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Activity of *Clitoria ternatea* and *Embilica officinalis* in Diabetic Animal Models

Morampudi Ravi Kiran^{1*}, Neelakandan Vishali² and Naveen Kumar B.³

¹Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India

²Department of Anatomy, Vels Medical College, Chennai, Tamil Nadu, India

³Department of Anatomy, Mamata Academy of Medical Sciences, Hyderabad, India

*Corresponding Author

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Abstract: The ethanolic extract of flowers of *Clitoria ternatea* (CT) and fruits of *Embilica officinalis* (EO) had hypoglycemic and antioxidant properties in streptozotocin-induced diabetic rats. Malondialdehyde (MAD), superoxide dismutase (SOD), glutathione reductase (GSH-Rd), and reduced glutathione (GSH) levels were evaluated in the plasma to determine the level of oxidative stress. A significant ($P < 0.05$) decline in fasting blood glucose was seen after oral administration of the CT and EO at a dose of 100 mg/kg b.w. Additionally, it raised MAD and GSH-Rd levels in hepatic and renal tissues while drastically reducing the levels of antioxidant enzymes, such as SOD and GSH. The results strongly indicate that the ethanolic extract of CT and EO treated groups may successfully normalize the decreased antioxidant status in streptozotocin-induced diabetes. The extract reduced the incidence of diabetes complications and quickly displayed protective effects against lipid peroxidation by scavenging free radicals.

Keywords: *Clitoria ternatea*, *Embilica officinalis*, Oxidative stress, Anti-diabetic

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Introduction

Diabetes mellitus (DM) is a chronic condition brought on by genetic or acquired deficiencies in pancreatic insulin production or due to the ineptitude of the insulin (Yazdan *et al.*, 2005). Multiple pathophysiological problems as well as irregularities in carbohydrates, lipids, proteins, and metabolism are characteristics of DM (Dheer and Bhatnagar, 2010; Mohammadi *et al.*, 2011; Attanayake *et al.*, 2013). According to the World

Health Organization (WHO), 451 million more people will develop diabetes worldwide by 2017 as the frequency of the disease rises. This "record" is anticipated to rise to 693 million cases by 2045, with a USD 850 billion projection for worldwide healthcare spending on diabetics in 2017 (Cho *et al.*, 2018).

The therapeutic benefits of medicinal plants include their affordability, safety, and efficacy in

treating a wide range of illnesses. Alternative treatments for DM and associated co-morbidities that are safe and effective could come from natural materials. Such solutions must first be tested in relevant animal models to determine their potential effects. Several medications are used to manage DM, however complete diabetes control is infrequently attained (Damasceno *et al.*, 2008). The side effects of chemical hypoglycemic agents have all been cited as reasons for encouraging the use of medicinal plants as alternative treatments (Maiti *et al.*, 2005). In recent years, traditional medicines with roots in medicine have been increasingly important in managing diabetes mellitus (Cheng *et al.*, 2013).

Free radicals, on the other hand, are biologically significant molecules that are created in the body during regular metabolic processes. "Oxidative stress" refers to increased free radical production in the body. Cellular components may become oxidatively damaged by the extremely reactive radicals (Baynes, 1991).

Atherosclerosis, cancer, neurological diseases, and diabetes are just a few of the illnesses that can arise from the production of free radicals and lipid peroxidation (LPO). Additionally, it's crucial for the growth of both macrovascular and microvascular problems associated with diabetes. Due to the harmful impact that free radicals play in biological systems, radical scavenging activities are crucial. Diabetes also causes alterations in the tissue quantity and activity of antioxidant enzymes (Aruoma *et al.*, 1991).

For the treatment of diabetes mellitus, there is currently no drug that provides satisfying and effective therapy. In order to find novel anti-diabetic medications with great therapeutic efficacy and few side effects, further research is required. It can be achieved by treating DM using plant-derived antidiabetic drugs, which are currently utilized in conventional medicine because they are secure, affordable, accessible, dependable, and extremely effective (Chauhan *et al.*, 2017). Several naturally occurring antioxidants found in plants have been demonstrated to lessen

oxidative stress and the onset of serious diseases (Kavitha, 2018).

