CHAPTER-6

DISCUSSION
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Traditionally, most of herbs are being used for the treatment of diabetes mellitus, from which merely some have been evaluated as per the modern system of medicine. From various parts of such plants only extracts have been prepared and evaluated for its antidiabetic activity on experimental diabetes in animals. Most of the reported plants seem to act directly on pancreas and stimulate insulin release in the blood. Some will favorably alter the activities of regulatory enzymes of glycolysis, gluconeogenesis and other pathways by acting directly on tissues like liver, muscle and fat (extra-pancreatic effect). Chemical constituents of these plants are known to possess wide range of medicinal properties.

The research was envisaged for antidiabetic and hypoglycemic effect with respect to the antioxidant properties (both in-vitro and in-vivo) of aerial parts of aqueous and ethanolic extracts of Grewia serrulata DC (AEGS &EEGS) on streptozotocin plus high fat diet induced diabetic rats. Phytochemical and toxicological studies were also performed on the plant to find out its bioactive principles, LD_{50} value and safety profile.

Total ash value assess the total amount of material remained after detonation and the amount of heavy metals and inorganic compounds present in the powder sample. Table infers that as the total ash content was 5 times greater than acid insoluble ash, the presence
of calcium oxalate crystals or acid soluble inorganic matter was indicated in *Grewia serrulata* DC

The water and volatile content of a crude drug were determined by test for loss on drying. High water content will deprecate phytochemical constituents followed by hydrolysis and enhance growth of microorganisms. Hence there should be a set of confines for water content for a plant under research. In our investigation the percentage yeild of loss on dying was found to be 3.26% (w/w).

Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol and water soluble extractives values were found to be higher than ether soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non polar components yields higher extractive value as compared to water.

The various extract's yield values were illustrated and high yield was attained by ethanol solvent i.e., 20 %( w/w). It specifies that extractive power depends on the solvent polarity amongst which ethanol has fine polarity as evidenced by the depicted data.

Literature survey states that presence of alkaloids, flavonoids, glycosides, terpenes, steroids, polysaccharides, phenols, coumarins and proteins in the plant extract contribute to pharmacological activities such as antidiabetic, hypoglycemic, antihyperlipidemc and antioxidant properties. Preliminary phytochemical evaluation report illustrates that ethanolic extract of aerial
parts of *G. serrulata* (EEGS) showed the existence of glycosides, saponins, phytosterols, flavonoids, phenols, steroids, terpenoids and aqueous extract (AEGS) showed the presence of carbohydrates, saponins, phytosterols, flavonoids, steroids and terpenoids as phytoconstituents. The extracts of petroleum ether, benzene, chloroform and acetone reveal the absence of many chemical constituents. Hence, keeping all this in view, research work was focused on the above mentioned constituents in both extracts, for the evaluation of hypoglycemic, antidiabetic and antioxidant potential.

Toxicity study of a new compound must be done accurately for the selection of the dose, used for its pharmacological screening. This study is carried out on animals in the laboratory with a very sophisticated procedure.

In drug evaluation, LD$_{50}$ estimation (lethal dose for 50% of the test group) is regularly an early step in the evaluation of the toxic properties of a substance. It is a primary analysis of toxic indications, gives details about health risks that are likely to arise from short-term drug exposure and is one of the very first screening studies performed with all substances.

In this study, both the extracts at the dose of 2000mg/kg indexed neither visible signs of toxicity nor mortality and observations did not point out any proofs of substance related toxicity. The no-observed-adverse-effect level (NOAEL) was noticed at the dose of 2000mg/kg. As the LD$_{50}$ of extracts in rats was higher than 2g/kg, it
is categorized under category 5 of GSH as per OECD guidelines

Based on the LD$_{50}$ value, 1/5$^{th}$ and 1/10$^{th}$ of its value was chosen for pharmacological studies.

The aqueous and ethanolic extracts of aerial parts of *Grewia serrulata* DC were evaluated for safety profile by sub-acute toxicity study following OECD TG-407 in wistar albino rats of both sexes. Since clinical sign assessment plays a major part in toxicological evaluation, twice a day throughout the research mortality and morbidity had been recorded. Both extracts did not show significant change in their body weight gain, food and water usage contrasted to that of control. Justified by the outcomes, it reveals that aerial parts of *Grewia serrulata* DC did not negatively impact the basic metabolic process of the experimental rats.

