IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 787

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Preliminary Phytochemical & Anti-diabetic Activity of Leaves and Flowers of *Rungia repens* Nees

Prakash Pant [1*], Rahul Trivedi [2]

1. Research Scholar, Faculty of Pharmacy, Mandsaur University, Mandsaur (MP), 458001
2. Faculty of Pharmacy, Mandsaur University, Mandsaur (MP), 458001

ABSTRACT

AIM- The aim of the present investigation is to study the Preliminary Phytochemical & Anti-diabetic Activity of Leaves and Flowers of *Rungia repens* Nees. **MATERIAL & METHODS-** The leaves and flowers of *Rungia repens* Nees were collected from outfield and also purchased from local markets during the month of July that shows the green color with rough surface. The correctly identified plant leaves and flowers were dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. The mare was dried in air to remove traces of petroleum ether. Defatted drug was subjected to extraction with chloroform, ethyl acetate, ethanol, methanol and aqueous in soxhlet apparatus, the extraction was completed in 17-18 hrs. Preliminary phytochemical screening was performed for the presence of various active phytochemicals. Acute oral toxicity test was carried out according to the OECD guideline No. 423. After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes. **RESULTS-** The study on selected plant material, shows, that the difference of two consecutive weighing after drying for 30min. and cooling for 30 min. in a desiccators- 0.09 & 0.23 gm. The Experimental results on selected plant material shows – volume occupied by 1 gm of plant material= 1.62, 1.45 ml. Petroleum ether, chloroform, ethyl acetate, ethanolic extracts had moderately significant effects (p<0.01) on 14th and 21st days. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the p<0.001 in 14 days whereas methanolic extract (200 & 400 mg/kg) reduced the glucose level highly significant with p<0.001. **CONCLUSION-** The present investigation comprises of the phytochemical and pharmacological investigations of leaves and flowers of plant for the antidiabetic activity.

KEYWORDS-Preliminary Phytochemical, Anti-diabetic Activity, Leaves and Flowers, *Rungia repens* Nees, Streptozotocin (STZ)

INTRODUCTION

Diabetes is a significant, chronic ailment that profoundly affects the quality of life and overall welfare of people, families, and communities across the globe. It ranks as one of the leading ten contributors to adult mortality, responsible for an approximate four million fatalities globally in 2017. Diabetes is a persistent health condition arising from inadequate insulin production by the

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

pancreas or the body's impaired utilization of the insulin it generates. Insulin, a vital hormone for blood glucose regulation, is at the core of this malfunction. Elevated blood glucose, known as hyperglycemia, is a prevalent consequence of unmanaged diabetes, progressively causing severe harm to multiple bodily systems, particularly the nerves and blood vessels (Deepthi *et al*, 2017)

Diabetes Mellitus (DM), commonly known as diabetes, was historically associated with the presence of "sweet urine" and muscle atrophy. Insulin, a hormone produced by the pancreas, plays a crucial role in regulating blood sugar levels, ensuring they remain stable. In individuals with diabetes, either there is insufficient or no production of insulin, leading to elevated blood sugar levels known as hyperglycemia (Bordoloi & Dutta, 2014).

In Ayurveda and other traditional medicinal systems, several medicinal plants have been traditionally employed for the management of diabetes and its complications. However, their purported efficacy claims and underlying mechanisms have yet to be scientifically validated. Consequently, the current study aimed to investigate the antidiabetic activity of these traditional remedies. The aim of the present investigation is to study the preliminary phytochemical & antidiabetic activity of leaves and flowers of *Rungia repens* Nees.

MATERIAL & METHODS

Collection and authentication of the plant leaves & barks:

The leaves and flowers of *Rungia repens* Nees were collected from outfield and also purchased from local markets during the month of July that shows the green color with rough surface. The plant leaves and barks were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plant was identified by the Botanist, Research Officer; Botany (Scientist C) at Central Council for Research in Ayurveda, Govt. of India and herbarium specimen was submitted in Department of Pharmacognosy.