Some anti-diabetic medications, according to experience in the use of herbal medicine, were ineffective when taken alone but had great therapeutic effects when combined. In Ayurvedic medicine, *Clitoria ternatea* (CT) and *Embilica officinalis* (EO) are two of the most commonly used treatments for diabetes mellitus. The first plant CT in the current study is an herbaceous perennial from the Fabaceae family. It is referred to as Shankpushpi. Antidepressant, anticonvulsant, anti-stress, memory enhancer, as well as anxiolytic and sedative qualities are among CT's pharmacological effects (Mukherjee, 2008). It has a long history of usage as a treatment for a number of illnesses, including rheumatism, urinogenital disorders, bronchitis, purgatives, diuretics, anthelmintics, and diabetes mellitus. Additionally, it is frequently used to treat gonorrhea and infertility (Khare, 2010).

The second plant, EO, from the Euphorbiaceae family, is used extensively in Ayurveda and is said to be beneficial for various illnesses, including diabetes. In addition to growing in tropical and subtropical areas, the EO fruit is indigenous to India. Minerals, amino acids, and vitamin C are all present in the EO fruit. Free radical scavenging and antioxidant properties of EO (D'souza, 2014), anti-hyperglycemic and lipid-lowering properties of EO (Akhtar *et al.*, 2011), neuropathic pain by reducing oxidative stress in experimental diabetic rats (Tiwari *et al.*, 2011), and glucose homeostasis, and metabolic parameters (Patel *et al.*, 2013) have been reported.

It has been claimed that EO includes tannins, which are well known for their potent antioxidant action and can ameliorate glycemic status and oxidative stress in type 2 diabetic mice. These studies also discussed EO's ability to treat diabetes (Ansari *et al.*, 2014). The exact biological mechanism(s) underlying EO's blood glucose-lowering activity, such as cell hypertrophy or hyperplasia or other mechanisms, are not yet known because antioxidants by themselves do not

lower blood glucose levels. Ellagic acid (EA), gallic acid, emblicanin A and B, as well as vitamin C, are all abundant in EO extract. Gallic acid is a strong antioxidant that is frequently employed as the reference substance in investigations to scavenge free radicals.

One of the main substances in EO is EA, a derivative of gallic acid (Amakura *et al.*, 2000). It possesses antioxidant properties and has been investigated for its antidiabetic potential. Only the anti-diabetic impact of EA has been studied, and plausible conclusions are presented without understanding the exact mechanism of action. The aim of the current study was to determine if the two plants *Clitoria ternatea* (CT) and *Embilica officinalis* (EO), have any influence on the development of diabetes in rats after streptozotocin (STZ)-induced diabetes and compare their effects to those of the metformin standard treatment.

Materials and Methods

Preparation of the extracts:

The flowers of CT and fruits of EO were obtained locally, cleaned and were dried for two weeks in the shade and later ground into a fine powder. In order to use them, the powdered ingredients were kept in airtight containers. A Soxhlet extractor was used to soak 500 g of dried, coarsely ground materials in 1 liter of ethanol for 1 day. The extracts were stored at 45 °C to evaporate the remaining solvent. The yields of the alcohol extract we made from the CT and EO were determined to be 26.48% (w/w) and 29.83% (w/w), respectively.

Preliminary phytochemical screening:

According to the established protocols, the ethanolic extracts of CT and EO were screened for the presence of various phytochemical components like alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, and fixed oils.

Animals:

This study used male albino rats weighing 200–

250 g. The rats were kept in typical cages with a 12-h light/12-h dark cycle, 20°C, and 65% humidity. Rats were fed with water and regular food pellets. The study was approved by Institutional Ethics Committee and the care and use of all animals in accordance with the guidelines for the care and use of laboratory animals.

Acute oral toxicity study:

After the oral administration of plant extracts, the animals were monitored individually at least twice daily to look for any changes in grooming, hyperactivity, sedation, corneal reflex, urination, or mortality during the course of the 21-day experiment. This was done as part of acute oral toxicity research.

Diabetes induction:

For the objective of inducing DM, STZ was given once at a dose of 70 mg/kg body weight. Animals that had been fasting overnight were administered intraperitoneal injections. All treated animals were given access to food and drink. The estimated fasting blood glucose concentrations were also measured after the rats were allowed to stabilize for 4 days. In order to conduct the experiment, rats with moderate diabetes who also had hyperglycemia (i.e., blood glucose levels between 250 and 400 mg/dl) were used (Burcelin *et al.*, 1995).

Blood glucose levels were also assessed after STZ treatment to confirm that the rats were diabetic before oral administration of extracts and metformin. Blood glucose levels were measured after extract and metformin treatments at 0, 7, and 21 days. All of the animals received treatment for 21 days.

