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THE LEAF EXTRACT OF *MUNTINGIA CALABURA* EXHIBITS *IN VITRO* ANTIDIABETIC POTENTIALS BY THE SENSITIZATION OF INSULIN

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ABSTRACT
The objective of the current study was to evaluate the mechanism through which *Muntingia calabura* exerts its antidiabetic effects. **Materials &Methods**: The authenticated leaves of plant *Muntingia calabura* were dried and powdered. The powdered drug was defatted using petroleum ether, and extracted with ethanol. An initial phytochemical analysis of *Muntingia calabura*'s ethanol extract was performed. The rat hemi-diaphragm (skeletal muscle) model for glucose uptake was also employed to assess the potential of an ethanol extract of *Muntingia calabura* to improve peripheral tissue uptake of blood glucose. The albino rat that had been fasting the previous night was slaughtered and its hemidiaphragm was removed. The amount of glucose used by the tissue was calculated after weighing a portion of skeletal muscle and incubating it with glucose in Tyrode solution for 30 mins at 37^{o}C. **Results**: The skeletal muscle's ability to use glucose was greatly improved by the ethanol extract of *Muntingia calabura*, which also has the ability to make insulin more sensitive. **Conclusion**: The findings of the current study suggest that decreasing insulin resistance is one of the ways through which the ethanol extract of *Muntingia calabura* exerts its anti-diabetic effects.

KEYWORDS: Antidiabetic activity, *Muntingia calabura*, Hemidiaphragm, Glucose utilization.

INTRODUCTION
Diabetes mellitus develops as a result of disruptions in the body's metabolism brought on by an absolute or relative lack of insulin or by insulin resistance, which ultimately causes changes in the metabolism of the nutrients lipids, carbs, and amino acids.[1] According to a World Health Organisation (WHO) survey, India will have the highest prevalence of diabetes in the world by 2020, with an estimated 220 million people worldwide having the disease. Due to the great incidence of the condition and its long-term effects, there is therefore always room for the creation of anti-diabetic medications.[2] In those with insulin dependent diabetes mellitus (Type I or IDDM), a peptide hormone called insulin is administered, while in those with non-insulin dependent diabetic mellitus (Type II or NIDDM), oral hypoglycemic medications are used to convert hyperglycemia to euglycemia. Although there are a number of pharmacological medicines available for the therapy of diabetes mellitus, there is still no completely satisfactory medication for the effective control of this condition with minimal adverse effects. Therefore, the discovery and creation of novel therapeutic agents remain quite desirable.[3] Since herbal medicine plays a crucial role in this segment due to its minimal side effects, searching for more potent and less toxic hypoglycemic drugs from plant origin is under pipeline throughout the world in light of the toxicities effects and adverse reactions associated with the therapy using currently available oral hypoglycemic drugs and insulin.[4] The traditional Indian medical system of Ayurveda, which dates back to the time of Charka and Sushruta, has long advocated the use of a number of medicinal herbs for the proper control of diabetes with fewer adverse effects. Compared to synthetic pharmaceuticals, plant-based herbal treatments for diabetes mellitus, whether used alone or in combination with other medications, are much safer and less likely to cause side effects.[5]

Stimulation of insulin secretion, inhibition of intestinal glucosidase, and improvement of glucose utilisation by tissues are among the many strategies for managing diabetes mellitus.

The most traditional Indian medical system, Ayurveda, offers the important texts, such as the Vedas and Upanishads, which may contain plant medicines with radioprotective properties. Because they are viewed as effective and less hazardous than synthetic medications,

herbal remedies are a viable alternative. This gives reason to investigate the potential radioprotective properties of natural compounds. In Indian traditional medicine, the *Muntingia calabura* is a common herbal remedy used to cure a variety of ailments.[6,7] The plant is abundant in flavonoids and other phytoconstituents, and preclinical studies have linked it to a number of pharmacological effects. The plant has reported for its significant antioxidant property which is one of the essential approach to ameliorates free radicals which lead to fungal infection, bacterial infection, diabetes mellitus, cancer and etc.[8,9] In recent times, animal models have demonstrated the plant's ethanol extract's anti-diabetic capabilities. The current study was created to investigate the extract's mechanism of action using an in vitro experiment as part of ongoing research.

MATERIALS AND METHODS

Preparation of the ethanol extract

The leaves of *Muntingia calabura* was collected from local area in Bangalore and authenticated by Dr. V. Rama Rao, Research Officer (Botany), Central Ayurveda Research Institute. Plant matter was collected, dried, and stored in the shade. The powdered dried leaves are next treated with petroleum ether to remove fat from the coarse powder. The defatted powdered medication will undergo 48 hours of ethanol extraction in a Soxhlet system, and the residual marc will undergo an aqueous extraction process using chloroform water.[10]

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEMC) of *Muntingia calabura* was conducted as per procedure prescribed by Khandelwal.[11]

Evaluation of in vitro antidiabetic activity of extract of *Muntingia calabura*

Glucose uptake by isolated rat hemidiaphragm

Utilisation of glucose by rat skeletal muscle (hemidiaphragm) was assessed using techniques from prior studies.[12] The study consisting of four categories, with each group containing 6 graduated test tubes, were regarded as follows:

- **Category I:** Consists of 10 mL of 4% glucose in Tyrode solution.
- **Category II**: Consists of 10 mL of 4% glucose in Tyrode solutionand regular insulin suspension (1IU).
- **Category III**: Consists of 10 mL of 4% glucose in Tyrode solutionand 1.38 mL of EEMC (0.1% v/v).
- **Category IV**: Consists of 10mL of 4% glucose in Tyrode solutionand regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of EEMC (0.1% v/v)