The target organ system for toxic chemical substances and susceptible key for diseased functions both in human being and animals is the haemopoietic system.

During the study, extract treated groups did not revealed any variation in blood parameters. It indicates that *Grewia serrulata* DC has no effect on haematocytes and their production.

Astonishingly, in biochemical evaluation it was observed that serum glucose levels decreased in rats treated with extracts. It implies that *Grewia serrulata* DC could produce a slight hypoglycemic effect in normal rats. Many investigators during their research revealed that coumarin derivatives, flavonoids principles, terpenoids and secondary metabolites that includes arginine and glutamic acids possesses
antihyperglycemic properties in different experimental animal model.

Grewia serrulata DC of both extracts have an effect on lipid profile. A significant decrease in lipid levels was found in extract treated groups. Hence it infers that the plant possesses hypolipidemic activity and a few positive effects on the cardiac and vascular complications. Flavonoid existence in the plant may be the reason for lipid lowering activity. It was also observed that in AEGS of 800mg and EEGS of 400&800mg treated rats there was significant rise of protein levels in comparison to that of in control groups which was predicted due to its enhanced proteogenetic property.

The renal function was not disturbed in the rats treated with extracts as evidenced by its non significance in urea and creatinine levels compared to that of in control rats. Being creatinine a good sign of kidney activity, elevated levels of creatinine is observed unless if there is a noticeable break to renal functions. Normal structural features observed in the histological slides of kidney suggest the maintained renal reliability of extract treated rats.

Normally, hepatocyte damage is exemplified by an augmentation in the enzyme levels like LDH, ALP, AST and ALT etc. These enzyme levels were not altered during the study. An increase in serum ALP levels causes cholestatic liver disease. This parameter is insignificantly classified between the control and treated rats revealing that no feasible cholestasis took place at dosage levels tested. Medications like antibiotics and other chemical substances are identified to have an
effect on and alter bilirubin levels in blood circulation. Particular drugs and other substances are implicated to impact and affect circulating bilirubin. Increased bilirubin levels cause increased hemolysis\textsuperscript{144}. Both the extracts of \textit{Grewia serrulata} DC did perhaps not change the bilirubin levels in treated rats contrasted to the control.

Micrographic analysis of heart, lung, liver, spleen and kidney of the treated rats did maybe not show significant modifications in morphology showing the protective result of AEGS and EEGS on these tissues.

Herbal drugs are gaining a major role in testing various ailments across the world as they contain free scavengers. Presence of Phenolic and flavonoid contents in many plants is responsible for their dynamic antioxidant properties\textsuperscript{145}. Particularly phenols and flavonoids are effective antioxidants as they break the radical chains by donating electrons to radicals. Phenolic compounds will scavenge various reactive oxygen species and have shown to put forth a wide range of biological activities\textsuperscript{146}. Based on the obtained amount of total phenolic and total flavonoid contents, it was predicted that aerial parts of \textit{Grewia serrulata} DC possesses good antioxidant property which may play a key role for antihyperglycemic activity of the plant extracts.

Total antioxidant activity was also examined and results have confirmed the efficacy of the extracts for their antioxidant activity equi proportion to that of reference standard ascorbic acid.
DPPH being a steady free radical takes an electron or hydrogen radical to be a stable diamagnetic molecule. Natural antioxidants are evaluated for their free radical scavenging effect using DPPH at an absorbance of 517nm. Literature survey revealed that a positive correlation exists between free radical scavenging activity and total phenol content and hence DPPH free radical scavenging activity increases with the increase of phenol compound content. The DPPH free radical scavenging capability of the extract may be acknowledged to its hydrogen donating ability. Superoxide is the primary free radical in most biological systems. Though superoxide is unreactive radical contrasted to others, it can be decomposed to develop stronger oxidative species like singlet oxygen and hydroxyl radicals. It was found that aqueous and ethanolic extracts of aerial parts of *Grewia serrulata* DC (AEGS & EEGS) possess good scavenging activity against DPPH and superoxide radicals. Extracts were also evaluated for their nitric oxide radical scavenging activity. Reactive oxygen species (ROS) like superoxide anion react with nitric oxide and forms other reactive nitrogen species (RNS) like NO2, N2O4 and peroxynitrite. These two species combine and damage various acellular molecules and cellular components such as lipids, proteins, nucleic acids, carbohydrates. IC50 values of extracts infer that EEGS serves as potent scavenger for nitric oxide radical.