Experimental design:

The animals were allowed two weeks for acclimatization and then randomly divided into eight equal groups (n=6) as follows: (1) non-diabetic control group treated with 0.5 ml distilled water (NDC), (2) Non-diabetic + CT (NDC1), (3) Non-diabetic + EO (NDE1), (4) streptozotocin-

induced diabetic control group (DC), (5) Diabetic + CT, (DBC1), (6) Diabetic + EO (DBE1), (7) Diabetic + Vitamin C (DVC) and (8) Diabetic + Metformin (DM). Selection of the dose was based on acute oral toxicity studies. Each dose of extract was dissolved in 0.5 ml distilled water. Mean body weight recorded for all treatment groups at 0, 7, 14 and 21 days. The change in body weight was observed throughout treatment period in the experimental animals. Furthermore, for any signs of abnormalities throughout the duration of investigation, the rats were continuously observed.

Bio-chemical analysis:

All of the rats were killed by cervical dislocation at the end of the treatment. The animals' blood was taken via direct heart puncture. Centrifugation was performed to separate the serum and plasma, which was then kept at -20°C until the antioxidant enzymes were analyzed i.e., Malondialdehyde (Ohkawa *et al.*, 1979), Superoxide dismutase (Marklund and Marklund, 1974), GSH reductase (Staal *et al.*, 1969) and reduced GSH (Moron *et al.*, 1979).

Statistical Analysis:

All values are expressed as mean $\pm$ standard deviation for groups of six animals each. Statistical analyses were performed by Student's t-test. The significance level was set at ($P < 0.05$).

Results

Preliminary phytochemical screening:

Phytochemical screening of medicinal plants is important for identification of new sources of therapeutic and industrial importance. In the present analysis, the ethanolic flower extract of CT observed the presence of various phytochemicals such as proteins, carbohydrates, glycoside, resins, alkaloid, steroid, tannin, and phenols (Gupta *et al.*, 2010). Whereas ethanol extracts of EO revealed existence of alkaloids, tannins, saponins, steroids, phenols, glycosides, flavonoids, ellagitannins, chebulinic acid, chebulagic acid, corilagin, emblicanin A and B, geraniin, isocorilagin,

pedunculagin, phyllanemblinins A-F, and punigluconin (Fatima *et al.*, 2014).

Acute oral toxicity study:

Animals showed good tolerance to testing single doses of ethanolic extract of CT and EO in doses as high as 2 g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable signs of toxicity such as irritability, tremor, labored breathing, staggering, convulsion and mortality after daily administration orally for 15 days. So, the extract is safe for long term administration.

Body weight:

Decrease in body weight due to derangement of metabolic pathways is a common feature in diabetes and even observed with the chronic administration of metformin which causes the loss of lipids in body. In the present study, ethanolic extracts of CT and EO to diabetic rats (Groups DBC1 and DBE1) decreased body weight significantly ($P < 0.05$) which was comparable to control (Fig. 1).

Antidiabetic activity:

Fasting blood glucose levels in the control remained unchanged during the course of the experiment. In diabetic groups, level of fasting blood glucose was significantly ($P < 0.05$) higher as compared to normal control group. On the other hand, administration of ethanolic extract of CT and EO for 21 days was found to lower the blood glucose level (Figs. 2, 3).

Anti oxidant activity:

Oxidative stress assessment was performed by recording the activities of anti-oxidative enzymes i.e. malondialdehyde (MAD), superoxide dismutase (SOD), glutathione reductase (GSH-Rd) and reduced glutathione (GSH). The diabetic rats showed significant ($P < 0.05$) decreased level of antioxidant enzymes i.e. GSH and SOD; raise in MAD and GSH-Rd in rat blood. Treatment with CT and EO significantly ($P < 0.05$) increased GSH and SOD in addition to decrease in MAD and GSH-Rd in