Determination of Physico chemical parameters

Ash Values

The ash content of crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. (Mukherjee, P.K., 2002)

a *Determination of total Ash value*

Accurately weight about 3 gm of air dried drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

with reference to the air dried drug. The determination of total ash value was calculated by using following formula. (Mukherjee, P.K., 2002)

b *Determination of acid insoluble Ash value*

The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug. (Mukherjee, P.K., 2002)

c *Determination of water soluble Ash value*

The total ash obtain was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water ignited for 15 minutes. The weight of insoluble matter was subtracted from the weight of total ash. The differences in weight represent the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. (Mukherjee, P.K., 2002)

Determination of Extractive values

Extractive values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. (Mukherjee, P.K., 2002)

a *Determination of Alcohol Soluble Extractive Value*

5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105^0c and weighed. The percentage of ethanol soluble extractive value was calculated with reference to air dried drug.

b *Determination of Water Soluble Extractive Value*

5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105^0c and weighed. The percentage of water soluble extractive value was calculated with reference to air dried drug.

Determination of Loss on Drying

Weight about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^0c or 105^0c. Cool in a desiccators and watch. The loss in weight is recorded as moisture. (Mukherjee, P.K., 2002)

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Determination of Moisture Content :

About 10g of leaves (without preliminary drying), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105^{o}C for 5 hours and weighed. Drying was continued and the root was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators, did not show more than 0.01g difference.

Determination of Swelling Index (WHO, 2000):

Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25 ml glass stoppered measuring cylinder. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material. (Mukherjee, P.K., 2002)

Successive extraction methods: (Mukherjee, P.K., 2002)

The correctly identified plant leaves and flowers were dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. These should be extracted with different solvent in order to their increasing polarity to get the correct and dependable retention factor. Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mare was dried in air to remove traces of petroleum ether. Defatted drug was subjected to extraction with chloroform, ethyl acetate, ethanol, methanol and aqueous in soxhlet apparatus, the extraction was completed in 17-18 hrs. The extract was dried & stored in dark place.

The % Yield of the Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Methanol, & Aqueous extract of *Rungia repens* Nees was calculated by using the following formula.

$$\% \text{ Yield} = \frac{\text{Net weight of powder in gram after extraction}}{\text{Total weight of leaf powder in gram taken for extraction}} \times 100$$

Phytochemical Screening (Kokate, C.K., 1996**;** Khandelwal, K.R., 2006**)**

Preliminary phytochemical screening was performed for the presence of various active phytochemicals.

Antidiabetic study of different extracts

Experimental Animals

Wistar Albino rats of either sex (150 to 200 g) were purchased from the CPCSEA approved vendor New Delhi. They were maintained under standard laboratory conditions at 25 ± 2°C,

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

relative humidity (50 ± 15%) and normal photoperiod (12-hour light-dark cycle) were used for the experiment. Commercial pellet diet (MFD, by Nav Maharashtra Chakan Oil Mills ltd., New Delhi, India) and water were provided ad libitum throughout the course of study.

Selection of Dose

Acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of different extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks. Hence in our studies we selected 1/10 and 1/5th dose i.e. 200 and 400 mg/kg dose.

Preparation of Doses

Doses equivalent to 200 mg and 400 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Streptozotocin (STZ) induced diabetes in rats

After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was observed by moderate Polydipsia and marked Polyuria. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study. (Ali *et al*., 2009)

Experimental Design of antidiabetic study of leaves of *Rungia repens* Nees (RR)

In order to assess the anti-diabetic activity, the animals were divided in fifteen groups of six animals in each group.

Group 1: Normal control, 0.9% NaCl-treated animals
Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)
Group 3: Treated with Pet. Ether extract of leaves of *RR* (200 mg/kg body weight)
Group 4: Treated with Pet. Ether extract of leaves of *RR* (400 mg/kg body weight)
Group 5: Treated with chloroform extract of leaves of *RR* (200 mg/kg body weight)
Group 6: Treated with chloroform extract of leaves of *RR* (400 mg/kg body weight)
Group 7: Treated with ethyl acetate extract of leaves of *RR* (200 mg/kg body weight)
Group 8: Treated with ethyl acetate extract of leaves of *FR* (400 mg/kg body weight)
Group 9: Treated with ethanolic extract of leaves of *RR* (200 mg/kg body weight)

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Group 10: Treated with ethanolic extract of leaves of *RR* (400 mg/kg body weight)
Group 11: Treated with methanolic extract of leaves of *RR* (200 mg/kg body weight)
Group 12: Treated with methanolic extract of leaves of *RR* (400 mg/kg body weight)
Group 13: Treated with aqueous extract of leaves of *RR* (200 mg/kg body weight)
Group 14: Treated with aqueous extract of leaves of *RR* (400 mg/kg body weight)
Group 15: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Experimental Design of antidiabetic study of flowers of *Rungia repens* Nees (RR)

In order to assess the anti-diabetic activity, the animals were divided in fifteen groups of six animals in each group.

