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In-vivo Anti-diabetic activity of the polyherbal formulation

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ABSTRACT

This study aimed at investigating in vivo anti-hyperglycaemic activity of ethanolic extract of various crude drugs used in the ethanolic plant extract polyherbal formulation (EPEPF) were Wrightia tinctoria, Terminalia chebula, Plumbago zaylanica, Persea macarantha, Coscinium fenestratum, Phyllanthus emblica and Zingiber officinale in (EPEPF) induced diabetic mice. The anti-hyperglycaemic property was measured through oral administration of plant extract powder formulation that is polyherbal formulation at doses of 200 and 400mg/kg body weight and Knowledge measures of blood glucose levels every hour for up to 24 hours a glucometer was used to determine hyperglycaemic levels. The poly-herbal formulation showed a dose reliant on anti-diabetic activity in Streptozotocin-induced diabetic mice. The plant's extract powder poly-herbal formulation was significantly hyperglycaemic levels it reduces the blood sugar level. The report proves to the poly herbal formulation reduces oxidative stress. Feature in this formulation developed for preclinical studies and will use in anti-diabetic drugs.

Keywords: Polyherbal formulation, Plants extract, antidiabetic, Diabetic, blood glucose.

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INTRODUCTION

Active constituents in the polyherbal combination act in as synergistic manner [1,2]. For more than thousands of years, Nature has been one of the main traditional methods of herbal medicine and the source of medical agent. Active constituents' presence in poly herbal formulation against plenty of disease and having various mechanism it produces synergic effect. Two or more plant's extracts produce poly-herbal formulation it indicates the synergic and antagonistic effect in presence of chemical constituents [3,4,5]. The individual plant extract has their own mechanism [6,7]. The complication of the research poly-herbal formulation has one or more plant extract and plenty of chemical compound some chemical compounds produce synergic effect it enhances therapeutic activity but its unfortunately produce toxic level it will be identified and. Polyherbal formulas combine in a dynamic way to create maximum therapeutic benefits with minimal side effects [8,9].

Insulin level degreased or lack of insulin performance on its objective tissue or both it called Hyperglycaemia. This disorder would be explaining doubt less sly as one of the major morbidities causes all around the world [10,11]. As per the report of WHO by year 2025 there will be 300 million people affected with chronic diabetic condition [12].

Being one of the causes of mortality, diabetes spread across the world alarmingly. Worldwide diabetes prevalence increased from 4.6% to 8.4% from 1980 to 2014 among adults over the age of 18, as per the reports by the World Health Organization;

The report also states that by 2030 diabetes deaths will be the seventh leading cause of death. Over 400 plants have been found to cure diabetes [13].

Numerous herbal remedies as single agents or in altered oral preparations have been suggested for hyperglycemic due to the fact that they are less harmful than oral anti-diabetic agents such as sulfonylurea,

metformin etc. [14]. Herbs like Wrightia tinctoria, Plumbago zaylanica, Terminalia chebula, Persea macarantha, Phyllanthus emblica, Coscinium fenestrum and Zingiber officinale are mentioned in ethnomedicinal literature for various therapeutic properties which include hypoglycaemic, hypolipidemic, antioxidant and other therapeutic properties. It is hypothesised that these herbs when used as a combination may have a synergistic effect and can be employed in managing diabetes and its associated conditions. These herbal drugs are considered to be safe and do not have many side effects compared to synthetic drugs.

MATERIALS AND METHODS

Plants Collection and identification

The all-botanical source was collected from in and around Tiruchirappalli, Tiruchirappalli District of Tamil Nadu, India. The taxonomic distinguishing proof was produced using BSMPUS, Government of India, Tirunelveli District, Tamil Nadu. The botanical source was removed water content under the shadow, separated the plant dust, crushed by a mechanical processor and went through a 40-lattice shifter. The plant's powdered materials were used to store in a hermetically sealed holder [15, 16].

Preparation of Extracts

The ethanolic solvent having highly penetrated plants' crude powder releases high concentrate greasy material it has plenty of chemical constituents The ethanolic solvent extract collected from plants it using a cold maceration process. A rotary evaporator and lyophilizer are used to dry and freeze the extracted change powder store [17,18].

The extraction of the above plants is carried out using a cold maceration process and the ethanol is used as a solvent. The ethanol extract was used as a rotary evaporator and lyophilizer to dry and freeze the extracted ethanol solvent powder.

