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Evaluation and Investigation of the Antidiabetic Effect of Aqueous and Hydroalcoholic Extract of *Elaeocarpus ganitrus* (Rudraksha)

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ABSTRACT: This experimental study investigated the antidiabetic effect of aqueous and hydroalcoholic extract of the *Elaeocarpus ganitrus* (Rudraksha) plant, which is usually used to cure many diseases due to its electromagnetic property. Diabetic was induced in fasted rats by inducing a single intraperitoneal injection of 150 mg Kg^{-1} of Alloxan monohydrate. Two doses (550 and 1050mg Kg^{-1}) of these aqueous and hydroalcoholic extracts of *Elaeocarpus ganitrus* were administrated orally to diabetic rats. The standard control group receives distilled water only. After half a month (15 days) of treatment, body weight gain, blood glucose level, Serum insulin other body metabolites were studied and evaluated for Diabetic. It was noticed that at tested doses, aqueous and hydroalcoholic extract of *Elaeocarpus ganitrus* decreased blood glucose levels significantly after half month of administration. The aqueous extract reduces body weight gain, contrary to the hydroalcoholic section. Increase Serum insulin level compared in the group, treated group and diabetic control group. The hydroalcoholic extract reduces the monohydrate concentration in Serum. Values of total cholesterol and triglyceride are similar in all groups. This study demonstrated the potential antidiabetic property of aqueous and hydroalcoholic extract of *Elaeocarpus ganitrus.* thus justifying its traditional usage. The purpose of this research is to understand the antidiabetic effects of Rudraksha and gain an understanding of the challenges faced by novice researchers and students when examining the antidiabetic effects of the *Elaeocarpus ganitrus* plant. Research has improved the globe by addressing the issue of therapeutic Rudraksha use that is currently present.

Keywords: Rudraksha, *Elaeocarpus ganitrus*, blood glucose, insulin, antidiabetic effect.

INTRODUCTION

The study investigated the antidiabetic effect of *Elaeocarpus ganitrus* (Elaeocarpaceae). Diabetes is a chronic disease caused by insufficient pancreatic insulin production or ineffective systemic insulin utilization (Bilous *et al.*, 2021). The insulin hormone regulates blood sugar levels. Hyperglycemia, or increased blood sugar, is a common side effect of uncontrolled diabetes which, over time, seriously harms our body parts, including the blood vessels and neurons (Kifle *et al.*, 2022). If someone has diabetes, the patient has too much sugar in their blood (Roglic, 2016). Uncontrolled diabetes frequently results in hyperglycemia or elevated blood sugar, which causes substantial harm to many different body systems, including the neurons and blood vessels, over time. Having too much glucose in the blood might cause health concerns over time. Although there is no cure for diabetes, one can manage diabetes and stay healthy (Papatheodorou *et al.*, 2016).

There are three main types of diabetes:

- Type 1
- Type 2
- Gestational diabetes

Type 1 diabetes can manifest at any age; children and adolescents are the most commonly affected by this type of disease. When a person has type 1 diabetes, bodies produce very little insulin, necessitating daily insulin injections to keep blood glucose levels under control. On the other hand, Insulin resistance occurs in type 2 diabetes, preventing the body from effectively using insulin (Rubin & Peyrot 1999). To fulfil the daily insulin demand, the Pancreas produces more insulin until it cannot meet demand (Hule *et al.*, 2011). The subsequent decrease in insulin synthesis results in elevated blood sugar. The pregnancy-related production of insulin-blocking substances results in gestational diabetes. Pregnancy is the only time when gestational diabetes occurs. People with previous pre-diabetes problems and a family history of diabetes are more likely to experience it. Numerous cardiac issues, neuropathy, nephropathy, retinopathy, hearing loss and Alzheimer's disease are significantly more common in diabetic persons (Zimmet *et al.*, 2014).

Elaeocarpus ganitrus. *Elaeocarpus ganitrus* plant is found in India and the north-eastern region of Asia. It is a species of flowering plant belonging to the Elaeocarpaceae family and a significant medicinal plant

with several therapeutic applications in the conventional medical system (Arivu & Muthulingam 2017). It has been utilized in many world regions to treat various health issues. Traditional remedies for liver ailments, stress, anxiety, depression, palpitations, nerve pain, epilepsy, and migraines are cured using these plants' leaves and seeds (Soman & Surya 2018). According to several *in-vitro* studies, an extract from *E. ganitrus* leaves showed considerable antioxidant activity. Alkaloids, tannins, flavonoids, steroids, glycosides, saponins, phytosterols and other components have all been found in phytochemical screenings of different preparations of this plant (Hardainiyan *et al.*, 2015).

