. After the stabilization of color, around 20 minutes later in dark, reading was noted down. Ascorbic acid was set as positive control drug in the antioxidant test. Absorbance was measured

using the spectrophotometer (LAB INDIA, Model 3200) at 517nm wavelength. All values were documented, and the percentage inhibition determined using the below formula:

% Inhibition = [(Absorbance of control Sample - Absorbance of Test Sample)/Absorbance of control Sample] X 100

Cytotoxicity evaluation by MTT assay

On HepG2 cells, the cytotoxicity of *Grewia abutilifolia* leaf extracts was tested. DMEM-High Glucose (#AL111, Himedia), Fetal Bovine Serum (#RM10432, Himedia), MTT Reagent (5 mg/mL) (# 4060 Himedia), DMSO (#PHR1309, Sigma), Camptothecin (#C9911, Sigma), and D-PBS (#TL1006, Himedia) were used to cultivate the cells. 200 µL cell suspension of HepG2 cell line was poured in a 96-well plate at required cell consistency (20,000 cells per well) and cultured for 12h. Cells were sustained in various dilutions of extract, such as 25, 50, 100, 200, and 400 g/mL and were maintained in 37 °C incubators with 5% CO2. Used medium was discarded after incubation, and 20 L of MTT reagent was added to the cells and incubated for 2 hours in a CO_2 incubator. At 570 nm characterization process was carried out. All experiments were consummated in triplicate. % cell viability calculated by a standard formula.

Acute toxicity evaluation for Toxicology

All experiments were consummated on experimental rats, sanctioned by Institutional Animal Ethics committee, Pinnacle Biomedical Research Institute (PBRI), Bhopal having registration No. 1824/PO/Rc/S/15/CPCSEA. Eighteen rats with either sex (nulliparous in terms of female), weight 200-225g were used for the study. After all necessary requirements for welfare and ethical use of animals, eighteen rats were divided into 3 groups (where n=6): Group I (control), Group II (Aqueous alcoholic extract), and Group III (Ethyl acetate extract). The study procedure adapted as per the OECD (Organization for Economic, Co-operation and Development) Guidelines, 423 with four doses 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. FOB (functional observational battery) parameters were appraised at 0,1,7 and 14th day at various times i.e. 2:30 pm, 3:30 pm, 4:30 pm, and 5:30 pm (As per Indian Standard Time) after a single administration of various doses (Olayode et al., 2020; Upadhyay et al., 2019).

FOB for Experiment

FOB is neurobehavioral screening methods widely used to identify potential neurotoxic effect or activity related parameter of new drugs, or extract in rat. FOB was performed using the both extracts of *Grewia abutilifolia*, with single dose at different days with various time intervals and results of the samples were compared with control group.

Antidiabetic study

The antidiabetic studies were carried out using below mentioned tests:

Alpha-Glucosidase Inhibition Activity:

Various concentrations of the plant extracts i.e. 62.5, 125, 250,500 and 1000 µg/mL along with 1 mL of starch solution (2% w/v Maltose/ Sucrose) and 0.2 M Tris buffer of pH 8.0 were incubated (5 min at 37^{o}C). After the incubation process, the reaction was initiated with 1 mL of the alpha-glucosidase enzyme (1 U/mL), then incubate for 40 min at 35°C. Termination of the reaction was consummated by adding 2 mL of 6N HCl. Voglibose was used as standard positive control drug (Manikandan et al., 2016). Color intensity was measured at 540 nm using an ELISA reader (BIOTEK ELX 800). Percentage inhibition (I%) calculation formula as below:

I%=100-(As-Ab)/(Ac-Ab) X 100

As = Average absorbance of the Sample
Ab= Average absorbance of the Blank
Ac =Average absorbance of the Control.

Digestive enzymes inhibition (Alpha-amylase activity):

0.1% w/v starch solution was prepared by stirring 0.1g of potato starch (100 mL of 16 mM, sodium acetate buffer). Solution of enzyme was developed by mixing 27.5 mg of Alpha-amylase in 100mL of DDW, on other hand, colorimetric reagent was prepared by mixing sodium potassium tartrate solution and 3, 5-di nitro salicylic acid solution (96mM). Finally, the starch solution was incubated with standard and test samples, and the alpha-amylase solution was allowed to act (alkaline conditions at 25^{o}C). The sample's absorbance was measured at 540 nm using an ELISA reader after 3 minutes (Shettar et al., 2017). Percentage inhibition (I%) was calculated by the below-mentioned formula:

I%=100-(As-Ab)/(Ac-Ab) X 100

As = Average absorbance of the Sample
Ab =Average absorbance of the Blank
Ac =Average absorbance of the Control.