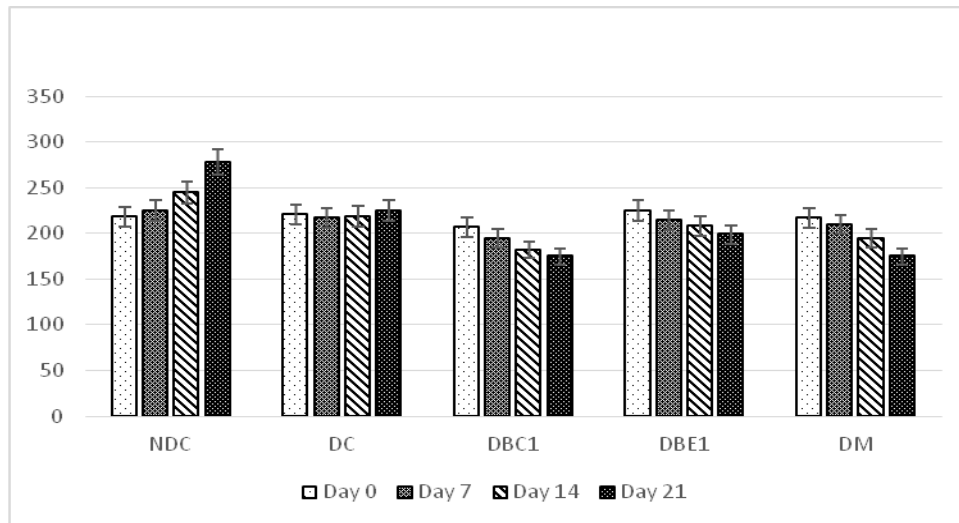


Fig. 1: Effect of CT and EO on mean body weight.

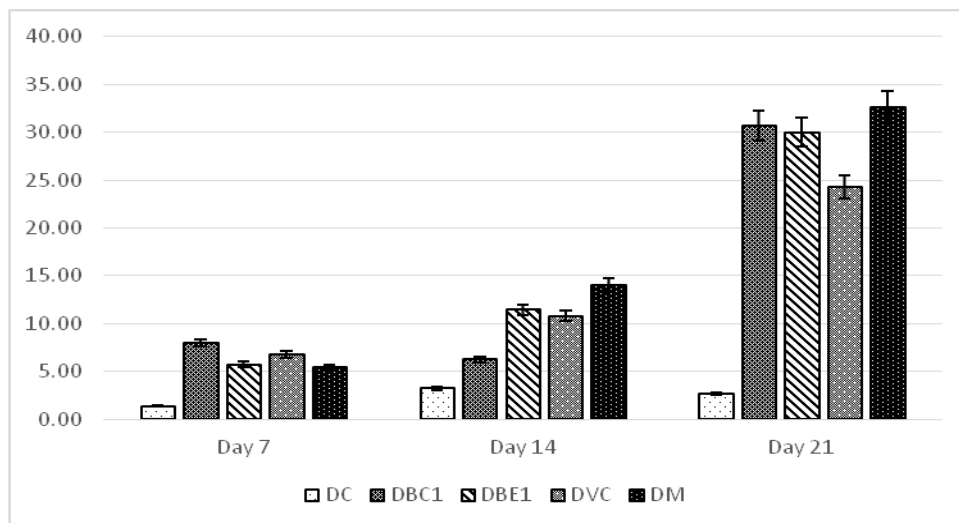


Fig. 2: Effect of CT and EO on sugar levels in diabetic groups.

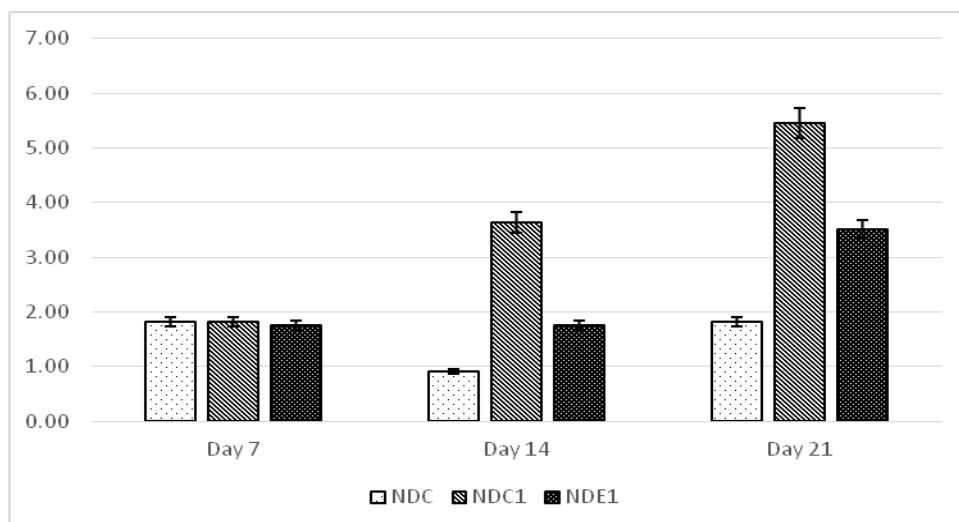


Fig. 3: Effect of CT and EO on sugar levels in Non-diabetic groups.