Fig. 1. *Elaeocarpus ganitrus* plant.

MATERIAL AND METHOD

Collection and authentication of Plant material. Plant Material: The seed of *Elaeocarpus ganitrus* was collected. The collection of authenticated *Elaeocarpus ganitrus* (Rudraksha) was done by purchasing online from the Rudra Center (https://www.rudraksha-ratna.com/). Preliminary X-ray analysis was conducted and noticed that Rudraksha seed internal structure has five compartments, which signifies that Rudraksha seeds obtained from *Elaeocarpus ganitrus* are five-face genuine Rudraksha seeds.

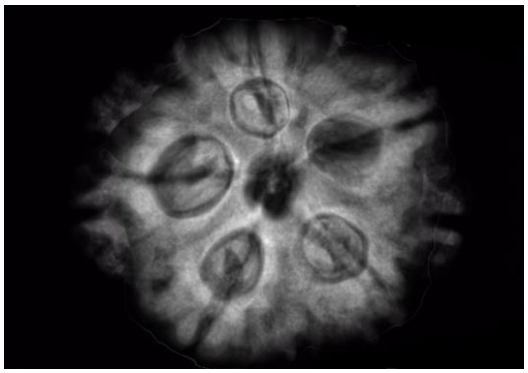

Fig. 2. X-Ray analysis of Rudraksha seed showing genuine five face.

We further confirmed its originality by CSIR-NISCAIR's, Research & Academics in Science & Technology, Communication New Delhi.

Authentication No: (NISCAIR/RHMD/Consult/2019/3436-37).

The seed was cleaned with water and cut into small pieces, dried and extracted with water or ethanol (1:1v/v) dilution. The seed powder was soaked in water or ethanol-water (2L) and heated in the water bath at 80°C for 1hr for aqueous extract. The exact quantity was macerated for 72 hr in ethanol /water solution (2l) for the hydroalcoholic section. The crude extracts were filtered with what-man filter paper and evaporated in a vacuum at 40°C using a rotary evaporator. The yield of the preparation was 16% (w/w) for aqueous extract (AE) and 13% (w/w) for Hydroalcoholic Extract (HAE)

Chemicals. Alloxan monohydrate, DPPH (2,2-diphenyl-1-picrylhydrazyl), and AAPH (2,2' amino propane dihydrochloride) were purchased from Bilwal Medchem and Research Laboratory Pvt. Ltd, Vidhyadhar Nagar, Jaipur.

Bilwal Medchem and Research Laboratory
CiN: U24290R12016PTC054867(CPCSEA),
Registration No:2005/PoRCBt/S/18/CPCSEA

Animals: Male Wister rats (200-250g) were obtained from Bilwal Medchem and Research Laboratory, Pvt. Ltd, Vidhyadhar Nagar, Jaipur CiN: U24290R12016PTC054867(CPCSEA),

Registration No: 2005/PoRCBt/S/18/CPCSEA. These rats were fed with Conventional chow (toxin-free following EU regulation) and tap water. Rats were acclimatized for seven days under standard environmental conditions of temperature, relative humidity and dark light cycle. The animals were randomly divided into different groups for the experiment and were deprived of food except for water 16h hours before the experiments. All animal testing was conducted acceding to the law set by CPCSEA.

Initiation of diabetes in Rats: Diabetes induced in fasted rats (12hr) by a single intraperitoneal injection of 120mg Kg^{-1} of Alloxan monohydrate. Alloxan was freshly dissolved in distilled water and the injection volume was 20ml Kg^{-1}. The diabetic state was assessed by measuring the non-fasting blood glucose level 10 days after the Alloxan injection. Rats with blood glucose levels in a range of 200-500 mg dL^{-1}, with polyuria and glucosuria, were selected for the experiment. Blood glucose level was measured with a Glucometer on the tail vein (Ravi *et al.*, 2013).

Experimental design:

Rats were divided into seven groups of seven rats.

Group 1 (Gr.1) Normal control rats received distilled water alone.

Group 2 (Gr.2) Diabetic control rats received distilled water alone.