Group 1: Normal control, 0.9% NaCl-treated animals
Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)
Group 3: Treated with Pet. Ether extract of bark of *RR* (200 mg/kg body weight)
Group 4: Treated with Pet. Ether extract of bark of *RR* (400 mg/kg body weight)
Group 5: Treated with chloroform extract of bark of *RR* (200 mg/kg body weight)
Group 6: Treated with chloroform extract of bark of *RR* (400 mg/kg body weight)
Group 7: Treated with ethyl acetate extract of bark of *RR* (200 mg/kg body weight)
Group 8: Treated with ethyl acetate extract of bark of *RR* (400 mg/kg body weight)
Group 9: Treated with ethanolic extract of bark of *RR* (200 mg/kg body weight)
Group 10: Treated with ethanolic extract of bark of *RR* (400 mg/kg body weight)
Group 11: Treated with methanolic extract of bark of *RR* (200 mg/kg body weight)
Group 12: Treated with methanolic extract of bark of *RR* (400 mg/kg body weight)
Group 13: Treated with aqueous extract of bark of *RR* (200 mg/kg body weight)
Group 14: Treated with aqueous extract of bark of *RR* (400 mg/kg body weight)
Group 15: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Blood collection and biochemical estimations in serum

On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer (Easy Gluco, Morepen Laboratories Ltd.; New Delhi). (Tripathi & Chandra, 2010)

Statistical Analysis

Data were expressed as the mean standard error of mean (S.E.M.) of the means and statistical analysis was carried out employing one-way ANOVA. Differences between the data were considered significant at $P < 0.05$.

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

RESULTS & DISCUSSION

Determination of physicochemical parameters

Table No. 1: Physico-chemical parameters of *Rungia repens* Nees

S. No.	Determination	*Rungia repens* Nees	
		Leaves	**Flower**
1	Total ash	8.0	8.6
2	Acid insoluble ash	3.12	2.17
3	Water soluble ash	4.96	3.89
4	Alcohol soluble extract value	5.40	4.45
5	Water soluble extract value	3.90	3.40
6	Loss on Drying		

Determination of Moisture content

The study on selected plant material, shows, that the difference of two consecutive weighing after drying for 30min. and cooling for 30 min. in a desiccators- 0.09 & 0.23 gm for leaves and flower of *Rungia repens* Nees.

Determination of Swelling Index

The Experimental results on selected plant material shows – volume occupied by 1 gm of plant material= 1.62, 1.45 ml for leaves and flower of *Rungia repens* Nees.

% Yield Determination and Characteristic views of *Rungia repens* Nees extracts

Table 2: %Yield (w/w) of extracts *Rungia repens* Nees

S No.	Solvent	***Rungia repens* Nees**	
		Leaves	**Flower**
1	Petroleum ether	5.29	3.12
2	Chloroform	5.18	4.15
3	Ethyl Acetate	5.14	6.34
4	Ethanol	11.28	9.56
5	Methanol	5.28	8.56
6	Aqueous	9.80	4.67

Table No. 3: Characteristic view of *Rungia repens* Nees extracts

1. Characteristic view of petroleum ether extract-

Nature	Powder
Colour	Greenish
Odour	Characteristic

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Taste	Bitter

2. Characteristic view of chloroform extract-

Nature	Powder
Colour	Dark greenish
Odour	Characteristic
Taste	Bitter

3. Characteristic view of ethyl acetate extract-

Nature	Powder
Colour	Blackish-green
Odour	Characteristic
Taste	Bitter

4. Characteristic view of ethanol extract-

Nature	Viscous
Colour	Blackish-green
Odour	Characteristic
Taste	Bitter

5. Characteristic view of methanol extract-

Nature	Viscous
Colour	Blackish-green
Odour	Characteristic
Taste	Bitter

6. Characteristic view of aqueous extract-

Nature	Powder
Colour	Blackish-green
Odour	Characteristic
Taste	Bitter

Phytochemical Screening

Phytochemical screening of different extracts showed the presence of different phytochemical.