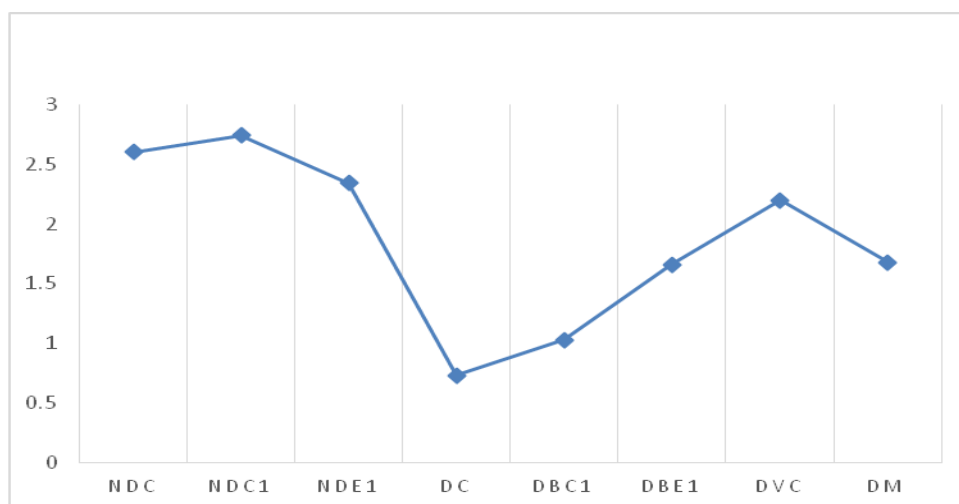


Fig. 4: Effect of CT and EO on GSH.

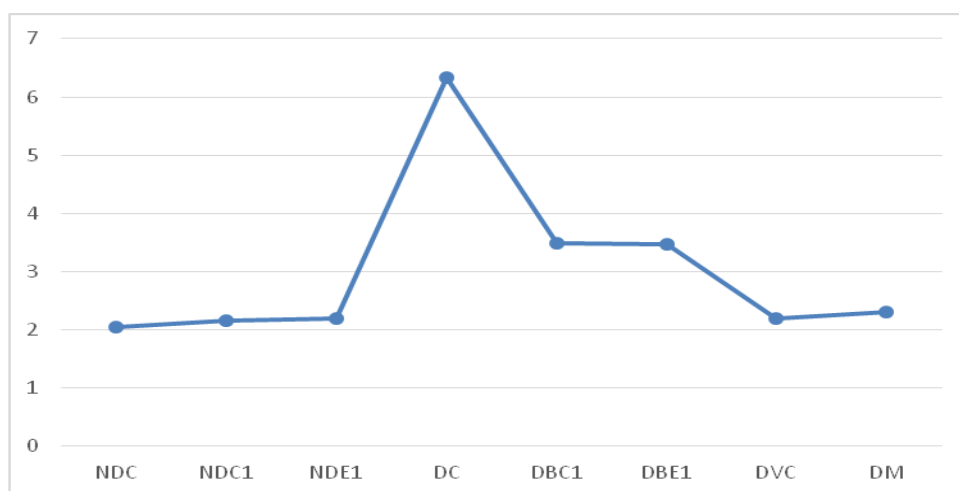


Fig. 5: Effect of CT and EO on MDA.

rat blood. The CT and EO was found to possess antioxidant effect in a dose dependent manner.

Reduced glutathione:

GSH has a multifaceted role in antioxidant defense. It serves as both a cosubstrate for glutathione peroxidases' detoxification of peroxides and a direct scavenger of free radicals. GSH levels of experimental diabetic animals treated with alcoholic extract of *CT and EO* showed significant decline than control groups (Fig. 4) ($P < 0.05$).

Malondialdehyde:

Oxidative stress biomarker malondialdehyde (MDA) was measured in plasma homogenates of control, untreated diabetic and diabetic animals

treated with reference drug and experimental groups treated with alcoholic extract of *CT and EO*. In the plasma of DC rats, lipid peroxidation levels as evidenced by MDA determination increased significantly as compared to normal control group ($p < 0.05$). In diabetic rats treated with metformin and vitamin C a significant decrease in MDA was observed (Fig. 5). In diabetic rats extract of CT and EO treatment significantly inhibited the increase in MDA.