Group 3 (Gr. 3) Diabetic Rats, treated with 550 mg Kg^{-1} AE

Group 4 (Gr. 4) Diabetic rats, treated 1100 mg Kg^{-1} AE

Group 5 (Gr. 5) Diabetic rats, treated with 550 mg Kg^{-1} HAE

Group 6 (Gr. 6) Diabetic rats, treated with 1100 mg Kg^{-1} HAE

Group 7 (Gr.7) Diabetic rats, treated with 100 mg Kg^{-1} Metformine (Standard oral Hypoglycemic agents)

The plant extract was taken in distilled water for solubility and fed orally to animals by gastric incubation for 15 days. All the drugs were given as a single dose at sunrise. Body weight and blood glucose levels were estimated on days 1, 7, and 15. After the

last dose, animals were tranquillized with ether. Blood samples were collected by the oral sinus puncture, centrifuged at 500*g, and Serum was kept at -20°C. Total Cholesterol (TC) and Triglycerides (TG) were estimated using reagent kit ELISA by enzymatic method. Malondialdialdehyde concentration was measured spectrophotometrically.

Table 1: Effect of aqueous extracts (AE) and hydroalcoholic extract (HAE) on body weight gain (BWG).

Body weight (g)			
Groups	**Day 1**	**Day 15**	**BWG %**
C	170.44±1.98	239.25±4.17	39.88
DC	217.89±13.9	251.78±15.87	15
AE550mg kg^{-1}	191.56±18.2	202.65±36.36	6.5
AE1100mg kg^{-1}	234.72±11.8	238.54±12.3	6.4
HAE 550 mg kg^{-1}	148.8±6.87	186.56±12.56	26.67
HAE 110mg kg^{-1}	217.67±8.9	255.88±16.93	17.21
Met 110mg kg^{-1}	227.72±15.3	254±23.16	8.95

C=normally controlled rats DC= Diabetically controlled rats AE = Aqueous extract of 550 and 1100 mg Kg^{-1} *Elaeocarpus ganitrus* fed with diabetic rats HAE=Hydro alcoholic extract of 550 and 1100 mg Kg^{-1} *E. ganitrus* fed with diabetic rats Met: Metformine 110 mg Kg^{-1}fed with diabetic rats. The data were expressed as Mean ±SEM (n =7), followed by ANOVA and Fisher LSD Test at 5%.

The body weight gain (BWG) % represents

(*Day15*-Day_1) Day_1*100

Table 2: Effect of different doses of aqueous extracts (AE) and hydroalcoholic extract (HAE) on blood glucose level.

Blood glucose level (mg dL^{-1})			
Groups	**Day.1**	**Day 7**	**Day 15**
C	91.52±3.24	89.25±5.27	82.58±3.32
DC	328.89±31.92^{a3}	341.78±35.23 ^{a3}	375.5±35.23 ^{a3}
AE550mg kg^{-1}	345.41±33	236.45±38.	210.36±39^{b}
AE1100mg kg^{-1}	352.62±36.30	223.81±47.65	265.5±38.7^{b}
HAE 550 mg kg^{-1}	306.8±6.87	186.56±12.56	26.67
HAE1100 mg kg^{-1}	283.00±38.12	262.89±43.67	188.67±19.88^{b3}
Met 110mg kg^{-1}	277.72±19.3	334±53.16	225±95^{b2}

C=normally controlled rats DC= Diabetically controlled rats AE = Aqueous extract of 550 and 1100 mg Kg^{-1} *E. ganitrus* fed with diabetic rats HAE=Hydro alcoholic extract of 550 and 1100 mg Kg^{-1} *E. ganitrus* fed with diabetic rats Met: Metformine 110 mg Kg^{-1}fed with diabetic rats. The data were expressed as Mean ±SEM (n =7), followed by ANOVA and Fisher LSD Test at 5%. a^3 P<0.001 vs Normal Control, b^1 P<0.05, b^2 P<0.01 and b^3 P<0.001 vs Diabetic control.

Table 3: Effect of aqueous extracts *E. ganitrus* (AE) and *E. ganitrus* hydroalcoholic extract (HAE) on serum insulin, MDA, triglyceride, and total cholesterol.

