ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 3, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

EFFECT OF ETHANOLIC EXTRACT OF *CALOPHYLLUM INOPHYLLUM* LEAVES ON OXIDATIVESTRESS COMPLICATIONS IN MOUSE MODEL

VARSHA G[1], UMA MAHESWARI B[1], RAMASAMY M[1], KARUNANITHI M[2]*

[1]Department of Bioengineering, School of Chemical and Biotechnology, SASTRA University, Thirumalaisamudram, Thanjavur - 613 401, Tamil Nadu, India. [2]Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur - 613 401, Tamil Nadu, India. Email: karuna@carism.sastra.edu

Received: 09 February 2016, Revised and Accepted: 10 February 2016

ABSTRACT

Objectives: To find out the effect of the extract of *Calophyllum inophyllum* leaves on the streptozotocin-induced oxidative stress complications in mice.

Methods: The plant extract was first checked for its radical scavenging activity by the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method and L-ascorbic acid was used as the standard. DPPH assay activity of the plant extract was found to be close to the standard drug ascorbic acid. Acute toxicity was conducted as per OECD 425 guidelines. From the results obtained, 250, 300, and 350 mg/kg dose were chosen for further experimentation. After 16 days of drug treatment, glucose, cholesterol, triglyceride and enzymatic antioxidants levels were estimated in serum samples.

Results: *C. inophyllum* leaves extract has significantly reduced the glucose, cholesterol, triglyceride and enzymatic antioxidants levels. Hence, this proves that the plant has anti-diabetic property. However, vitamin E had no effect on the triglyceride level. Antioxidant activity was monitored by superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde assay and it was found that the plant extract has effectively increased the antioxidant activity as the dose increases.

Conclusion: *C. inophyllum* leaves extract have anti-diabetic activity and effective in curbing the oxidative stress complications.

Keywords: *Calophyllum inophyllum*, Oxidative stress complications, Anti-diabetic activity, Free radicals.

INTRODUCTION

Herbal medicine also is known as phytomedicine known to use part of plant or whole for medicinal purpose. Various parts of the plant are used for medicinal purposes in diverse forms such as capsules and in powder form. This turned out to be one of the safe and natural ways of treatment traditionally. Off late, the World Health Organization has found that nearly three-fourth of the population depends on herbal medicine for cure.

Oxidative stress is basically instigated by disparity in the production of levels of oxidants and antioxidants. The reactive oxygen species levels can be retained with the help of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). In the case of diabetes, the free radical level is increased as a result of increased peroxidation. One such byproduct of lipid peroxidation is malondialdehyde (MDA). Increased CAT activity is due to the high hydrogen peroxide ratio. This will prevent the SOD inactivation and that increases in SOD activity. The decomposition of hydrogen peroxide is enhanced by GSH-Px. In the diabetic state of an animal antioxidant enzymes and MDA levels are raised up. This causes damages to all cell components including DNA and lipids [1].

Various other factors, such as non-enzymatic protein glycosylation and rescued ascorbic acid levels, are also a reason for oxidative stress apart from the production of the free radical. Besides GSH, there are also other various other defense mechanisms against free radicals such as CAT, SOD, and GSH. Humans possess natural antioxidant defense system in their body to protect against free radicals production as a result of oxidative stress. The biological system consists of both endogenous and exogenous antioxidants [2]. *Calophyllum inophyllum* belongs to a family of mangosteen. Common name of this plant in Tamil is pinnay or punnai. The leaves when checked for their active compounds were identified as having phenols and hydrocarbons which exhibit antioxidant activity [3]. So far no scientific studies were performed in streptozotocin (STZ) induced diabetic in rodent. Hence, an attempt is being made to screen anti-diabetic potential for the above-mentioned plant extract.

