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Original Article

EVALUATION OF ANTICHOLINERGIC, ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *OCHNA OBTUSATA* DC USING *IN VITRO* ASSAYS

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ABSTRACT

Objective: To study the antidiabetic, anticholinergic and antioxidant activity of leaf extracts of *Ochna obtusata* DC. using *in vitro* methods.

Methods: Alcoholic extract and a decoction of *Ochna obtusata* DC. leaf was screened for antidiabetic, anticholinergic and antioxidant activity by using *in vitro* methods. Total phenolic and total antioxidant capacity of *Ochna obtusata* DC. leaf extracts were quantified as gallic acid equivalents, using the spectrophotometric method.

Results: The ethanol extract and water decoction showed strong inhibition to 1,1-diphenyl-2-picrylhydrazyl radical (IC_{50} 0.3±0.01 µg/ml, 90±5.1 µg/ml), Nitric Oxide (IC_{50} of 43±1 µg/ml, 82±1 µg/ml) hydrogen peroxide (IC_{50} of 45±2.1 µg/ml, 92±1.3 µg/ml), hydroxyl radical (IC_{50} of 22±2.3 µg/ml, above 200), lipid peroxidation (IC_{50} of 4.37±0.5 µg/ml, 1.68±0.3 µg/ml)equivalents of gallic acid (EGA), whereas the Acetylcholinesterase (IC_{50} of 0.5±0.2, 40±2.12) and α-Glucosidase IC_{50} of 3.5±.04 µg/ml, 45±3.03 µg/ml against the standard drug (acarbose equivalents) respectively. The present investigation revealed that the alcoholic extract of *Ochna obtusata* DC (except lipid peroxide) is effective than water decoction.

Conclusion: The present observations suggest that *Ochna. obtusata* DC leaves may be considered as a new source for anticholinergic, antidiabetic and antioxidant constituents for therapeutic use.

Keywords: *Ochna obtusata* DC, Acetylcholinesterase, α-Glucosidase, Antioxidant

INTRODUCTION

The genus *Ochna* (Ochnaceae) represented by more than 80 species distributed in tropical Asia, Africa, and America [1]. *Ochna obtusata* DC. (Telugu: Kukkamogi, Erravuddi; English: Golden champak) is a medium-sized tree widely grown throughout the hilly tracts of South India [2]. It is one of the potential medicinal plants and used for the treatment of various ailments in Indian traditional system of medicine. The roots of *Ochna obtusata* DC. were used in the treatment of menstrual complaints, asthma, constipation, poisonous bites, and also showed very strong analgesic and anti-inflammatory activity [3-4]. The stem bark was used for bone fractures, swellings, and as digestive tonic [5-8]. The leaves were used to cure, fever, inflammation, cholera, dysentery, dry cough, bronchitis, sores, cancer, lumbago, snake bite, menstrual complaints, asthma and epilepsy [9-11].

In view of its wide importance in folklore as well as an Ayurvedic system of medicine, *Ochna obtusata* DC. has been undertaken for antioxidant and other biological studies using *in vitro* assays. Free radicals or reactive oxygen species are implicated in various immune-modulatory diseases have attracted great attention in recent years. They are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS) in the human body by various endogenous systems, when exposed to different physicochemical conditions or pathophysiological states [12]. Free radicals can adversely alter lipids, protein and DNA and have been implicated in the etiology of several human diseases like diabetes, oxidative stress including Alzheimer's disease (AD) [13].

Several experimental pieces of evidence support the involvement of free radicals in inducing diabetes, and mainly in the development of diabetic complications [14], in addition to cardiovascular diseases, autoimmune and neurodegenerative disorders, inflammations, etc [15]. Hence, antioxidants may offer resistance to oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, etc, and they prevent deadly diseases. Many commercial antioxidants namely, butylated hydroxyl toluene (BHT), L-Ascorbic acid and α-tocopherol are available, but they show side effects under certain conditions [16]. Several natural products from dietary components such as β-carotene, vitamin C, E and medicinal plant products serve as antioxidants which are believed to be non-toxic and less side-effects [17].