Table 4: Preliminary Phytochemical test for different extracts of *Rungia repens* Nees extracts

S.No.	Test	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Aqueous
1.	Carbohydrate						
	➢ Molish test	-	-	-	-	+	+
	➢ Felling test	-	-	-	-	+	+
2.	Glycosides						
	➢ Bronteger test	-	-	+	-	+	+

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

3.	Alkaloid ➢ Mayer test ➢ Hager test	 - -	 + +	 + +	 - -	 + +	 - -
4.	Phytosterol + Triterpinoids ➢ Salkowaski test	-	+	-	+	+	-
5.	Protein + Amino acid ➢ Biuret test ➢ Ninhydrin test	 - -	 - -	 - -	 - -	 - -	 - -
6.	Phenolic test ➢ Ferric test ➢ Lead acetate test	 - -	 + +	 + +	 + +	 + +	 - -
7.	Flavonoids ➢ Alkaline test	-	-	+	+	+	+
8.	Saponin ➢ Foam test	-	-	-	-	+	+
9	Mucilage ➢ Iodine test ➢ Ethanol test						+ +

Note: (+) *ve* indicates positive result, whereas (–) *ve* indicates negative result

Antidiabetic study of leaves of *Rungia repens* Nees (RR)

Effect on Blood glucose level

The induction of diabetes with streptozotocin increases the blood glucose level significantly (p<0.001) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the p<0.001 in 14 days whereas methanolic extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with p<0.01 & p<0.001. Petroleum ether, chloroform, ethyl acetate, ethanolic extracts had moderately significant effects (p<0.01) on 14th and 21st days. However, aqueous extracts didn't show any significant decrease in glucose levels.