The total volume of the test tubes was created by individually combining the amounts of each assay tube to create a total volume of 4 mL. Wistar albino rats of all ages were kept on a 24-hour fast before being slaughtered under very light anaesthesia. The diaphragms of experimental animals were quickly cutted with little damage and splitted into 2 equal halves. Two diaphragms from the same animal were not used for the same set of studies. In each study category, about xix diaphragms were used. The collected skeletal muscles (diaphragm) were shook at a rate of 140 CPM while being incubated at $37^{O}C$ for around 30 minutes in a 100% oxygen atmosphere. The ratio of the initial and final glucose concentrations in the incubated medium was used to calculate the quantity of glucose that was utilised for every gramme of tissue.[13,14]

RESULTS

Preliminary phytochemical investigation

The percentage yield of the EEMC was discovered to be 9.24% w/w. In the ethanol extract of Muntingia calabura, early phytochemical analysis revealed the presence of polyphenols, flavonoids, tannins, steroids, alkaloids, and carbohydrates.

Effect on peripheral glucose uptake

The ethanol extract of *Muntingia calabura* greatly improved the rat hemidiaphragm's utilisation of glucose in the current investigation, and its efficacy was comparable to that of the standard agent Insulin. EEMC and insulin work well together and have demonstrated synergistic effects. According to the findings, either insulin and EEMC alone for 30 minutes significantly increased glucose absorption by 3.32 and 2.96 times, respectively. When insulin and EEMC were added to the incubation media, the rate of glucose utilisation in the rat hemidiaphragm increased by 3.58 times compared to the untreated control animals, but there was no appreciable increase compared to the insulin alone treated group [Table 1 and Figure 1]. When compared to the vehicle control, the skeletal muscle of the rats used glucose significantly more than any other category.

Table 1: Effect of EEMC on glucose uptake by isolated rat hemi-diaphragm.

S. No	Glucose uptake for 30 mins (mg/g)
Control	82.55±1.32
Insulin	273.35±6.11**
EEMC	244.84±4.5**
EEMC + Insulin	295.32±13.8**

Values are mean ± SEM (n=6). ** p< 0.01 as compared with control

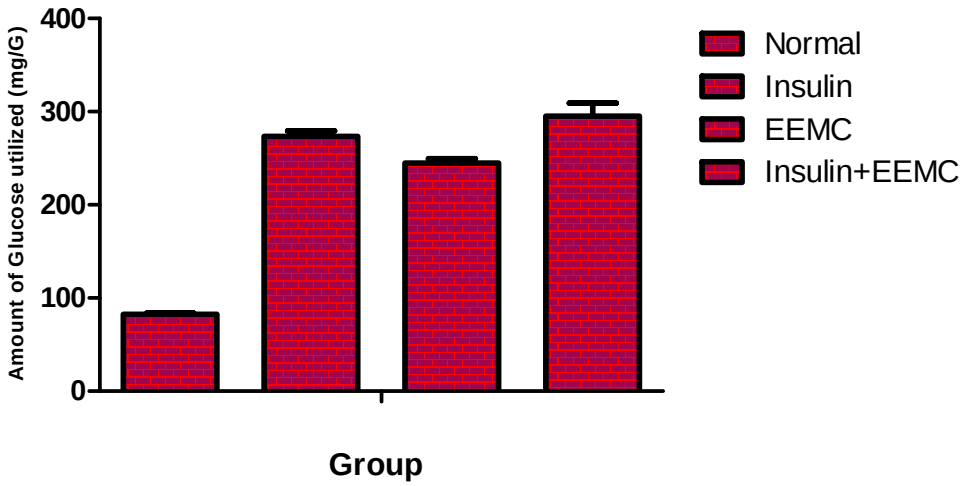

Figure 1: Effect of EEMC on glucose uptake by isolated rat hemi-diaphragm.

DISCUSSION

The prevalence of diabetes mellitus (DM), a metabolic, multifactorial, and debilitating condition, is rising globally. It can cause multiple organ failures, peripheral neuropathy, retinopathy, nephropathy, hyperlipidemia, and different cardiovascular problems, among other complications.[15,16] Enhancement of glucose by peripheral tissues is a key mechanism in one of the novel treatment approaches to managing diabetic mellitus. Skeletal muscle makes up between 30-40% of the entire body, making it one of the most important target tissues for insulin's activity, which improves the utilisation of glucose at the peripheral level. It is commonly known that insulin and anti-diabetic medications increase peripheral cells' and tissues' ability to use glucose.[17] The main discovery of the current study is that EEMC have significant actions that are similar to those of insulin, as demonstrated by the stimulation of glucose uptake from the rat's hemidiaphragm, which is made up of muscle tissue that is crucial for insulin-regulated glucose discharge. Compared to insulin, the EEMC significantly increased the absorption of glucose by isolated rat muscle hemidiaphragm. The results of the normal group of glucose utilisation by rat peripheral tissue seem to indicate that EEMC has an effect on those tissues, and they are consistent with past studies.[22] In the current investigation, the EEMC shown its ability to reduce insulin resistance by boosting the uptake of glucose by peripheral tissues, and the extract demonstrated its potentials that may represent a potential mechanism of action for its ability to combat diabetes.

CONCLUSION

The results of the current study suggest that decreasing insulin resistance is one of the ways through which the ethanol extract of *Muntingia calabura* exerts its anti-diabetic effects. However, more research is required to identify and quantify the precise elements present in the ethanol mextract of *Muntingia calabura* that may be in charge of the positive effects on the health conditions related to diabetes mellitus.

CONFLICT OF INTEREST

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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