Glucose uptake in skeletal muscle cell:

L6 myotubes cells cultured in 6-well plate at a density of 2 x 10^5 cells/2 mL in DMEM with CO_2, overnight at 37 °C. After a day's duration, discarded the spent media and treated with test and standard compound, 2 mL glucose-free culture medium containing 100 µM 6-NBDG (Time of incubation is 2-4 hrs.). After completion of the reaction, remove media then wash with PBS solution and add 200 µL trypsin. Incubate for 3 to 4 hrs. at 37°C. Add 2 mL of DMEM culture in 12 x 75 mm tubes. After centrifugation carefully aspirates the supernatant. Analysis carried out after resuspension in 0.5-1 mL PBS. Analysis accomplished with flow cytometer at 465 nm and 540 nm respectively (Magnone et al., 2020; Aneesa et al., 2019).

GLUT4 Uptake and translocation Activity:

HepG2 cells were incubated in 6 well plate at density 2 x 10^5 cells/2 mL with desirable conditions (CO_2 with temperature 37°C). Over a day incubation, aspired spent media with test and standard (prepared in 2mL glucose-free culture media). After 2 hr incubation, the media was removed and washed with PBS. After removing PBS, added 2 mL culture media and culture cells further in12 x 75 mm tubes. Centrifuged and precisely aspirated supernatant. The cells were resuspend in PBS(0.5-1mL), incubated in Mouse Anti-Human Glut4 Monoclonal Antibody for 30min (On ice) and add 2mL buffer (FACS). After rewashing again incubate for 30min in dark with 5µL fluorescein isothiocyanate (FITC Goat Anti-Rabbit IgG). Analysis was done through Flow Cytometer (Becton-Dickinson, San Jose, CA) at 488nm via Cell Quest Pro Software (Wang et al., 2020).

Statistical Analysis

All experiments were repeated three times, with results represented as mean standard deviation (SD). IC50 was calculated by MS excel . Kruskal-Wallis analysis was used in FOB with the help of Sigma Plot software 12.3 version.

Result and Discussion

Free Radical scavenging activity [DPPH ((1,1-diphenyl-2-picrylhydrazyl) Test]

The *in-vitro* antioxidant activity of ethyl acetate and aqueous-alcoholic extract of *Grewia abutilifolia* are presented in **Figure 1**. The results showed that both extracts scavenge the radical in a concentration- reliant mode. In inclusion, comparable scavenging activities of both extracts were observed with those of the standard compound. However, ethyl acetate extract showed higher DPPH scavenging activity compared to aqueous alcoholic extract. It was perceived that the concentration that can scavenge 50% of the radical (IC_{50}) values of both the extracts were 58.841 µg/mL (Ethyl acetate Extract) and 76.303 µg/mL (Aqueous alcoholic Extract). IC50 value for ascorbic acid was 82.974 µg/mL.

Cytotoxicity evaluation by MTT assay

Cellular toxicity was assessed by the MTT assay utilizing HepG2 cells having concentration i.e. 25, 50, 100, 200, 400 ug/mL (Plant extracts and positive control, Camptothecin) for 24hrs incubation.

Observation of statistical data of cell cytotoxicity study revealed that extract does not show any significant cytotoxic properties in the concentrations ranging from 25ug/mL to 400ug/mL, respectively in the incubation period of 24hrs. The Results suggested that the test extracts are having IC_{50} value for aqueous alcoholic extract was 16mg/mL and ethyl acetate extract have 35.28mg/mL.**Table 1** shows % cell viability of test extracts compared to 15 µM standard Camptothecin drug. Hence results showed that extracts not exhibited significant toxicity to HepG2 cells. **Figure 2** contribute extracts non-toxic nature with HepG2 cells.

Acute toxicity evaluation for Toxicology with safety contour

Test drugs (aqueous alcoholic extract and ethyl acetate extract) showed various effects on FOB, initial undesirable effects in terms of behavioral and neurotoxin effects were observed by FOB. *Grewia abutilifolia* shows various effects on different time intervals (0, 2, 4, 6, 8 hrs) and on 1^{st}, 7^{th} and 14^{th} day. Oral administration of 5, 50, 300 and 2000 mg/kg dose was found with no adverse effects including alertness, tremors, convulsion, right reflux, griping reflux, corneal reflux, pupils reflux, urination, salvation, skin colour, lacrimation and hyper activity. Apart from these, based upon the Rat Grimace Scale (RGS) scale for the animals which involve eye, nose, ear and whisker changes, there was no indications of toxicity observed. Conclusively no remarkable changes were noticed in FOB with different extracts of *Grewia abutilifolia* as juxtaposed to control group. Detailed observations are depicted in the **Table 2.**

Antidiabetic study

Digestive enzymes inhibition (Alpha-amylase activity):

Digestive enzymes inhibition studies by ELISA reader suggested that aqueous-alcoholic extract and Ethyl acetate extract shows good alpha Amylase enzyme inhibition activity in the concentrations range of 62.5-1000 ug/mL. IC_{50} value of standard drug voglibose was 431.57µg/mL and IC_{50} values of aqueous-alcoholic and ethyl acetate extracts were 585.7 µg/mL and 241.18µg/mL, respectively (**Table 3 & Figure 3**).