Superoxide dismutase (SOD):

SOD was assessed in plasma of control and experimental diabetic animals treated with alcoholic extract of CT and EO which showed

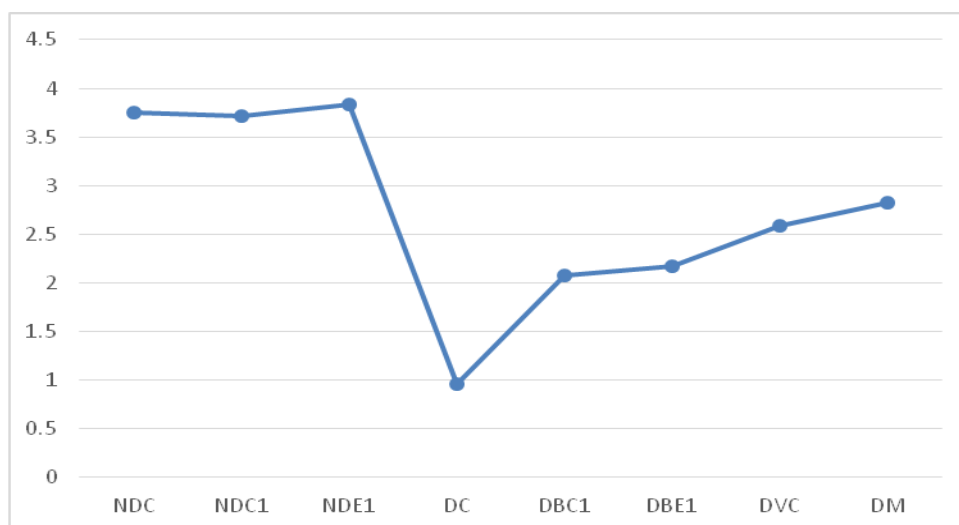


Fig. 6: Effect of CT and EO on SOD.

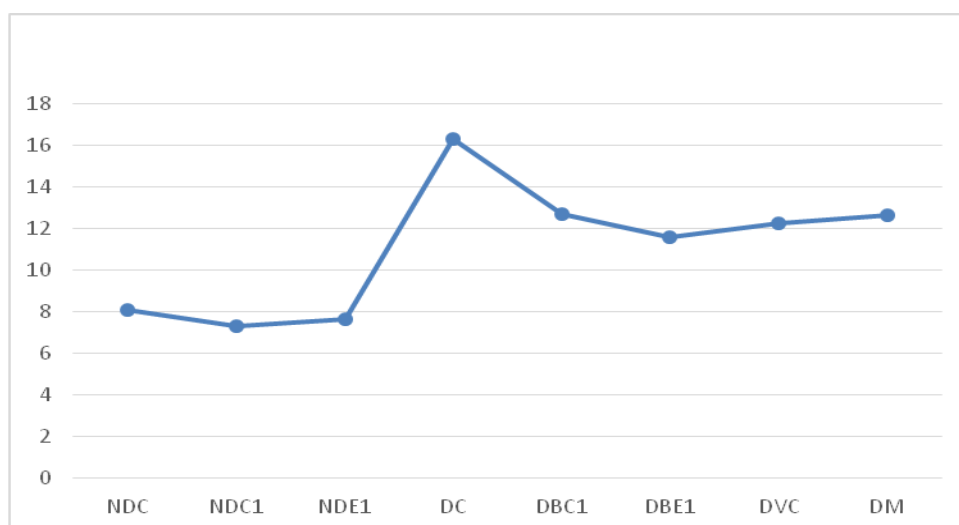


Fig. 7: Effect of CT and EO on GSH-Rd.

significant decline than normal control groups (Fig. 6) ($P < 0.05$).

Glutathione Reductase (GSH-Rd):

In the plasma of DC rats, elevated GSH-Rd increased significantly as compared to normal control group ($P < 0.05$). In diabetic rats treated with metformin and vitamin C a significant decrease in MDA was observed (Fig. 7). In diabetic rats extract of CT and EO treatment significantly inhibited the increase.