From the thorough review of the literature, it was noticed that *Ochna obtusata* DC.leaves show multiple beneficial activities which might be linked with antioxidant properties directly hither to not evaluated so far. Hence, the present investigation was carried out to evaluate the extracts obtained from the leaves of *Ochna obtusata* DC. for antioxidant, anti-acetyl cholinesterase and anti-α-glucosidase properties. To combat the side effects of synthetic drugs, the natural products especially, medicinal plants serve as a good resource for such beneficial activities. An acetylcholinesterase inhibitor (AChEI) or anti-cholinesterase is a chemical or a drug that inhibits the acetylcholinesterase enzyme from breaking down acetylcholine, thereby enhancing the level and duration of action of the neurotransmitter acetylcholine. Acetylcholinesterase inhibitors are used as weapons in the form of nerve agents, as insecticides and applied medicinally to treat myasthenia gravis (increase neuromuscular transmission), glaucoma, postural tachycardia syndrome, as an antidote to anticholinergic poisoning, reverse the effect of non-depolarising muscle relaxants, neuropsychiatric symptoms of Alzheimer's disease, particularly apathy, increases chances of lucid dreaming (by prolonging REM sleep), Lewy Body dementia and Parkinson's disease. AChEIs are primarily used to treat the cognitive (memory and learning deficits) symptoms of dementia. These symptoms are attenuated due to the role of acetylcholine in cognition in the CNS.

The report also suggests that AChEIs may attenuate psychotic symptoms (especially visual hallucinations) in Parkinson's disease in addition to treat cognitive impairments in patients with schizophrenia [18-24]. Alpha-glucosidase inhibitors used for diabetes mellitus type 2 which are oral anti-diabetic drugs which

prevents the digestion of carbohydrates (such as starch and table sugar) into simple sugars (monosaccharides). Hence, α-glucosidase inhibitors play an important role in controlling blood sugar particularly postprandial hyperglycemia [25]. So the present investigation finds importance in the treatment of several neurogenreic diseases, diabetes and in oxidative stress with *Ochna obtusata* DC. in addition to providing scientific validation of the traditional usage.

MATERIALS AND METHODS

Plant material

The leaves of *Ochna obtusata* DC.were collected from hilly tracts of Tirumala, Chittoor district, Andhra Pradesh, India. The voucher specimen (#27034) has been deposited in the herbarium at Sri Krishnadevaraya University (SKU), Ananthapuramu, India. The plant material was identified by using authenticated regional floras [26-30].

Preparation of plant extracts

The leaves were washed with distilled water and dried in the shade at room temperature. Ten grams of shade dried, pounded plant material was taken and soaked in the ethanol or distilled water and boiled carefully at 50° C for 3 h and the extracts were filtered and the filtrate was concentrated, stored at low temperature and used for the assays.

Quantification of total polyphenols

Total phenol content of leaf extracts was quantified using Folin-Ciocalteau reagent [31] gallic acid was used as standard drug. The ability to reduce the phosphotungstate-phosphomolybdate complex was considered and the resultant color was measured using gallic acid standard. The colorimetric method is based on the reduction by phenolics to blue colored products in alkaline conditions. Ten µl of alcoholic extract or decoction were taken and 125 µl of Folin-ciocalteau reagent was added. After 10 min 300 µl of 20 % aqueous sodium carbonate solution was made up to 1 ml with distilled water and the reaction mixture was incubated in dark for 2 h. The absorbance was recorded spectrophotometrically at 725 nm and the total phenol concentration (TPC) was quantified from the calibration curve prepared, in the same conditions with gallic acid (standard) and expressed in milligrams of gallic acid equivalent (GAE)/g of dry plant extract.

Total antioxidant capacity

For the total antioxidant capacity assay, 10 µl of the extract was mixed with1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid. The antioxidant capacity of extracts was estimated by the phospho molybdenum-reducing assay [32].

DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging activity

The free-radical scavenging activity of plant extracts was measured by the decrease in absorbance of a methanol solution of DPPH [33]. A stock solution of DPPH (33 mg/l) was prepared in methanol and 5 ml of this stock solution was added to 100 µl of the plant extract solution at a concentration of 1000 µg/ml. After 30 min, absorbance was measured at 517 nm and compared with the ascorbic acid as a standard (50µg/ml). Scavenging activity was expressed as the percentage inhibition.

Hydrogen peroxide scavenging activity

This was performed by the ferrous ion oxidation-xylenol orange (FOX) assay [34]. One volume of reagent 2 was added to 9 volumes of reagent I Reagent 1 (4.4 mM butylated hydroxytoluene (BHT) in HPLC-grade methanol) reagent 2 (1 mM xylenol orange plus 2.56 mM ammonium ferrous sulphate in 250 mM H_2SO_4) to make the working FOX reagent. The extracts of different concentrations were mixed with 10 µl of 40 mM H_2O_2 and incubated in dark for ten minutes, and 1 ml of xylenol orange reagent was added. The disappearance of color was considered as a positive reaction, and the test samples were spectrophotometrically read at 548 nm and compared with a standard antioxidant (vitamin C).

Hydroxyl radical scavenging activity

Hydroxyl radical (OH) scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by the Fe^{3+}–ascorbate-EDTA–H_2O_2 system according to the method [35] Arouma et al., 1987 O.I. Arouma, M. Grootveld and B. Halliwell, The role of iron in ascorbate-dependent deoxyribose degradation, *J. Inorg. Biochem.* (1987), pp. 289–299. Depending upon the use of EDTA, this method comprised non-site and site-specific scavenging of OH radicals. For non-site specific hydroxyl radical scavenging assay, the Haber-Weiss reaction mixture (1 ml) contained 2-deo-xyribose (10 mM), Fe(III) chloride (10 mM), EDTA (1 mM), and H_2O_2 (10 mM) without or with the test extracts (10–100 µg/ml) in 50 mM potassium phosphate buffer, pH 7.4. EDTA was replaced with the same amount of buffer in case of site-specific hydroxyl radical scavenging assay. The reaction was triggered by adding ascorbic acid (1 mM) which served as a reducing agent by reducing Fe^{3+} to Fe^{2+} ions and subsequent incubation of the mixture for 1 h at 37 °C. Solutions of Fe(III) chloride, ascorbic acid and H_2O_2 were prepared in distilled water just prior to use. To 1 ml solution of above mixture, TBA in 25 mM NaOH (1 ml, 0.5%) and TCA (1 ml, 10% w/v aqueous solution) were added. The mixture was heated for 90 min on a water bath at 80 °C and the amount of pink chromogen produced was spectrophotometrically measured at 532 nm. Ascorbic acid was used as a standard compound.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by spectro-photometric method [36, 37]. Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with a concentration of the extract (100µg) prepared in methanol and incubated at 25 °C for 30 min. A control reaction without plant extracts or standard compound but with an equivalent amount of methanol was taken has been considered as a complete reaction. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1%sulphanilamide, 2% phosphoric acid and 0.1% N 1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546 nm and percentage scavenging activity was measured with reference to standard (ascorbic acid).

Anti-lipid peroxidation assay in rat liver tissue homogenate

Preparation of liver homogenate

Normal albino rat of the Wistar strain (250 g) was sacrificed and perfused the liver with 0.15KCl and homogenized at 0–4 °C and centrifuged at 800×g for 15 min, and the clear cell-free supernatant was used for *in vitro* lipid peroxidation assay.

The protective effect of the extracts was determined by mixing different concentrations of the extracts with 0.15 M KCl and rat liver homogenate. Peroxidation was initiated by adding 100 µl of 10 mM ferric chloride. After incubation at 37 °C for 30 min., lipid peroxidation was monitored by the formation of thiobarbituric acid reactive substances (TBARS). The amount of malondialdehyde formed was estimated by spectrophotometrically at 548. TBARS were estimated by adding 2 ml of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.5% TBA, and 0.5% butylated hydroxytoluene (BHT) to the reaction mixture, followed by heating at 80 °C for 60 min.