METHODS

Preparation of ethanolic extract of *C. inophyllum*

The plant materials were collected from Kabaleeshwarar Temple, Chennai. The leaves were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. The cold extraction method was carried out in preparing the plant extracts. 100 g of powdered crushed leaves was mixed with 500 ml of ethanol in a flask. All the filtrate obtained from filtration was kept in refrigerator. A measure of 500 ml of ethanol was again mixed with the same leaf extract and left undisturbed for 24 hrs. The filtrate was then collected and stored in a refrigerator at the temperature of 0-5°C. The same procedure was performed for another time. The filtrates which were filtered are put together and placed it in a rotary evaporator to take away ethanol if present. The concentrated filtrate which then obtained was stored until the next process of the experiment [4].

Induction of diabetes

The experimental animals fasted overnight were given a single intraperitoneal injection of STZ, dissolved in citrate buffer 0.01 M, pH 4.5 at a dose of 55 mg/kg body weight. 5% glucose solution was mixed with the drinking water and given to the animals to surpass the drug- induced hypoglycemia. About 48 hrs post treatments, blood glucose levels were measured, and those mice which had a blood glucose level greater than 200 mg/dl were taken as experimental animals having diabetes. Treatment with our plant extract and standard drug was begun 48 hrs after STZ injection.

Experimental procedure

After induction of diabetes by STZ, the mice which showed diabetes were alienated into groups of six where each group has six animals each.

Group I (Control): Normal control (saline 0.9%)
Group II: STZ treated control (55 mg/kg)
Group III: STZ (55 mg/kg)+Vitamin E (400 mg/kg)
Group IV: STZ (55 mg/kg)+Plant extract (Dose I: 250 mg/kg)
Group V: STZ (55 mg/kg)+Plant extract (Dose II: 300 mg/kg)
Group VI: STZ (55 mg/kg)+Plant extract (Dose III: 350 mg/kg).

The experiment was continued for 16 days and blood was collected on the 17th day from the retro-orbital plexus under mild anesthesia from overnight fasted animals and fasting blood sugar was estimated. Serum was separated from blood by centrifugation at 3500 rpm for 15 minutes. It has been analyzed for enzymatic antioxidants and also for serum glucose, cholesterol, triglycerides. The kits for glucose, cholesterol, and triglyceride were purchased from Biosystems Diagnostics Pvt. Ltd, Tamil Nadu, India.

The radical scavenging activity of the plant extract was performed by 2,2-diphenyl-2-picryl hydrazyl hydrate (DPPH) method. IC_{50} value which is nothing, but the quantity of the extract required to inhibit the concentration of the free radicals by 50% was graphically determined by a linear regression method using MS-Windows based GraphPad InStat software.

The assay of copper-zinc SOD activity was determined by the procedure of Kakkar *et al*. [5]. The activity of GSH-Px was assayed by the method of Rotruck *et al*. [6]. MDA was estimated by the thiobarbituric acid assay by the method of Beuge and Aust [7]. Reduced GSH was estimated by the method of Moron *et al*. [8]. The activity of CAT was assayed by the method of Beers and Sizer [9].

Statistical analysis

All the data were expressed as a mean±standard deviation for six animals each in six groups. Moreover, the data were examined using one-way analysis of variance. All those values which are expressed showed significant ($p<0.05$).

RESULTS

DPPH radical scavenging or the antioxidant activity of leaf extract of *C. inophyllum* and ascorbic acid (taken as the standard) are presented in Table 1. The plant extract has expressed a noteworthy dose-dependent inhibition of DPPH. This influence of antioxidants on DPPH is due to its ability to donate hydrogen [10]. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 0.46 µg/ml and 0.26 µg/ml, respectively. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

The serum biochemical parameters such as glucose, cholesterol, and triglyceride are given in Table 2. The values of glucose, cholesterol, and triglyceride were reduced significantly in groups treated with plant extract compared to the negative control which is STZ treated. The standard vitamin E also showed a reduction in glucose and cholesterol level. However, vitamin E showed no significant reduction in triglyceride levels. Table 3 shows the effect of *C. inophyllum* extract on levels of SOD, CAT, GSH-Px, reduced GSH, and levels of MDA produced as a result of lipid peroxidation. It has been shown that groups treated with plant extract at the dose of 350 mg/kg increased levels of enzymatic antioxidants when compared to negative control and also the standard group treated with vitamin E also increased the levels of enzymatic antioxidants. Increased MDA levels are showed in the negative control group. Administration of plant extract showed a significant reduction in levels of MDA at the dose of 350 mg/kg.