Animal

After being acclimated with humidity (± 1%) in a 12-hour light / dark cycle in a room for 7 days at room temperature (25 ± 2°C) and healthy. Over 7 days of weight and hygiene is observed for albino adult male Wister [16]. They are given access to water with a standard pellet diet ad libitum Testing of animals was carried out according to the guidance of the Supervisory Committee (CBCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Bharathidasan University Tiruchirappalli (Approved number: BDU/IAEC/P13/2018)

Acute toxic study of ethanolic extracts powder polyherbal formulation from various plants extract

Healthy adult male Wister albino rats were subjected to acute toxicity studies as per OECD-420 guidelines (Organisation for Economic Co-operation and Development) for the following Study. For each phase of the study, five animals used ethanolic extract powder polyherbal formulation. Animal weights are given the same dose of extract. The dose of crude extract was gradually increased by 5 mg/kg, 300, and 2000mg/kg body weight. The animal dose fixed based on the mortality at one step will decide the next step. The procedure flow chart described the procedure followed for each of the starting doses [19,20].

Experimental design

Anti-diabetic activity of the ethanolic extracts powder of Wrightia tinctoria, Terminalia chebula, Plumbago zaylanica, Persea macarantha, Zingiber officinale, Phyllanthus emblica and Coscinium fenestratum was investigated in Streptozotocin stimulate diabetic mice. The Mice were selected randomly and divided into four groups. Hyperglycaemic study of the extracted plant powder of poly-herbal formulation by administering orally. 5 groups each with 5 animals were selected for the study. Untreated study group, I was given 0.0001Liter of sodium chloride. The same concentration of normal Saline was administrated to hyperglycaemic group II also. The following group was the positive control group which was given standard glibenclamide in 0.0001Liter of sodium chloride the subsequent hyperglycaemic study group was given EPEP Fat the dose of 0.2gm per kilogram of body weight in 0.0001liter of normal saline. The next study group, group V was given double the dose (400mg/kg) of EPEPF in the same concentration of NL. Hyperglycaemia was induced in Wistar rats by intraperitoneal injection of STZ; which was treated with the standard drug as well as EPEPF with a low dose and high dose [21,22].

Induction of hyperglycaemia

Hyperglycaemia was induced intraperitoneally through the administration of freshly prepared Streptozotocin 45mg/kg body weight. The blood glucose levels were noted after two days of administration of STZ. Mice with glucose levels above 0.0111 mol/litre were considered hyperglycaemic and were accurate for using bioassay. The mice were made to fast for 8-12 hours before executing the experiment. During fasting the mice were allowed only for free access to water. [23,24].

Blood glucose assay

Blood was collected from the tail portion. Before collection of the blood, the tail part was sterilized with ten percentage of alcohol. The experimental procedure was done after the first, second, third, fourth, sixth and twenty-fourth hours. Blood glucose monitoring was carried out with the help of a glucometer [25].

Data analysis

The data obtained was escorted to the Microsoft Excel Spread Sheet where it was cleaned and then transferred to Statistical Package for Social Sciences Software (SPSS) for statistical analysis. The results of statistical analysis were expressed as Mean ± Standard Deviation (SD). One-way ANOVA and post-ANOVA (Tukey's post hoc test) were used to compare the means of the untreated group of normal mice with diabetic groups of mice treated with normal saline, conventional drug and plant extract at various dosages. Statistical significance was set at $p \leq 0.05$[26].

Result and discussion:
Toxicological Evaluation of Polyherbal Formulation (EPEF)

Table: 1 Effect of Ethanolic extract powder Polyherbal Formulation (EPEF) on acute toxicity test.

Observation	***Effects of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg, 2500mg/kg***								
Gross activity	***Up to 3 hrs***	***3 ½ hrs***	***4 hrs***	***4 ½ hrs***	***5 Hrs***	***5 ½ hrs***	***6 hrs***	***12 Hrs***	***24 Hrs***
Respiration	+	+	+	+	+	+	+	+	+ +
Writhing	-	-	-	-	-	-	+	+	+
Tremor	-	-	-	-	-	-	+	+	+ +
Convulsions	-	-	-	-	-	+	+	++	++
Hind limb paralysis	-	-	-	-	-	-	-	-	-
Sense of touch and sound	+	+	+	+	+	+	+	+	-
Salivation	-	-	-	-	-	+	+	++	++
Diarrhoea	-	-	-	-	-	-	-	+	++
Mortality	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***1x***

+ Normal Effect + + High Effect – No effect

Fasting blood glucose:

Table:2 Effect of ethanolic extract powder Polyherbal Formulation (EPEF) on level of fasting blood glucose in control and experimental rats.

S.no	Groups	FBG
1	Control	092.86± 0.09
2	Diabetes Induced	205.06± 0.12
3	Glibenclamide	103.73± 0.08
4	PHF 200	137.42± 0.23
5	PHF 400	130.07± 0.08

p<0.001,p<0.01,p<0.05,ns-non significant.

Data is expressed as mean±SD. (n=6, animals in each group) Values are statistically extremely significant at p<0.001.