Alpha-Glucosidase Inhibition Activity:

IC_{50} values for Alpha-glucosidase inhibition activity were determined for the sample's solutions of *Grewia abutilifolia*(Given in **Table 4 & Figure 4**). IC_{50} value for aqueous alcoholic extract and ethyl acetate extract were found to be 526.43 ug/mL and 334. 38 ug/mL respectively, while standard drug voglibose showed IC_{50} of 15ug/mL. The results depicted that a decrease in inhibitory activity was observed while increasing fraction polarity.

Glucose uptake in skeletal muscle cell:

The monitoring carried out by flow cytometry recommends that in HEPG2 cells, the Relative Mean Fluorescence Intensity of 6-NBDG is very less in Untreated Cells (9.98MFI) as compared to Metformin (100uM; 55.21MFI). The aqueous-alcoholic and ethyl acetate extract at the concentration of 400ug/mL show to be 35.58 and 48.33(MFI), respectively of 6-NBDG Expression in terms of Mean Fluorescence Intensity.

The observations suggested that the test extracts may have possible therapeutic and anti-diabetic capacity and could be explored further for the anti-diabetic ability in the *in-vivo* models (**Fig 5** and **Fig 6**)

GLUT4 Uptake and translocation Activity:

The GLUT4 Uptake and translocation study performed by the flow cytometry in L6 cells divulged that relative mean fluorescence intensity of GLUT4 was established to be less in untreated cells (7.04MFI) in comparison Metformin (37.42MFI) at the tested 100 µM. Test aqueous alcoholic and ethyl acetate extracts at 400µg/mL showed the intensity of 21.81 and 24.62 MFI, respectively. The outcome of the study revealed that the test extracts showed comparable results to control drug and have anti-diabetic potential and can be explored further for the anti-diabetic drug potential (**Fig. 7** and **Fig. 8**).

Current finding in context to published relevant studies

We analyzed the previous published studies on various species of *Grewia* genus, reported for various activities viz. antioxidant activity, toxicity, cell proliferation inhibition and antidiabetic activity. Relative comparison has been performed of our findings with the published reports and same discussed below:

In a study by Rao et al., 2018, *Grewia villosa* root extract showed DPPH scavenging activity with IC_{50} value of 185 µg/mL and 162 µg/mL, for Ethyl acetate and hydroalcoholic (70% Ethanol), respectively (Rao et al., 2018). In another, study Ethyl acetate extract of *Grewia hirsute* leaf extract showed DPPH scavenging activity with IC_{50} value of 34.72 µg/mL (Hutke et al., 2020). A study on *Grewia asiatica* by Gupta et al., 2007 showed scavenge of 50% of the radical at concentration of 26.17±1.49 µg/mL (Gupta et al., 2007).The findings of our study on leaves extract of *Grewia abutilifolia,* revealed IC_{50} of 58.841 µg/mL and 76.303 µg/mL for ethyl acetate and aqueous alcoholic extract, respectively, which found to in compliance other published reports. High content of antioxidants like flavonoids and polyphenolic compounds may be attributed for the good antioxidant activity (Adebiyi et al., 2017; Rani et al., 2021).

Various of species of genus *Grewia* like *G. arborea*, *G. tiliaefolia*, G. *tiliaefolia, G. serrulata*, *G. tenax* are already explored by the different researchers for cytoxicity studies, in majority of which, no significant toxicity was reported. Key findings of such studies are summarized in the below section (Selvaraj et al., 2017; Rajavel et al., 2017; Chandiran et al., 2013;Al-Said et al., 2011)