It is observed that vitamin E taken as a standard as well as the three different doses of plant extract have played their roles in decreasing the level of MDA, in comparison to the STZ induced diabetic control group ($p<0.05$). However, the plant extracts have elevated GSH levels in the experimental animals on comparability with STZ induced diabetic control group ($p<0.05$). Vitamin E has also remarkably increased the GSH level.

Table 1: DPPH radical scavenging activity of plant extract and standard (ascorbic acid)

Concentrations (µg/ml)	Plant extract (% of inhibition)	Ascorbic acid (% of inhibition)
20	29±11.13	41±9.4
40	51.21±6.47	68.10±8.6
60	60.85±7.31	84.64±7.9
80	86.36±4.54	98.23±5.4
IC_{50} (µg/ml)	46	26.50
Correlation co-efficient (r)	0.9869	0.9866

Results were expressed as mean±standard deviation for triplicates.
DPPH: 2,2-diphenyl-2-picrylhydrazyl hydrate

Table 2: Effect of *Calophyllum inophyllum* extract on serum glucose, cholesterol and triglyceride levels

Group	Serum glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)
Control	83.25±18.51	121±20.07	117±41.39
Negative control	138.0±15.44	195±30.64	194.3±37.90
Vitamin E	102.0±15.87	115±16.82	151.2±11.29
Dose I	117.8±9.67	135±11.06	135±11.06
Dose II	95.25±10.70	124.2±9.51	90.75±10.77
Dose III	73.50±3.96	124±8.54	43.80±18.31

The values are expressed as mean±standard error

Table 3: Effect of *Calophyllum inophyllum* extract on enzymatic antioxidants

Group	MDA (mmole/dl)	GSH (mg/dl)	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)
Control	8.37±0.59	6.25±0.39	8.16±0.66	8.00±0.56	8.64±0.55
Negative control	12.33±0.88	2.50±0.15	4.76±0.38	4.66±0.33	5.78±0.36
Vitamin E	08.89±0.85	5.75±0.23	8.12±0.49	7.82±0.37	8.23±0.43
Dose I	8.81±0.63	6.25±0.39	7.48±0.60	7.33±0.52	6.26±0.40
Dose II	9.69±0.69	5.00±0.31	6.80±0.55	6.66±0.47	7.22±0.46
Dose III	8.81±0.58	7.50±0.47	8.16±0.66	8.64±0.61	8.67±0.55

The values are expressed as mean±standard deviation. MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

It was observed that there seems to be an elevation in SOD, CAT and GPx activities when compared to the STZ induced diabetic control group ($p<0.05$). Dose III of plant extract (350 mg/kg) has showed remarkable antioxidant activity. The effect of Vitamin E in increasing the SOD, CAT, and GPx levels has proved its well-recognized role as an antioxidant.

Influence of lowering levels of reactive free radicals and concurrent drop in the enzymatic antioxidants might enhance the membranes affinity to lipid peroxidation and cause pancreatic beta cell destruction apart from other related organ damage [11,12]. A high level of lipid peroxide tops the list of principal causes of diabetic complications [13]. Therefore, an ideal anti-diabetic drug should have hypoglycemic effects as well as antioxidant properties. MDA is formed as a result of lipid peroxidation and its produced under the influence of soaring levels of free radicals that are unscavenged and has high potential to cause damage to protein and most importantly inactivation of membrane-bound enzymes, thereby playing a significant role in the damage of pancreas associated as one of the consequence of diabetes [14].