Based on the in vitro studies, it was assumed that AEGS and EEGS provides protection against oxidative damage influenced to biological molecules.
Free radical scavengers like enzymes such as SOD and CAT system protect biological system against the harmful impacts of activated species.

Superoxide radicals (O$_2^-$) are converted into H$_2$O$_2$ plus O$_2$ by SOD thus; hence, it additionally participates with other anti-oxidant enzymes, in the enzymatic defense against oxygen poisoning. In the current investigation, there's an enhance of SOD activity in a dosage reliant way suggesting that EEGS at both doses features an efficient protective result in response to ROS.

Present research also lead the depletion of lipid peroxidation as seen by the significant decrease in the TBARS levels of the liver and kidneys in treated rats. Catalase levels in both tissues and SOD levels in liver had been discovered to be greater in EEGS treated rats at dosage 400mg than that of standard Vitamin E. Hence, EEGS is found to possesses strong anti-oxidant properties.

Base on the obtained results from investigations for the antioxidant property of aqueous and ethanolic extracts of aerial parts of *Grewia serrulata* DC both in vitro and in vivo, it was assumed that ethanolic extract was discovered to have extremely great antioxidant properties compared to that of aqueous extract. Hence, keeping this in view further research was carried out selecting ethanolic extract of aerial parts of *Grewia serrulata* for its hypoglycemic and antidiabetic activity.
The ethanolic extract shows significant enhancement in glucose tolerance in glucose fed hyperglycemic normal rats. A single dosage of two levels of ethanolic extract shows significant hypoglycemic action than that of aqueous extract in streptozotocin- induced hyperglycemic rats. During our previous research work phytochemical investigations revealed presence of flavonoids, terpenoids and sterols that illustrates their strong antioxidant properties and this chemical composition of plant may also be the reason for its hypoglycemic activity.

An immense reservoir of biologically active substances with different chemical structures and illness preventive properties is the one and only plant kingdom. For this reason herbal drugs have actually gotten greater interest as an alternative to allopathic medication and the need for these herbal treatments features significantly increased recently. The current research had been directed to investigate the antidiabetic activity of ethanolic extract of aerial areas of *Grewia Serrulata* DC (EEGS) on high fat diet (HFD)-streptozotocin (STZ) induced diabetic rats.

The examination of body weights of all the animals was done on day 1, 7, 14, 21 and 28 of the study period. Rats belonging to all groups did not show any significant changes in the body weight. There was no mortality or signs of toxic reactions in animals and maintained their health status during the study period.
Most of plants have actually been reported for their hypoglycemic activity and the probable mechanism underlines may be an insulin release from pancreatic β-cells or secretion of bounded insulin or their insulin like actions \(^1\). Hypoglycemic effect of EEGS may be due one of the above said reasons. In the present study the resultant decrease in insulin levels could probably be due to the insulin sensitizing activity of the extract.

An enhance in the mobilization of free fatty acids from the peripheral storage space location leads to an abnormally large concentration of hepatic and plasma lipids in diabetic patients because hormone sensitive lipase is hindered by the insulin. The distinct hyperlipidemia that differentiates the diabetic condition is treated as a significant unrestrained count of lipolytic hormones (glucagon and catecholamine) on the adipose reserve space \(^2\). It is mentioned that lipoprotein lipase activity deficit in diabetics may give to a crucial enhance of triglycerides in blood with insulin administration; lipoprotein lipase activity is improved and leads to decrease of plasma triglyceride levels \(^3\). EEGS management almost reversed these results as it reduced triglyceride and total cholesterol levels, LDL levels, and improved HDL, particularly in combination. In this context, EEGS had been discovered to be effective as pioglitazone in bringing down the plasma lipid profile in the diabetic rats.
Serum urea and creatinine are raised in diabetic hyperglycemia and are considered as significant indicators associated to renal disorder. In addition, the protein glycation in diabetes will cause enhanced purine liberation that acts as a major source for the production of uric acid responsible for xanthine oxidase activity and muscle wasting.