	Insulin	**MDA**	**TG**	**TC**
Groups	**ngmL^{-1}**	**Pmolml^{-1}**	**(gL^{-1})**	**(gL^{-1})**
C	4.5±0.55	6.7±0.27	1.43±0.2	0.78±0.045
DC	0.81±0.12^{a3}	8.8±0.43 ^{a1}	1.16±0.41	1.15±0.1
AE550mg kg^{-1}	4.41±073^{b2}	6.7±0.66	1.52±0.45	0.86±0.45^{b}
AE1100mg kg^{-1}	2.62±0.73^{b}	7.41±0.65	1.16±0.45	1.05±0.07
HAE 550 mg kg^{-1}	2.48±0.83^{b}	6.3±0.54^{b}	1.18±0.23	1.07±0.06
HAE1100 mg kg^{-1}	2.55±0.52^{b}	6.6±0.4^{b}	0.93±0.23	0.87±0.068
Met 110mg kg^{-1}	1.52±0.39	6.5±0.56^{b}	1±0.34	0.67±0.08

C=normally controlled rats DC= Diabetically controlled rats AE = Aqueous extract of 550 and 1100 mg Kg^{-1} *E. ganitrus* fed with diabetic rats HAE=Hydro alcoholic extract of 550 and 1100 mg Kg^{-1} *E. ganitrus* fed with diabetic rats Met: Metformine 110 mg Kg^{-1}fed with diabetic rats. The data were expressed as Mean ±SEM (n =7), followed by ANOVA and Fisher LSD Test at 5%. a^3P<0.001 a^1P<0.001 a^3P<0.001 vs Normal Control, b^1 P<0.05, b^2 P<0.01 vs Diabetic control.

DISCUSSION

In this experimental study, Alloxan-induced diabetes in rats approximates an insulin-dependent diabetic state with non-fasting blood glucose between 250 and 550mg dL^{-1}. Several studies have been conducted in *in-vitro* and *in-vivo* to understand the cytotoxicity effect of this drug. Alloxan-induced diabetes is insulin-dependent diabetes that manifests in animals after Alloxan administration or injection. Alloxan specifically interacts with pancreatic beta cells, generating oxygen-free Radicals (ROS) responsible for its toxic effect (Toppo *et al.*, 2015). It is well reported that the partial destruction of Beta cells causes a decrease in insulin secretion which is materialized by chronic hyperglycemia. The impact of Hydroalcoholic and aqueous extract in diabetic rats compared to controls show a significant reduction in diabetic blood glucose on the 15th day of treatment. The hydroalcoholic section was more effective at the end of the treatment in this animal study (Parmar *et al.*, 2010). Alloxan caused

body weight reduction, which was reversed by the hydroalcoholic extract at 550kg^{-1}. The diabetic group treated with aqueous extract showed a severe body weight reduction compared to diabetic controls. The effect of aqueous extract on body weight could be ascribed to tannins, being more plentiful in this extract than in hydroalcoholic extract. Several studies have revealed that tannins have an anti-nutritional function that causes rodent weight loss due to developing complex proteins in the intestinal lumen or reducing food intake (Zeashan *et al.*, 2008).

- **Effect of aqueous extracts and hydroalcoholic extract on body weight gain, blood glucose level, serum insulin, MDA, triglyceride, and total cholesterol.**

(Bellamkonda *et al.*, 2011; Majidi *et al.*, 2020)

Table 1 depicts the influence of AE and HAE on animal body weight gain. The proportion of body weight gain in controlled rats was more significant than in diabetically controlled rats. The ANOVA and Fisher LSD tests were used to compare the alcoholic and hydroalcoholic extracts, (550 and 1100 mg Kg^{-1}) *Elaeocarpus ganitrus*, administered to diabetic rats. The effect of different doses of aqueous extracts (AE) and hydroalcoholic extracts (HAE) on blood glucose levels is shown in Table 2. Blood glucose levels (mg dL^{-1}) were higher in normally managed rats than in diabetically controlled rats throughout time. The effect of *E. ganitrus* aqueous extracts and hydroalcoholic extract (HAE) on blood insulin, MDA, triglyceride, and total cholesterol is shown in Table 3. Diabetically controlled rats improved over usually kept rats throughout time.