In previous study by Selvaraj et al., 2017, ethanol extract of *Gmelinaarborea* (GA) and *Grewia umbellifera* leaves showed moderate cytotoxic effect on Vero cell line with IC_{50} value of 541.42 and 651.95 µg/mL, respectively (Selvaraj et al., 2017). Dicson and team performed toxicological studies using methanolic extract of *Grewia tiliaefolia* leaves, extract not exhibited any toxicity in blood mononuclear cells up to concentration of 2000 µg/mL. Moreover, no evidence of other toxicities like genotoxicity, and organ toxicity reported at the tested concentration (Dicson et el., 2015). Rajavel et al., 2017 performed cytotoxicity studies and anti-lung cancer activity of *Grewia tiliaefolia* (GT) extract. Benzene extract of GT leaf found to be non-toxic to normal human lung cells but inhibited the cells proliferation and also induced apoptotic cell death in A549 cell line. Benzene extract was found to be safe, and well tolerated *in-vivo*. Aqueous and ethanolic extracts of *Grewia serrulata* was found to be safe on Wistar Albino rats with no observed toxicity. LD_{50} of aqueous and ethanolic extracts was found be greater than 2000 mg/kg in female rats. Further, no abnormalities were detected in the extracts treated groups when compared with normal group in the Histopathological examination. In another study, 96% ethanolic extract of *Grewia tenax* fruits did not show lethality up to the dose of 2 g/kg in mice. Summarized finding of previous published reports on toxicity of *Grewia* genus supports outcomes of our study, in which cellular toxicity was reported at very high concentration, while no in vivo toxicity was observed at the tested concentration.

Through review of the published literature revealed that, no study is published on Grewia abutilifolia, assessing its effect on glucose uptake in skeletal muscle cell, GLUT4 uptake and translocation activity. In some studies, *Grewia nervosa* and *Grewia abutilifolia*are reported for α-amylase and α-glucosidase inhibition activity (Deo et al., 2016; Meena et al., 2017; Mamun et al., 2017). Apart from aforementioned enzymes inhibition, prominent content of flavonoids and other antioxidants may be contributing for the antidiabetic activity of *G.abutilifolia* (Al-Ishaq et., 2019;Rani et al., 2021).

Table 1: Table showing the Absorbance Readings at 570nm in ELISA Plate Reader of the Aqueous Alcoholic Extract and Ethyl Acetate Extract against the HepG2 Cell line

Concentration (uG)	% Cell Viability (Aqueous alcoholic Extract)	% Cell Viability (Ethyl acetate Extract)
Cell Control	100±0.0035	100±0.035

Std Control	50.23±0.0028	50.23±0.0026
25	99.84±0.0014	97.23±0.021
50	98.61±0.0021	95.91±0.0026
100	96.97±0.0028	93.52±0.0021
200	95.59±0.0028	92.29±0.0029
400	93.68±0.0014	90.28±0.0028

Table 2: Toxicity studies of Aqueous Alcoholic Extract and Ethyl Acetate Extract of *G.abutilifolia* at various time intervals.

FOB Test/ Group	Normal Control														
Weight															
Day	1 Day					7 Day					14 Day				
Hours	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
Alertness	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Tremors	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Righting Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Gripping Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Corneal Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Puplis Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyper Activity	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RGS Scale															
Eye Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nose Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ear Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whiskers Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FOB Test/ Group	Aqueous alcoholic extract																																																											
Weight	5 mg															50 mg															300															2000														
Day	1 Day					7 Day					14 Day					1 Day					7 Day					14 Day					1 Day					7 Day					14 Day					1 Day					7 Day					14 Day				
Hours	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
Alertness	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Tremors	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Righting Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Gripping Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Corneal Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Puplis Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyper Activity	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RGS Scale																																																												
Eye Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nose Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ear Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whiskers Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FOB Test/ Group	Ethyl Acetate extract																																																											
Weight	5 mg															50 mg															300															2000														
Day	1 Day					7 Day					14 Day					1 Day					7 Day					14 Day					1 Day					7 Day					14 Day					1 Day					7 Day					14 Day				
Hours	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
Alertness	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Tremors	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Righting Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Gripping Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Corneal Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Puplis Reflux	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyper Activity	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RGS Scale																																																												
Eye Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nose Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ear Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whiskers Changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Alpha-amylase inhibitory activity of Aqueous Alcoholic Extract and Ethyl Acetate Extract of *G. abutilifoli*

Test (ug/mL)	IC_{50} (ug/mL)
Voglibose	431.57±0.34
Aqueous alcoholic extract	585.7±0.20
Ethyl acetate extract	241.18±0.01

Table 4: Alpha Glucosidase inhibitory activity of the Aqueous Alcoholic Extract and Ethyl Acetate Extract of *G. abutilifolia*

Test (ug/mL)	IC_{50} (ug/mL)
Voglibose	290.15±0.2
Aqueous alcoholic Extract	526.43±0.01
Ethyl Acetate Extract	334.38±0.09