Hepatic serum biomarkers like SGOT and SGPT were estimated on day 28 and used for the evaluation of hepatic damage. Standard drug pioglitazone showed elevated levels of SGOT and SGPT which are known to cause hepatic damage. There is no significant difference in total protein content. Besides that, EEGS of 200mg and 400mg/kg b. w, at the end of the day of study, significantly (p<0.05 to p<0.01) raise the glycogen content of liver and kidney.

It was observed that there was better changes occurred in urine glucose of the rats treated with the pioglitazine and the extracts when measured on 0th, 7th, 14th, 21st, 28th day of treatment. EEGS of both doses could able to record obvious reduction in the level of urine glucose gradually throughout the experiment.

In histopathological analysis of the pancreas of the diabetic rats treated with standard pioglitazone, EEGS 200 & 400mg/kg were comparable to normal rats in terms of the overcoming moderate degenerative changes caused by diabetes. In diabetic rats treated with extracts, the liver and kidney architecture were appeared more or less like normal control.
Earlier research on anti-diabetic effect of ethanolic extract of EEGS in high fat diet (HFD)-streptozotocin (STZ)-induced diabetic rats has been reported 96. The analysis that comes about accomplished with incubating pancreatic islets to understand the exact mechanism of action of Grewia serrulata DC. This study proposed that Grewia serrulata DC have protective nature and stimulate the β-cells of islets of pancreas dose dependently. Glucose suppressing effect of Grewia serrulata DC could be due to excitement of surviving β cells of islets of Langerhans leading to more insulin release 157.

Certainly one of the therapy approaches for diabetes is to de-
crease the hyperglycemia that is post-prandial delaying the utilization of glucose through the inhibition of carbohydrate-hydrolyzing en-
zymes, such as α-amylase and α-glucosidase158, 159. α-glucosidases are enzymes that increase the absorption of digested glucose from nutri-
tional polysaccharides in the small intestine. The α -glucosidase inhibi-
tion of EEGS was indeed examined by determining the α-glucosidase inhibitory activity by the use of 4-Nitrophenyl-β-D-
glucopyranosiduronic acid (pNPG) as the response substrate. The crude enzyme solution of rat’s small intestine been utilized as a source of α-glucosidases, sucrase, maltase, and isomaltase. α-glucosidase catalyzes the final step of carbohydrate digestion and its particular inhibitors can delay the uptake of nutritional carbohydrates and hold back the postprandial hyperglycemia, and this can be a good activity discovery of diabetic drugs. But, it is confusing perhaps the mode of inhibition of a-amylase and α-glucosidase by EEGS is due to
competitive and noncompetitive techniques. The theory that α-amylase and α-glucosidase showed different inhibition that is unquestionable because of structural variations pertaining to the origins of the enzymes. The inhibition rate for α-glucosidase ended up being close to that of acarbose, and the inhibition rate for α-amylase additionally ended up being obviously nearer to that of nojirimycin. This suggested that EEGS had been an effective inhibitor of α-glucosidase and α-amylase enzymes.

Another important selecting of the work is that EEGS possesses significant insulin-like properties, as evidenced by the improvement of glucose up-take in the diaphragm, which represents muscle tissue cells that are a major place of insulin mediated glucose disposal. The estimation of glucose content in rat hemi diaphragm is a commonly utilized and a consistent technique in the in vitro research of peripheral uptake of glucose. In addition, EEGS of both doses considerably improves the uptake of glucose by isolated hemi diaphragm and is discovered to be less efficient than insulin. Ethanolic extract of both the doses revealed significant (p<0.05 to p<0.001) enhance in the peripheral glucose as evidenced in glucose absorption, by isolated rat hemi diaphragm either alone or in combination with insulin.

Additionally, insulin secretagogue effect of Grewia serrulata DC was evaluated by incubating pancreatic islets with diazoxide, a known inhibitor of insulin release via opening the K+-ATP channel. As diazoxide promotes the reactive oxygen species (ROS) generation by opening of mitochondrial K+ ATP channel contributes to inhibit the
release of insulin from pancreas \textsuperscript{161,162,163}. EEGS could possibly be produced insulinomimetic effect closing the potassium channel by preventing the generation of ROS with anti-oxidant activity. This in vitro part of the study obviously suggested that \textit{Grewia serrulata} DC reverse the influence of diazoxide on glucose induced insulin release at 10 min and 60 min revealing that \textit{Grewia serrulata} DC display potent insulin secretagogue effect by controlling the insulin release from islets of pancreas because of its anti-oxidant property.