- **Photo micrograph study of tissues of the Pancreas**

(Abdelrahim, 2013; Longnecker, 2014)

Figs. 3-6 shows the Photo micrograph study oftissues of the Pancreas of diabetic control rats, which shows the improvement in rats with diabetes by showing minor congestion in acinar cells and moderate islets of Langerhans. Fig. 3 of a pancreas cell from an ordinary rat (control) exhibits the standard histological organization of *Langerhans* (L) and acinar cells. Fig. 4 shows the Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by Alloxan which shows significant shrinkage of islets of Langerhans. Fig. 5 offers the Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by 550mg kg^{-1} aqueous extracts of *Elaeocarpus ganitrus*, which indicates relatively normal acilar cells and normal islets of Langerhans. Fig. 6 shows a Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by 550mg kg^{-1} extracts of hydroalcoholic extract *E. ganitrus*, HAE shows relatively small congestion in acinar cells and moderate islets of Langerhans.

In summary, after half a month of daily treatment, the insulin level in the groups treated with aqueous extract and hydroalcoholic extract was considerably higher than in the untreated control groups. The aqueous and hydroalcoholic extracts of *Elaeocarpus ganitrus* dramatically reduced blood glucose levels in the glucose tolerance test but did not affect fasted blood glucose levels, indicating that the extract works through a distinct pancreatic mechanism. The elevated insulin concentration is due to the consumption of glucose in peripheral tissues.

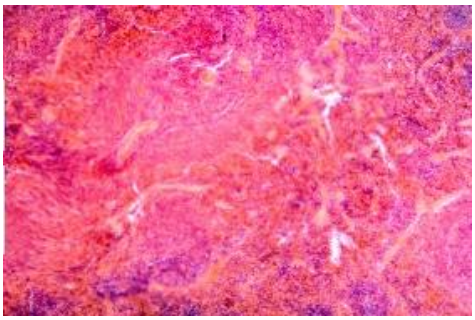

Fig. 3. Photo micrograph study of tissues of Pancreas of Normal Rat.

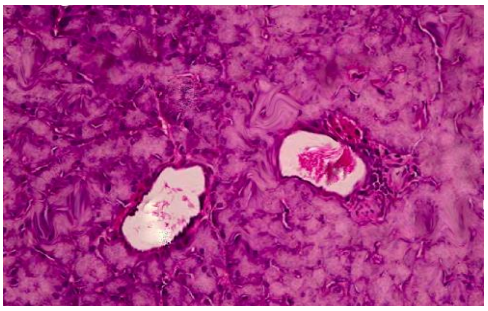

Fig. 4. Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by Alloxan which shows significant shrinkage of islets of Langerhans.

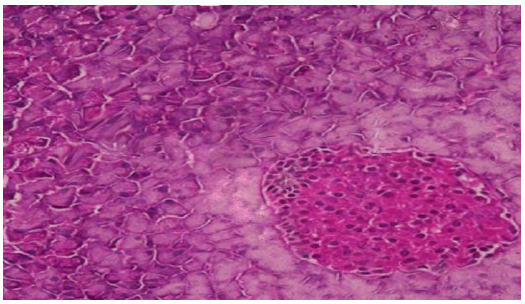

Fig. 5. Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by 550 mg kg^{-1} aqueous extracts of *Elaeocarpus ganitrus*, which shows relatively normal acilar cells and normal islets of Langerhans.

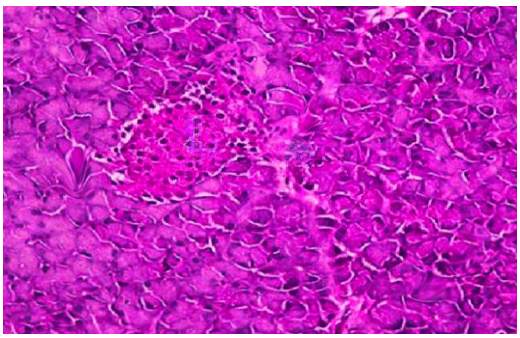

Fig. 6. Photo micrograph study of tissues of the Pancreas of diabetic control rats induced by 550mg kg^{-1} extracts of hydroalcoholic extract *Elaeocarpus ganitrus*, which shows relatively small congestion in acinar cells and moderate islets of Langerhans.

CONCLUSIONS AND FUTURE SCOPE

The experimental study concluded that the *Elaeocarpus ganitrus* extract exhibited antidiabetic activity by stimulating serum insulin levels in Alloxan-influenced rats. This exploratory study shows that *Elaeocarpus ganitrus* can help in the treatment of diabetes and the prevention of its complication. For a better understanding of the results, further scientific research may encourage new researchers in this field.

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Conflict of Interest. None.

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