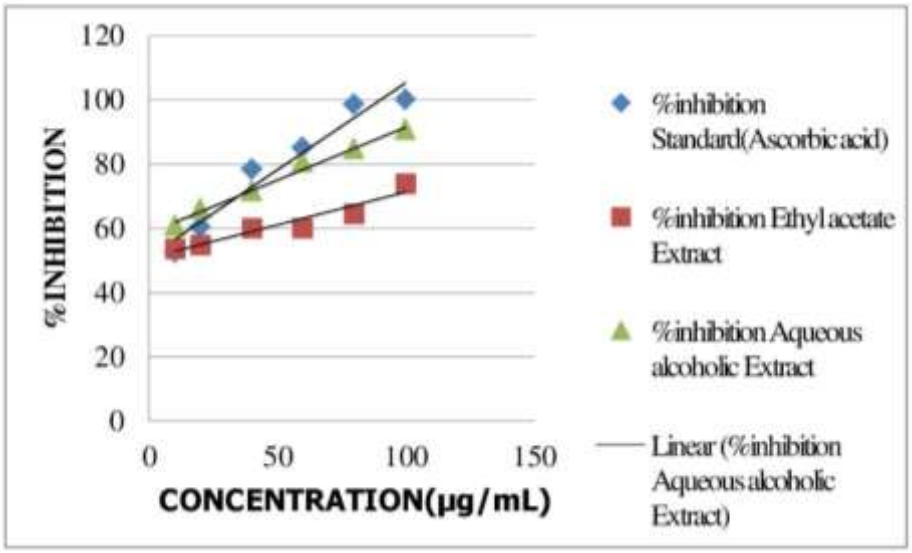


Figure 1: Free Radical scavenging activity [DPPH (1,1-diphenyl-2-picryl hydrazyl) Test]

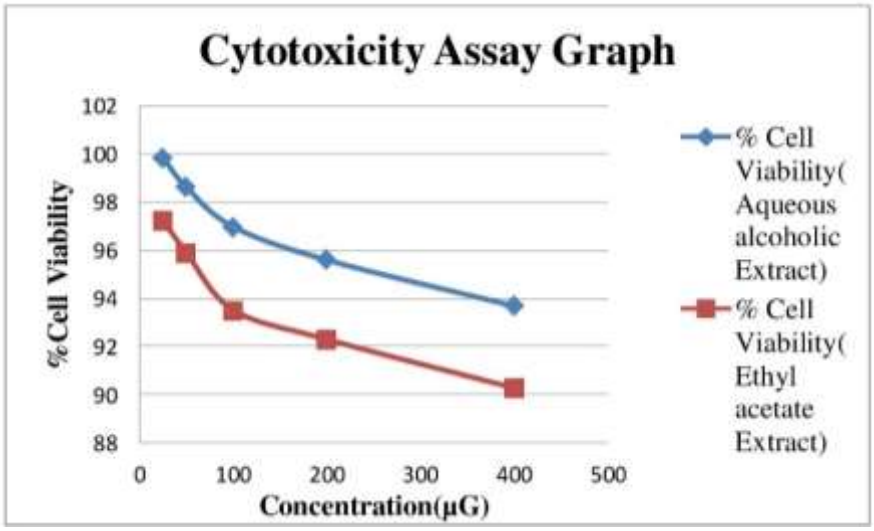


Figure 2: Scatter Graph show %cell Viability of HepG2 cell line against the sample

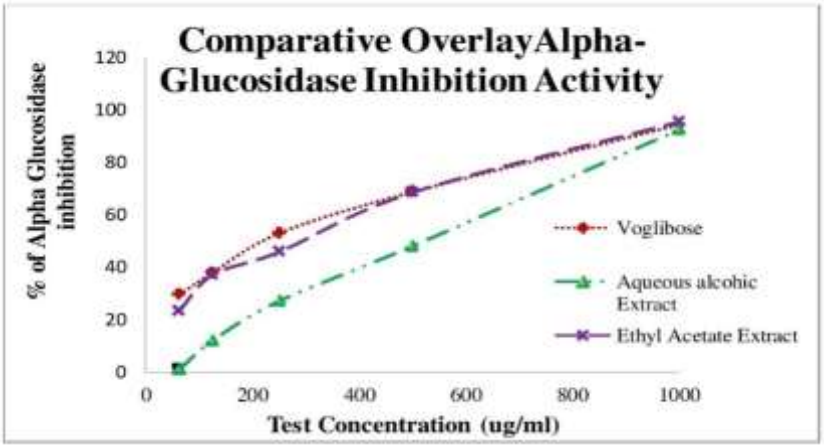


Figure 3: Overlay Scatter graph for % Alpha Glucosidase inhibition as per concentration of standard and test extract