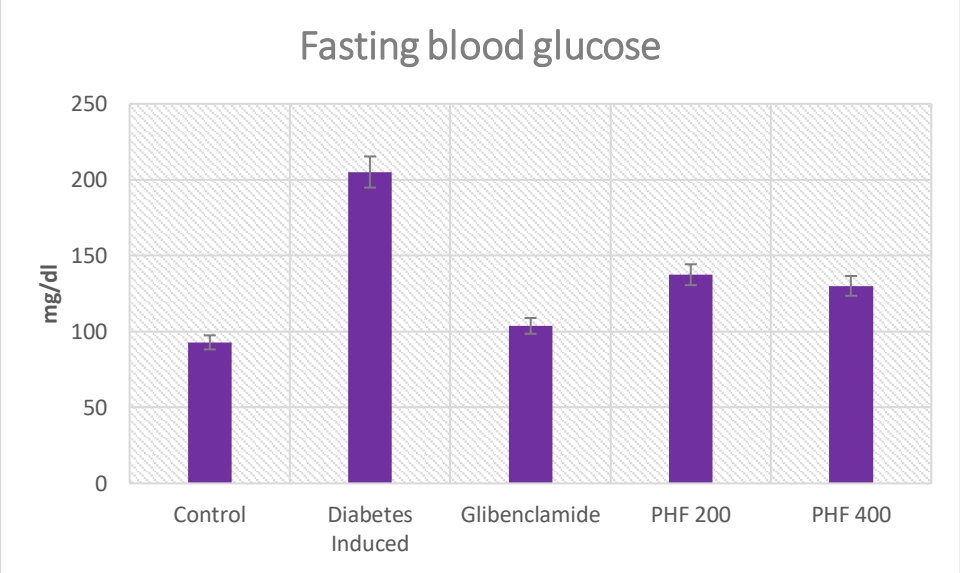

Fig: 1 Fasting blood glucose

Ethanolic extract powder Polyherbal Formulation (EPEF)on level HbA1C, Serum Glucose, insulin, and Pancreas weight in control and experimental rats.

Table 3: Effect of ethanolic extract powder Polyherbal Formulation (EPEF)on level HbA_1C, Serum Glucose, insulin, and Pancreas weight in control and experimental rats.

Groups	HbA1C	Serum Glucose mg/dl	Insulin	Pancreas wgt
Control	$4.9733 \pm 0.18^{b***}$	$92.33\pm 0.23^{b***}$	$22.8633\pm 0.19^{b***}$	$5.8433\pm 0.21^{b***}$
Diabetes Induced	$12.8567 \pm 0.11^{a**}$	$264\pm 0.08^{a**}$	$5.0667\pm 0.09^{a**}$	$8.6667\pm 0.19^{a**}$
Glibenclamide	$6.1833 \pm 0.17^{a*, b***}$	$114.67\pm 0.59^{ans, b***}$	$20.7333\pm 0.06^{ans, b***}$	$5.9967\pm 0.02^{ans, b***}$
EPEPF 200	$7.6633 \pm 0.29^{a*,b***}$	$129\pm 0.09^{a*,b***}$	$17.4233\pm 0.39^{a*,b***}$	$6.0133\pm 0.09^{a*,b***}$
EPEPF 400	$5.32 \pm 0.41^{ans, b***}$	$136.330.46^{*ans, b***}$	$20.0733\pm 0.46^{*ans, b***}$	$6.2488\pm 0.55^{ans, b***}$

[Values are mean ± SEM of 6 rats]

< 0.05, **< 0.01, *< 0.001*

*p values : ***< 0.001 **< 0.01, *< 0.05*

a →group II, III, IV, V,VI & VII compared with groups I.

b →group I, III, IV, V, VI & VII compared with groups II.