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

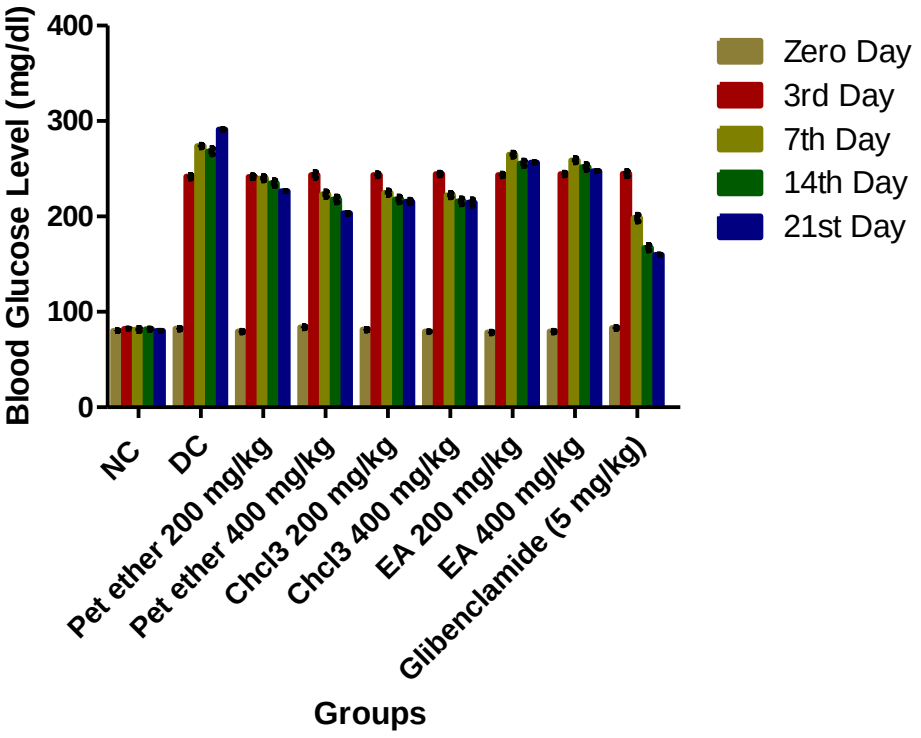

Figure No 1. Effect of different extracts on Blood glucose level

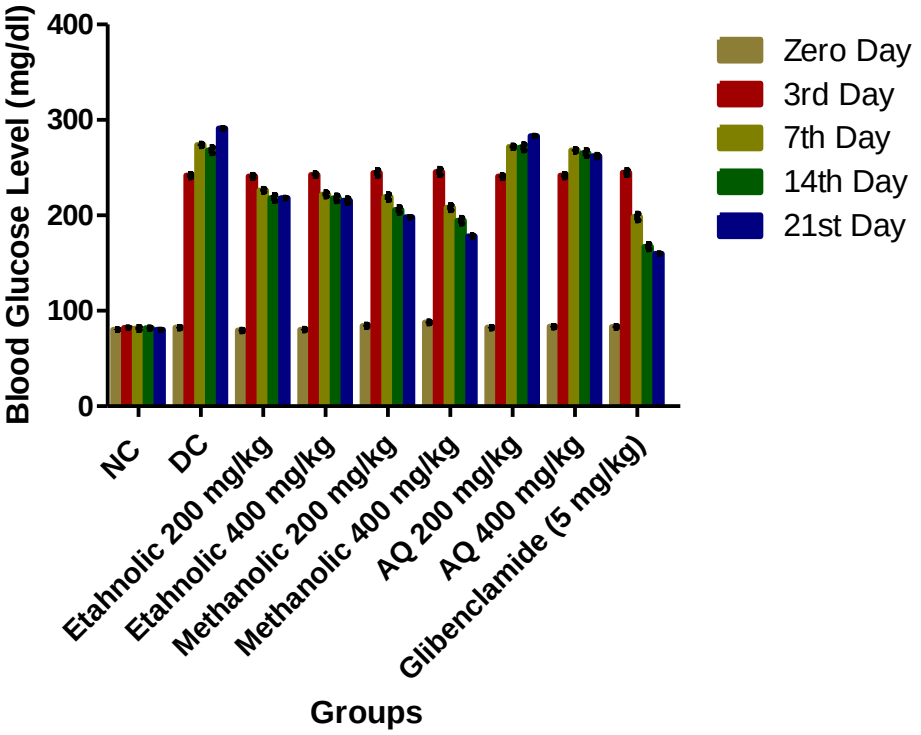

Figure No 2. Effect of different extracts on Blood glucose level

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Antidiabetic study of flower of *Rungia repens* Nees (RR)

Effect on Blood glucose level

The induction of diabetes with streptozotocin increases the blood glucose level significantly (p<0.001) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the p<0.001 in 14 days whereas methanolic extract (200 & 400 mg/kg) reduced the glucose level highly significant with p<0.001. Petroleum ether, chloroform, ethyl acetate, ethanolic extracts had moderately significant effects (p<0.01) on 14th and 21st days. However, aqueous extracts didn't show any significant decrease in glucose levels.