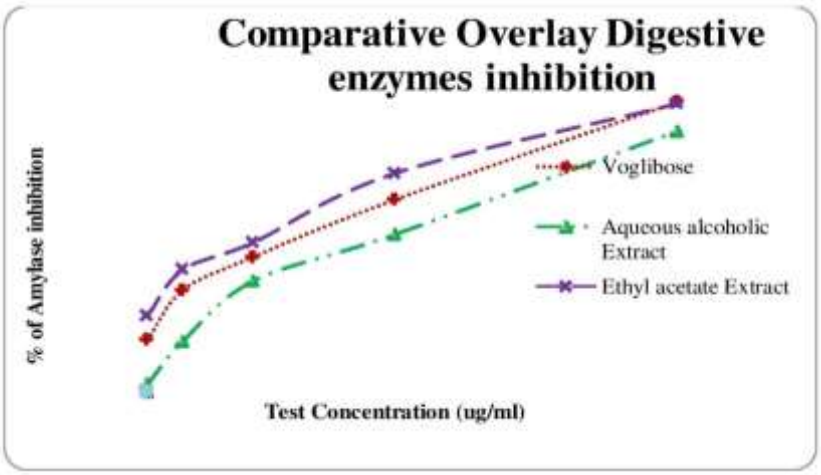
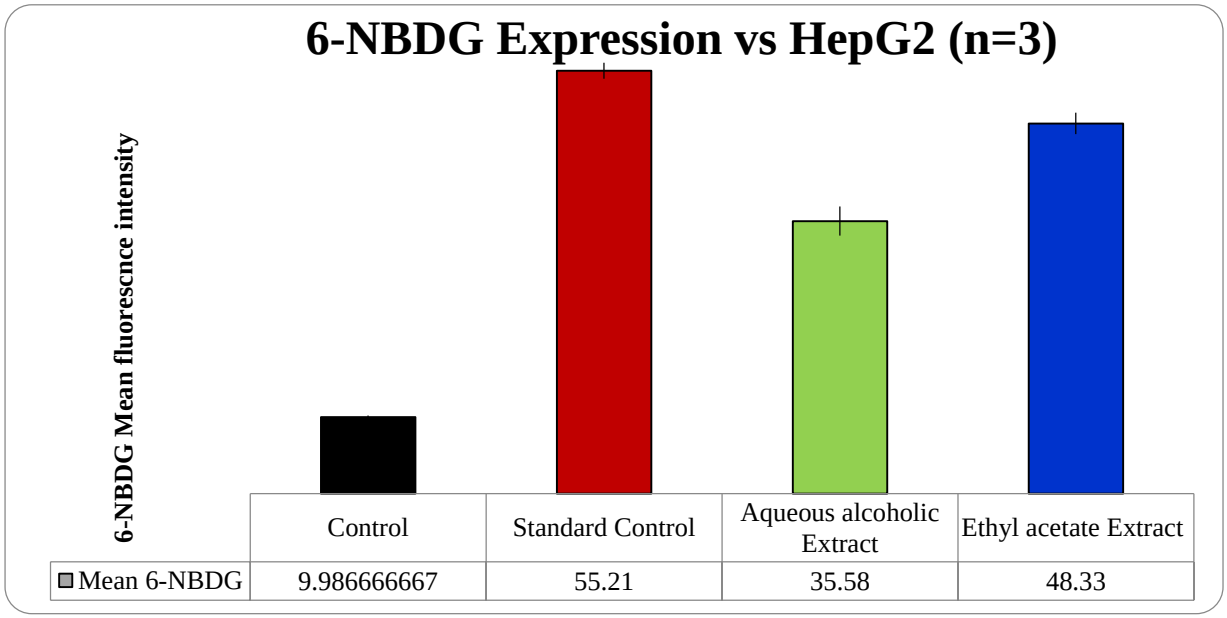


Figure 4: Overlay Scatter graph for Digestive enzyme inhibition as per concentration of standard and test extract



	Control	Standard Control	Aqueous alcoholic Extract	Ethyl acetate Extract
Mean 6-NBDG	9.986666667	55.21	35.58	48.33