The infra red spectrum had been recorded on FTIR Perkin Elmer at SAIF, IIT Madras, Chennai, Tamil Nadu, India. The FTIR spectroscopic evaluation exhibited characteristic broad peak focused at 3428 cm\textsuperscript{-1}, that’s characteristic of O-H stretching indicating the existence of hydroxyl group which had been corroborated by chemical identification tests; The absorption bands at 1577 cm\textsuperscript{-1} corresponds to CH2-stretching, at 1628 cm\textsuperscript{-1} as an outcome of CH=CH stretching, absorption at 2922 cm\textsuperscript{-1} is due to aliphatic C-H stretching, and the ring at 1048 cm\textsuperscript{-1} is as a result of the existence of cycloalkane.

The \textsuperscript{13}C-NMR spectrum revealed the presence of more or less 20-23 quantity of carbon atoms including two methoxy groups and a glucose molecule. The \textsuperscript{13}C-NMR has shown identifiable sharp sign at \( \delta \) 39.377-40.379 ppm, corresponds to the existence of two –CH\textsubscript{3} groups, which may be assigned to C-33 & C-35 positions that are attached to oxygen. The absorption peaks at \( \delta \) 177.911, 164.558, 161.701 representing 7, 1 & 3 carbons. The fifth & 9th carbon atoms are straightly attached to oxygen in their aromatic ring, since the Oxygen is electron
withdrawing the absorption peaks to these carbons is lower with 156.788 & 156.665 correspondingly. The absorption peaks for 14, 16, 8 12, 4, 13, 17, 21 & 2 are 148.906, 145.26, 133.790, 122.064, 121.646, 116.687, 115.680, 104.457, 101.340. This decrease in absorption may as a result of the Inductive impact of oxygen. The carbon atoms in glucose molecule show absorption peaks as 77.997, 76.958, 74.560, 70.389, and 61.432 for 23, 25, 22, 24 & 30 carbon atoms. These values may be because of to their attachment to oxygen atom since well as lack of aromaticity.

The 1H-NMR spectrum shown sharp signals for 6 hydroxyl groups consisting of δ 3.50-3.60 ppm it shows a singlet of 1 proton of hydroxyl group at para position of glucose, at δ 3.60-3.70 ppm showing 2 singlets of 2 protons showing 2 hydroxyl groups at meta positions of glucose, and 3 singlets from δ 5.1-5.2 ppm 2 singlets of hydroxyl groups of aromatic ring. It had additionally shown a sharp peak at δ 3.50-3.60 ppm showing a doublet of 6 protons of 2 methoxy groups. Furthermore it had shown a peak at δ 3.20-3.30 ppm of 2 protons in –CH2 in glucose and glucose –C-H exercises at δ 3.10, 3.20, 4.20, 4.80, 5.4 ppm and –C-H bonds in aromatic bands had shown at δ 6.10-6.50 ppm.

Thus from the above discussion, it could be revealed that the isolated molecule is containing one glucose molecule connected to a ring of benzopyranone with oxygen atom. The reported experimental outcomes at 13C-NMR, 1H-NMR and the mass spectra reported in the current research led us to formulate the molecular formula of the
component as the $C_{23}H_{24}O_{12}$, bearing the IUPAC nomenclature as: 2-(3,5-dihydroxyphenyl)-5,7-dimethoxy-3-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one.

The HFD-STZ-induced diabetic rat is the specific animal model of human NIDDM or type-II diabetes mellitus. The present research exposed that EEGS-C1 have actually strong hypotriglyceridemic and hypoglycemic properties when administered for two weeks to HFD-STZ-diabetic rats. The EEGS-C1 caused a significant time-dependent hypoglycemic result after a daily oral administration of 10 mg/kg b. w for 7 and 14 days. The EEGS-C1 administration considerably reduced the serum triglycerides. For a period of two weeks, the decrease in the serum triglycerides after the administration of EEGS-C1 to HFD-STZ-diabetic rats every day could be as a result of the decrease of serum non-esterified fatty acids (NEFA) in the HFD-STZ-diabetic rats.