ns- Non significant

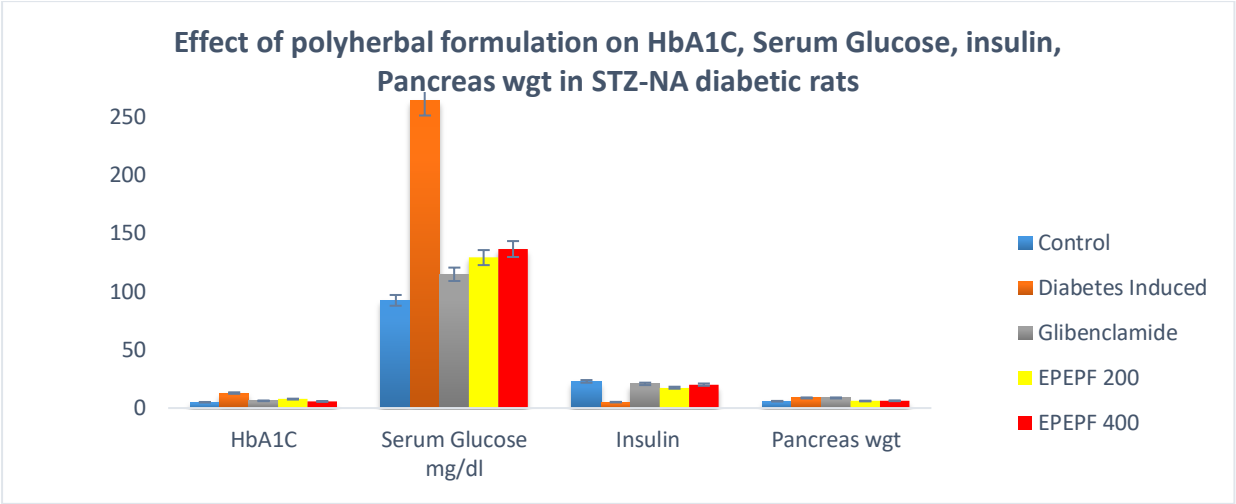

Fig:2 Effect of polyherbal formulation on HbA1C, Serum Glucose, insulin, and Pancreas weight in STZ diabetic rats

DISCUSSION

Type 2 diabetes is caused by the body's inability to respond to insulin. Unlike people with Type 1 Diabetes, most people with Type 2 Diabetes can still produce insulin, but not enough to meet their body's these features are the focus of intensive investigation. In this context, anti-diabetic oral administration drug-ethanolic plant extract powder Polyherbal Formulation (EPEPF) present interesting therapeutic properties. Diabetes mellitus is associated with the generation of ROS leading to oxidative damage, particularly in t liver and kidney. The elevated blood glucose levels in diabetes mellitus are thought to induce cell death through a free radical formation that occurs as a common sequel of diabetes-induced not-enzymatic modification of sugar moieties on protein and lipids. In the present study, the results of the acute toxicity study revealed that LD_{50} values of Polyherbal Formulation (EPEPF)) was high and apparently showed the safety of these same. The low death rate for the formulation of (EPEPF)was found at the doses of 2500mg/kg in addition to these,

In the beginning, those animals were kept under observation for four hours, and then at an interval of two hours for 24 hours to examine any change occurs (or) observed in the rat's behaviour such as respiration, writhing, CNS excitation & depression, reflexes, muscular weakness, salivation, diarrhoea, food intake and mortality.

The observation of cross behavioural studies revealed that after administration of Polyherbal Formulation (EPEPF) showed Table: 1sense of touch, salivary secretions, respiratory parameters and sound. The writhing reflex, tremor, convulsion and hind limb paralysis were absent. No alteration was found in GIT motility and induction of diarrhoea with this formulation. Overall results suggested the LD_{50} value as 5000mg/kg. Hence therapeutic dose was calculated as $1/10^{th}$ (500mg/kg of the lethal dose for the purpose of anti-diabetic investigations.) the dose fixed 400mg/kg high dose and 200mg/kg low dose.

Table: 2 (fig.1) shows (EPEF)Polyherbal Formulation (EPEPF) significantly improved glucose tolerance test up to 120 min. The administration of Polyherbal Formulation (EPEPF)to decrease the increased fasting blood glucose concentration to normal glycaemic concentration is an essential trigger for the liver and kidney to revert its normal homeostasis during experimental diabetes.

Table: 3 (fig: 2) shows significant decrease in serum blood glucose level in dose dependent manner. The Polyherbal Formulation (EPEPF) (200 mg/kg) administered shows7.6633in STZ-induced diabetic, respectively, in glycated haemoglobin HbA1C level whilst a decline of 5.32 were observed in animals treated with 400 mg/kg of Polyherbal Formulation (EPEPF) on day 0 to 21.

Table: 3(Fig.2) shows significant increase in Insulin when treated with polyherbal formulation (EPEPF). During diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated

haemoglobin. So total haemoglobin level decreased in STZ induced diabetic rats. Polyherbal formulation (EPEPF) administrated in STZ induced diabetic rats reversed the total haemoglobin level.

The result shows significant decrease in Pancreas weight when treated with polyherbal formulation (EPEPF).

Summary and Conclusion

Treating Diabetes with Hyperglycaemia, Glycorrhea, Hyperlipidaemia and Negative Nitrogen Balance Using Antioxidant Factors. Our aim was to evaluate the anti-diabetic effect of polyherbal formulation (EPEPF) in STZ-induced type 2 diabetic rats. From these results, we conclude that ethanol extract of polyherbal formulation (EPEPF) reduced the risk of hyperglycaemia and antioxidant stress to the pancreas. This drug is effective in treating diabetes due to the Western cause and associated errors. This extract, however, will be key to diabetes in future clinical trials to establish powerful phytomedicine. As we surmise this should be an indigenous product for diabetes mellitus if research would be continued further in future.

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