Figure 5: Overlay Bar Graph for 6-NBDG test of standard and test extract

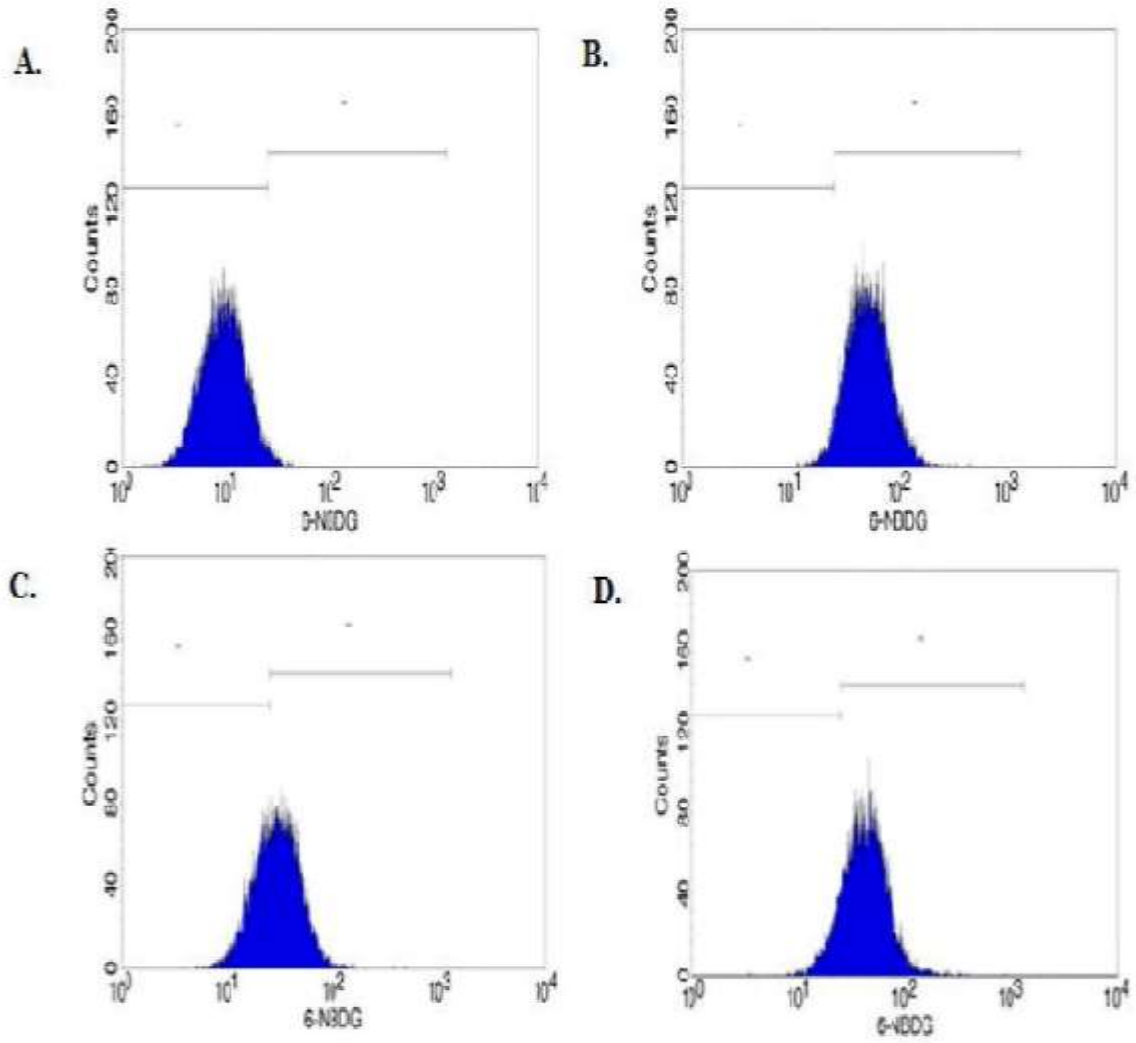


Figure 6: 6-NBDG expression study of the test extracts against HEPG2 cell line A: Control, B: Standard Control, C: Aqueous alcoholic extract, D: Ethyl acetate extract