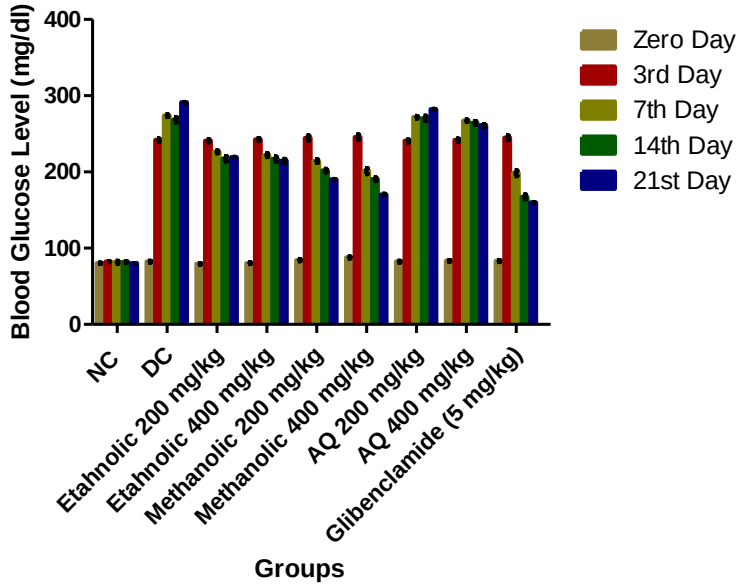

Figure No 3. Effect of different extracts on Blood Glucose Level

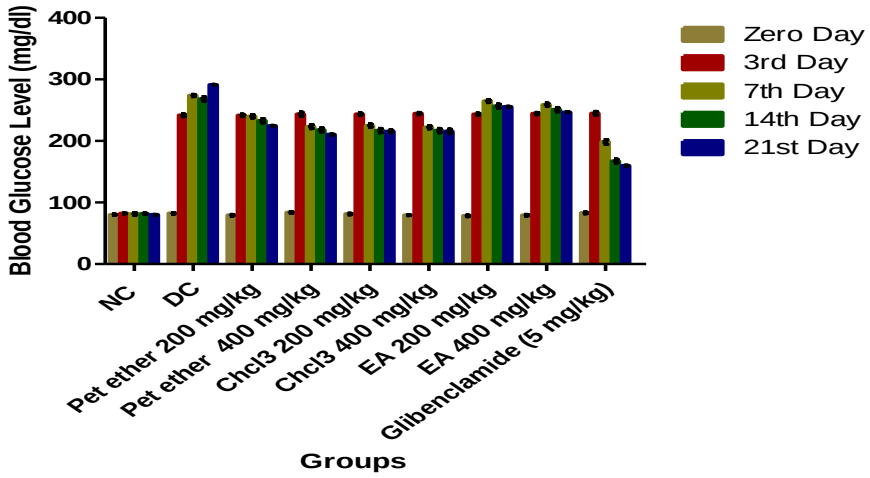

Figure No 4. Effect of different extracts on Blood Glucose Level

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

DISCUSSION

Prolonged exposure to free radicals is a pivotal cause of tissue stress and injury. The free radical permanent damage to tissue structures results from a permanent alteration in the molecular pattern of carbohydrates, lipids, proteins and even nucleic acid bases. In diabetes, the level of free radicals was reported to increase in alloxan and streptozocin treated rats an elevated level of free radicals was detected in several tissues including the kidneys (Shabeer *et al*., 2009).

Non-insulin dependent diabetes mellitus is a multifactorial sickness, which is characterized by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to be responsible for damage to cell membranes through non-enzymatic glycosylation of proteins, auto-oxidation of glucose or increase metabolism of glucose by the sorbitol–polyol pathway. Cell damages will in turn, result in elevated production of reactive oxygen species or ROS. High levels of ROS have been found to play a role in the pathogenesis of NIDDM (Al-Qattan *et al*., 2008).

Predictably, insulin dependent diabetes is treated with exogenous insulin and noninsulin dependent diabetes with synthetic oral hypoglycemic agents like sulphonylureas and biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatropy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in kidneys and severe vascular complications. Similarly, oral hypoglycemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and hemolytic anemia's, generalized allergic reactions, dermatological reaction etc (Mallick *et al*., 2007; Pepato *et al*., 2005).

Traditionally, there are various 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 these plants only plant extracts have been prepared and evaluated for its Antihyperglycaemic activity. 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 activity of different extracts (Leaves & flowers) procured by successive extraction methods and to find out or isolate the most possible active compounds from the active extracts showing the best activity.
Total ash value asses the total amount of material remained after detonation and the amount of heavy metals and inorganic compounds present in the powder sample. The total ash content was 5 times greater than acid insoluble ash, the presence of calcium oxalate crystals or acid soluble inorganic matter.

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

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. Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non polar components yields higher extractive value. Literature review states that the presence of alkaloids, flavonoids,glycosides,terpenes,srteroids,polysaccharides,phenols, coumarins and proteins in the plant extract contribute to pharmacological activities such as antidiabetic properties **(**Stahl, 1969**)**. Preliminary phytochemical evaluation of plants report illustrates that petroleum ether extract showed the existence of triterpenoids, steroids and fatty acids, chloroform extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids, terpenoids ethanolic and methanolic extract showed the presence of alkaloids, flavonoids and glycosides and aqueous extract showed the presence of carbohydrates, as phytoconstituents.

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 this study, all 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 was noticed at the dose of 2000mg/kg. The toxicity studies was determined by OECD guidelines 423.Based on the LD50 value, $1/5^{th}$ and $1/10^{th}$ (200 & 400 mg/kg) of its value was chosen for pharmacological studies.