AChE inhibition assay

Purified enzyme Acetylcholinesterase, obtained from Sigma-Aldrich Ltd. (type III, lyophilized powder, 425 U/mg, 687 mg/protein from the brain of electric eel) was dissolved in 1 ml of PBS buffer (50 mM, pH–8) to make 100 U/ml stock solution and further diluted with buffer to get 0.1 U/ml enzyme and used for inhibition studies. Assay based on Ellman's reagent, was modified to measure the acetylcholinesterase inhibition. The assay contained 1 ml of mixture of 0.25 mM Acetylthiocholine Iodide and 0.25 mM DTNB in 50 mM sodium phosphate buffer pH 8 and 0.1U of AChE and incubated for 20 min. The final volume was adjusted to 3 ml with 50 mM sodium

phosphate buffer pH 8. The enzymatic reaction of AChE was the hydrolysis of the acetyl group of ASCh and gave thiocholine (SCh) as the product and reacts with DTNB to form 5-thionitrobenzoate, a colored anion, which absorbed at 412 nm. The 1 µl of 0.1 U/ml AChE in 50 mM PBS buffer (pH 8) was added to the *Ochna obtusata* DC. Extracts in different concentrations and incubated for 10 min and 20 µl substrate was added, the absorbance was taken after 20 min. The effect of extracts on acetylcholinesterase activity (%) was calculated as the change in absorbance in a sample, when compared to the change in absorbance in blank wells (average, n=3). Spontaneous hydrolysis was subtracted from the rate of reaction [38-42].

α-Glucosidase inhibition assay

Plant extracts were pre-incubated with 10µg enzyme equivalents for 5 min and tested for activity of the enzyme by adding 100µl of the substrate, 0.1 mM ρ-nitrophenyl-α-D-glucopyranoside (*α-glucosidase*) and incubated at 35 °C for 10 min. The products were examined using spectrophotometrically [43-44]. The percent of inhibitions of the enzyme was calculated in relation to the activity without plant extracts (taken as 100%). Additional blank incubations with only plant extract but without enzyme source were also run. The final inhibitions shown by the extract were compared with the standard drug, acarbose. The concentration of the extract required to inhibit the activity of the enzyme by 50 % (IC_{50}) was calculated. Experiments were performed in duplicates, and IC_{50} values were calculated [45].

Calculation of scavenging activity

The percentage of scavenging/Inhibition activities of free radicals or target enzymes was calculated using the following formula:

$$\% \text{ scavenged free radicals} = [(A_0-A_1)/A_0] \times 100$$

where, A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the samples, and all the experiments were conducted for three times and the average taken and the standard error was calculated using Sigma stat 3.1 version and graphs were plotted using Sigma plot 9. The total antioxidant activity was compared to the ascorbic acid equivalents using standard graph.

RESULTS AND DISCUSSION

Total phenol and antioxidant assay

The results of the present investigation illustrated that the extracts of *Ochna obtusata* DC., used for various human ailments, was proved to possess beneficial biological activities in the prevention of free radical generated diseases including α–glucosidase and acetylcholinesterase activities. The phenolic compounds were estimated in alcoholic and decoction water 400±1 and 420±1.5 mg GAE/dried leaf of *Ochna obtusata* DC. The total antioxidant activity of the extracts calculated based on the formation of molybdenum complex which measured spectroscopically at 695 nm and represented as equivalents of ascorbic acid (EAA). The total antioxidant capacity of the alcoholic extract or decoction was found to be 240±0.5 mg or 100.6±0.3 mg EAA/g plant material.

Based on the preliminary test, it was confirmed that the extracts were possessed a good quantity of phenols and better antioxidant activity in ethanol as well as decoction. Hence, the extracts were further evaluated for pharmacologically important and specific radicals and the scavenging effects on Reactive Oxygen Species (ROS), Lipid Peroxides (LPO) and Reactive Nitrogen Species RNS including inhibitory studies of Acetyl cholinesterase (AChE) and α-glucosidase (AG).