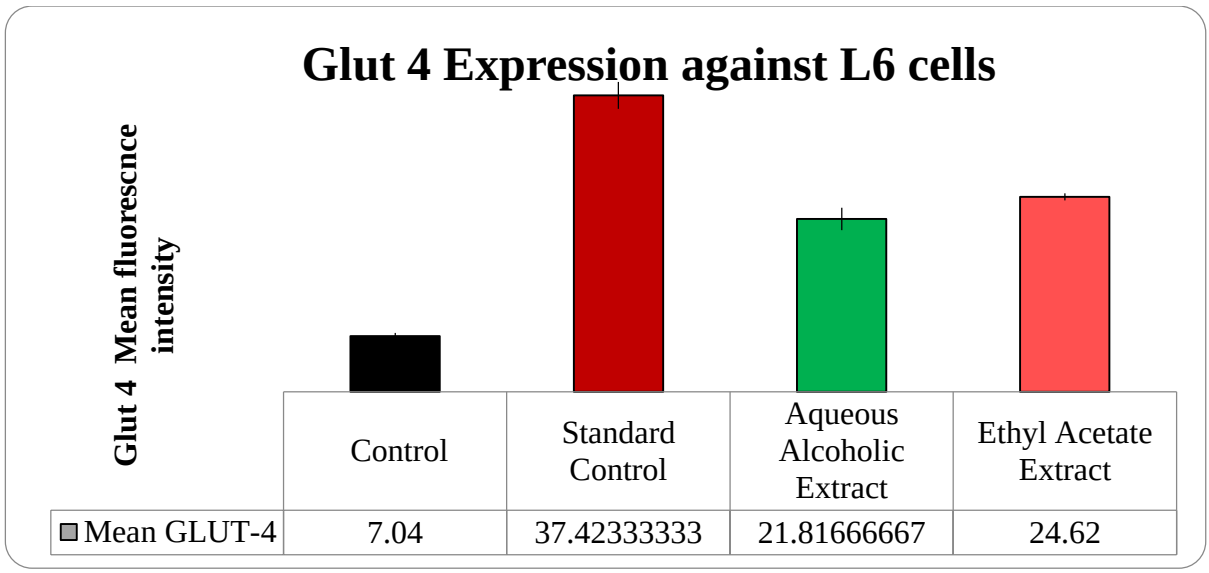


Figure 7: Overlay Bar Graph of Glut 4 expression against L6 cell lines

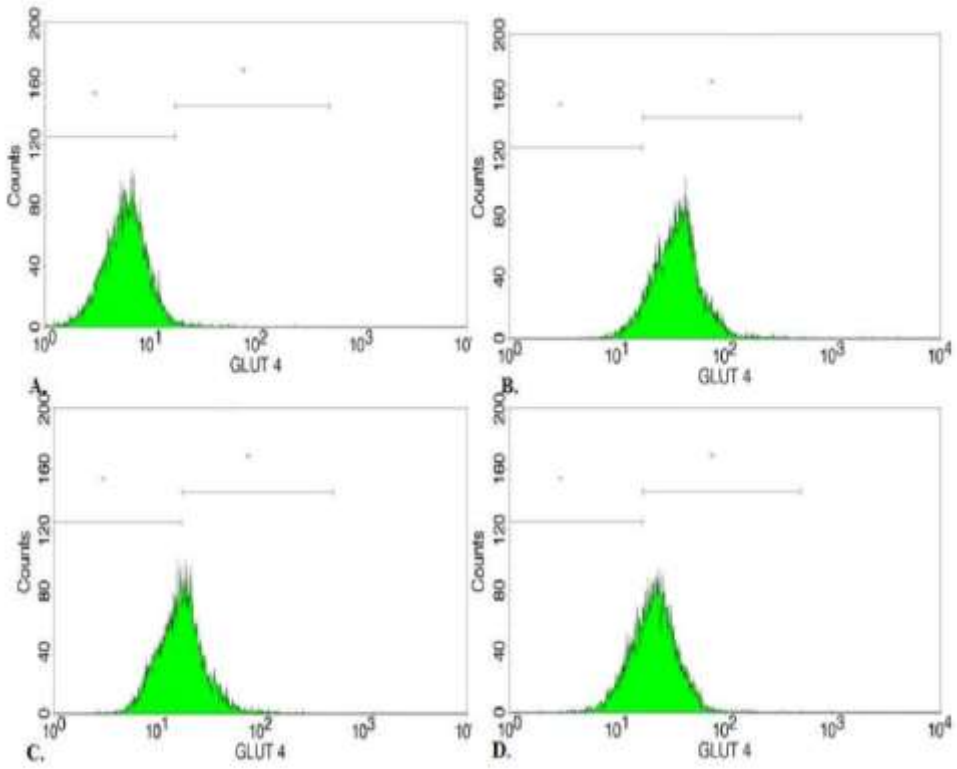


Figure 8: Gut 4 Expression study of Test Extract against L6 cell using BD FACS caliber.
A: Control, B: Standard control, C: Aqueous Alcoholic extract, D: Ethyl Acetate extract

Conclusion

DPPH test revealed the concentration-dependent antioxidant effects of extracts and, comparatively aqueous-alcoholic extract showed lower antioxidant activity than ethyl acetate extract. As per previous study carried out, species shows good antioxidant activity Plant material did not manifest any remarkable cytotoxic potential over cells at the highest tested concentration, with IC50 values of 16mg/mL and 35.28mg/mL for aqueous-alcoholic and ethyl acetate extract, respectively. To further extrapolate the toxicity studies in-vivo toxicity studies carried out on rodents. FOB was carried out to see the behavioral and neurotoxic effects of the drug. No significant change in FOB was seen as juxtaposed to the control untreated group. Based on the in-vitro tests performed for the anti-diabetic activity, it can be culminated that the extracts exhibited good anti-diabetic potential and can be explored for in-vivo studies. Based upon the outcome of glucose uptake assay and GLUT4 uptake and translocation activity, the possible therapeutic effects of plant extracts in diabetes can be proposed. The study backs up the traditional usage of *G. abutilifolia* leaves as an anti-diabetic drug, claiming that its effectiveness is related to flavonoids and phenols found in the leaves. As a result, this shrub could be a good source of potent and possibly novel anti-diabetic lead(s), which could be further studied to produce a series of chemicals for the creation of potent and safe anti-diabetic medications.

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Conflict of interest

The authors declare that there is no conflict of interest.

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