In this study, MDA levels have shown an appreciable decrease in STZ induced diabetic mice after the ethanolic extract and vitamin E supplementation. This implies that the *C. inophyllum* extract either reduces the harmful oxygen free radicals generated or perks up the action of antioxidant enzyme. It also appears to play a considerable part

in preventing or bringing down lipid peroxidation. Hence, *C. inophyllum* extract selected in our study expressed a direct and a circuitous defensive and a protective effect on diabetes by lessening the impact of oxidative stress complications.

DISCUSSION

The induction of experimental diabetes in rodents using chemicals which selectively destroy pancreatic B cells is very convenient and simple to use. STZ is widely used as a diabetogenic agent in rodents for screening antidiabetic activity of plant extract or phytochemicals [15]. STZ is taken up by pancreatic B cells via glucose transporter GLUT2 [16,17]. In this study, we have selected STZ for the induction of diabetes in rodents.

C. inophyllum leaves when checked for their active compounds were found to have phenols and hydrocarbons which exhibit antioxidant activity. Moreover, diabetic complications and its pathogenesis are substantially allied with oxidative stress and its severity causes cell damage. Hence, the present study aims at finding out the greater role of ethanolic extract of *C. inophyllum* leaves and its effects on oxidative stress complications in diabetic mice. No reports are available in literature highlighting this unique property of the plant and hence the investigation has been initiated in this study.

The serum samples obtained from the different animals from different groups were analyzed for serum glucose levels using the glucose kit in the autoanalyzer. It is evident from the graph that the plant extract has been really successful in reducing the serum glucose levels ($p<0.05$). Dose III at 350 mg/kg of the plant extracts has shown considerable reduction of serum glucose levels. This proves that the plant extract has anti-diabetic property. Vitamin E has also shown a decreasing effect on the glucose levels ($p<0.05$), which is comparable with previous study [18]. Hence, both vitamin E and the plant extract have proved effective in reducing the serum glucose levels in experimental animals. Serum cholesterol levels have been significantly reduced by the plant extract ($p<0.05$). However, vitamin E has been more effective in reducing the serum cholesterol levels. This can be assigned by the type of oxidation vitamin E inhibits. Vitamin E as an antioxidant, block only the oxidation by free radicals. This is one of the main reasons as to why vitamin E is unproductive against cholesterol oxidation as a result of other three types of oxidation [19]. Moreover, oxidation of low-density lipoprotein cholesterol in humans takes place over a prolonged period of time. But in studies, cholesterol oxidation is activated even before encountered by vitamin E, thereby inhibiting an ongoing oxidation. This could be the possible reason as to why vitamin E has reduced the serum cholesterol levels in the *in vitro* studies. It is observed from the graph that the plant extract has been effective in reducing the serum triglyceride levels ($p<0.05$) when compared with the STZ treated control. Vitamin E has not showed any remarkable effect in reducing the serum triglyceride levels. Dose III of the plant extract (350 mg/kg) has proved to be very effective in bringing down the serum triglyceride levels when compared to the other doses of the plant extract as well as vitamin E. All the values had a significance of $p<0.05$.

CONCLUSION

In conclusion, the current analysis proved that ethanolic extract of *C. inophyllum* leaves possess antioxidant activity and has exerted a protective effect on the STZ induced oxidative stress by providing a shielding effect to the organism by its ability to control the glycemic level and lipid peroxidation levels. The antioxidant potential of the plant extract can be attributed to its influence on the serum glucose, cholesterol and triglyceride levels. Hence, the plant extract is proved to hold oxidative scavenging effect mediated through glycemic and lipid control.

ACKNOWLEDGMENT

The authors are really thankful to School of Chemical and Biotechnology, SASTRA University for providing necessary lab facilities to carry out this work successfully.

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