Discussion

Many people in underdeveloped nations use

medicinal herbs as an alternate form of treatment. Numerous herbs are employed traditionally in India to treat diabetes mellitus. Only a few of these Indian medicinal plants have been thoroughly studied. Therefore, the goal of the current study was to investigate the antioxidant potential and hypoglycemic effects of CT and EO extracts against streptozotocin-induced diabetic rats. The results showed a significant ($P < 0.05$) decrease in blood glucose levels in the diabetic rats, which is comparable to that of the normal and diabetic control groups.

When the various phytochemical components

of the extracts of CT and EO were qualitatively examined, it became clear that the extracts of all of the aforementioned plants contained a wide variety of active chemicals. The CT and EO plants have become a very significant medicinal plant as a result of the presence of several potent secondary metabolites in the extracts.

The diabetic rats had significantly higher blood glucose levels than the non-diabetic rats during the oral glucose tolerance test. The decreased glucose tolerance in diabetic rats was significantly improved by oral administration of CT and EO at a dose of 100 mg/kg. In light of the aforementioned finding, the plant's hypoglycemic effects may derive from its insulin-like action, which involves working at the peripheral level to boost cellular glucose absorption or glycogenesis.

Several plants have been found to have hypoglycemic effects by causing insulin to release more readily, similar to metformin which modifies AKT signaling. Additionally, inhibiting AKT might improve peripheral glucose uptake. From the results it is assumed that the extract of CT and EO could be responsible for insulin sensitization and the observed restoration of metabolic activity.

Although diabetes and weight loss are linked, the effect of extract treatment on the diabetic group suggests that the effects of CT and EO may be attributed to an increase in gluconeogenesis and glycogenolysis as compared to the metformin group. Diabetes is associated with oxidative stress and a decline in antioxidant status, which can exacerbate the harmful effects of free radicals. The main enzyme that eliminates free radicals is called SOD. In diabetic rats, reduced levels of these antioxidant enzymes have been found in the tissues of the liver, kidney, and pancreas. This activity may have a number of harmful effects due to the buildup of superoxide anion (O^-) and hydrogen peroxide (H_2O_2), which in turn produce hydroxyl radicals ($OH^\bullet$), which start and spread lipid peroxidation.

SOD protects against oxygen free radicals by hastening the removal of superoxide radicals,

which damage cellular membranes and structures. In the current study, diabetic rats treated with extracts had considerably greater SOD activity. As a result, the extracts may lessen the possibility of enzyme glycation or they may lessen reactive oxygen free radicals while enhancing the activities of antioxidant enzymes. This finding demonstrates categorically that CT and EO have a free radical scavenging activity that may protect against pathological changes brought on by the presence of superoxide and hydrogen peroxide radicals.

A tripeptide antioxidant found inside of cells, glutathione guards against the harmful effects of lipid peroxidation. It serves as both a co-substrate for glutathione peroxidases' detoxification of peroxides and a direct scavenger of free radicals. It's likely that a significant rise in the aldehydic lipid peroxidation products has increased oxidative stress and consequently lowered GSH content. By controlling the redox status of the proteins in the membrane, GSH protects the cell membrane from oxidative damage during treatment with CT and EO extracts.

The most common oxidative stress biomarker used in conditions like cancer, COPD, asthma, and cardiovascular illnesses is MDA. Due to the phospholipid degradation in cellular membranes, MDA created during this lipid peroxidation. One of the most prevalent reducing thiols in most cells, reduced glutathione is kept in supply by the enzyme glutathione reductase. Glutathione serves important roles in the cellular regulation of reactive oxygen species (ROS) in its reduced state. The best conditions for redox control within a cell or for activating programmed cell death are determined by complex interactions between levels of ROS, levels of oxidized and reduced glutathione and other thiols, and antioxidant enzymes like glutathione reductase. ROS act as intracellular and extracellular signaling molecules. The lowering of GSH-Rd and MDA levels after treatment with CT and EO extract is thought to have a cytoprotective effect.

The decrease in the quantity of lipid peroxidation end products, a reliable indicator of

oxidative stress, in the treated group has been shown to assist the recovery of antioxidant enzymes with CT and EO. This evidence suggests that the above-mentioned phytoconstituents may be the cause of the actions of CT and EO extracts.

Conclusion

The results of the present investigation, which suggest that CT and EO extract has strong antioxidant activity, may explain why it has hypoglycemic properties. In order to identify the active ingredients responsible for antidiabetic activity and to understand its mechanism of action, more pharmacological and biochemical studies are needed.

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