The islet β-cells are susceptible to damage caused by oxygen free radicals (Prince and Menon, 1998; Cai *et al*., 2005) since the antioxidant defense system is weak under diabetic condition. The levels of antioxidant defense system are altered in streptozotocin-induced diabetic rats, which are in good correlation with the present observation. Non protein thiols like glutathione are one of the important primary defenses that counteract the oxidative stress. Decreased levels of serum glutathione in streptozotocin diabetic rats, which is in consistent with earlier reports (Cai *et al*., 2005). The observed decrease may be due to utilization of non protein thiols by increased oxygen-free radicals produced in hyperglycemic conditions associated with diabetes mellitus. The methanolic extract of *RR* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The antidiabetic effect may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration. Similarly, *RR* at the dose of 200 and 400 mg/kg body weight after treatment of 21 days produced a marked decrease in blood glucose levels in STZ models. As the results showed by *RR*, antidiabetic effect of *RR* may be due to increased release of insulin from β-cells.

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

CONCLUSION

The present investigation comprises of the phytochemical and pharmacological investigations of leaves and flowers of plant for the antidiabetic activity. The research work encompasses an in depth and systematic phytochemical and investigation of various extracts of leaves and flowers parts of the plant. These components could serve as lead molecules for development of prospective anti-diabetic agents. The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of diseases.

REFERENCES

1. Deepthi B, Sowjanya K, Lidiya B, Bhargavi RS, & Babu PS. "A modern review of diabetes mellitus: an annihilator metabolic disorder." Journal of In Silico & In Vitro Pharmacology. (2017): 3(1): 14.
2. Bordoloi R, Dutta KN. "A Review on Herbs Used in the Treatment of Diabetes mellitus." Journal of Pharmaceutical, Chemical and Biological Sciences. (2014): (2): 86–92.
3. Mukherjee, P.K., 2002. *Quality Control of Herbal Drugs-an Approach to Evaluation of Botanicals*. New Delhi, Business Horizons Pharmaceutical Publishers.
4. Kokate, C.K., 1996, *Practical Pharmacognosy*. Delhi, Vallabh Prakashan.
5. Khandelwal, K.R., 2006. *Practical Pharmacognosy*. Pune, Nirali Prakashan.
6. OECD Guidelines 2001. "Guidance document on acute oral toxicity testing" Series on testing and assessment No. 23, Organization for Economic Co-operation and Development, OECD Environment, health and safety publications, Paris Available from: http://www. Oecd.org/ehs [accessed 20 March on 2010].
7. Ali K M, Chatterjee K, De D, Bera T K, Ghosh D. Efficacy of aqueous extract of seed of *Holarrhena antidysenterica* for the management of diabetes in experimental model rat: A correlative study with antihyperlipidemic activity. International Journal of Applied Research in Natural Products 2009; 2(3): 13-21.
8. Shabeer J, Srivastava R S, Singh S K, Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan diabetic rats. Journal of Ethnopharmacology 2009; 124: 34–38.
9. Al-Qattan K, Martha Thomson a, Muslim Ali, Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. The European e-Journal of Clinical Nutrition and Metabolism 2008; 3: e62-e71.
10. Mallick C, Chatterjee K, Biswas M G, Ghosh D. Antihyperglycemic effects of separate and composite extract of *Musa paradisiacal* and leaf of *Coccinia indica* in streptozocin induced diabetic male albino rat. African Journal of Traditional, complementary and alternative medicine 2007; 4(3): 362-371.
11. Pepato M T, Mori D M, Baviera A M, Harami J B, Vendramini R C, Brunetti I L. Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. Journal of Ethnopharmacology 2005; 96: 43–48.

ISSN PRINT 2319 1775 Online 2320 787
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

12. Stahl, E. A Laboratory Handbook, 2nd Edition, Springer, New York, ISBN: 0387047360, 1969.

13. Prince P S M, Menon V P. Effect of *Syzigium cumini* in plasma antioxidants on alloxan-induced diabetes in rats, Journal of Clinical Biochemistry and Nutrition 1998; 25(2): 81–86.

14. Cai L, Wang J, Li Y, Sun X, Wang L, Zhou Z, Kang Y J. Inhibition of superoxide generation and associated nitrosative damage is involved in metallothionein prevention of diabetic cardiomyopathy. Diabetes 2005; 54: 1829-1837.