Table 1: IC_{50} values* for scavenging of ROS and Acetylcholinesterase and α-Glucosidase inhibition activity of *Ochna obtusata* DC.extracts

S. No.	Name of the radical scavenged/enzyme inhibition	Alcoholic extract (µg/ml)	Decoction (µg/ml)	Standards
1	DPPH radical	0.3±0.01	90±5.1	2.5±2.3[a*]
2	Hydrogen peroxide	45±2.1	92±1.3	15±1.03[a*]
3	Hydroxyl ion	22±2.3	>200	25±0.9[a*]
4	Nitric Oxide	43±0.86	82±1.2	17±1.06[a*]
5	Anti-Lipid peroxide	4.37±0.5	1.68±0.3	6±0.04[a*]
6	Acetyl cholinesterase	0.5±0.2	40±2.12	7.5±2.03[b*]
7	α-Glucosidase	3.5±.04	45±3.03	17.5±1.9[c*]

*IC_{50} values calculated as mean of triplicates, represented in micrograms of gallic acid equivalents of plant extracts. a, Ascorbic acid; b, Syldenaphyl citrate; c, Acarbose

All the assays were carried out in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). The differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05.

DPPH scavenging activity

The test samples of *Ochna obtusata* DC.showed good DPPH radical scavenging activity. But the IC_{50} values from the respective graph elucidating that alcohol extract inhibited very strongly than decoction (fig. 1).

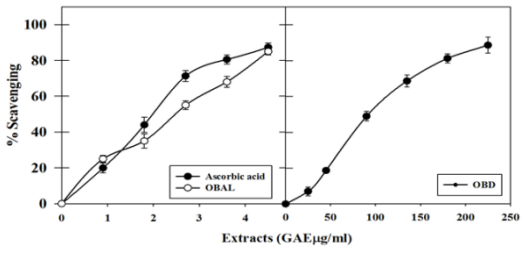

Fig. 1: DPPH radical scavenging activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata DC.*decoction (OBD). Standard compound, ascorbic acid was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

The IC_{50} values of alcohol extract, decoction and gallic acid are 0.3±0.01µg/ml, 90±5µg/ml and 2.52±2µg/ml respectively (table 1). This explains that alcohol extract is more potent in scavenging DPPH radicals generated *in vitro* than decoction and even commercial antioxidant compound (fig. 1).

Hydrogen peroxide scavenging assay

The leaf extracts executed a weak reducing capacity of extracts on the decomposition of H_2O_2 in a concentration-dependent pattern (fig. 2) at moderately high concentrations. Hydrogen peroxide is a weak oxidizing agent and can inactivate few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Two types of enzymes exist to remove hydrogen peroxide within cells namely catalases and the peroxidases which lead to ground state oxygen without any singlet oxygen. The extract might substantiate the enzymes during pathogenesis and helps in the decomposition of H_2O_2 which are believed to provide protection of health. In this event, it was noted that alcoholic extract with IC_{50} of 45±2µg/ml GAE was efficient over the decoction (IC_{50} of 92±1µg GAE) (table 1).

Hydroxyl radical scavenging assay

The ethanol extract and decoction of *Ochna obtusata* DC.leaves exhibited the scavenging activity of site-specific hydroxyl radicals in

concentration-dependent pattern. It is well observed that the ethanolic extract was the most active over the decoction as it was shown in fig. 3. In this assay, alcoholic extract with IC_{50} of 22±2 µg GAE was efficient over the decoction (IC_{50}–more than 200 µg GAE) in scavenging site-specific hydroxyl radical (table 1).

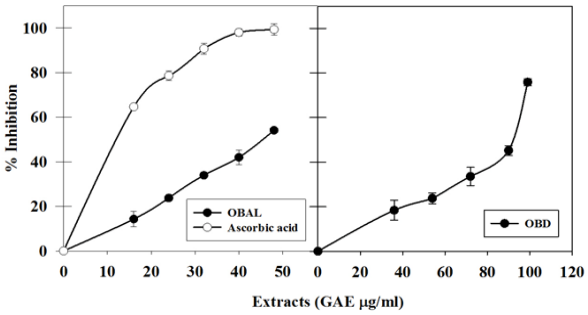

Fig. 2: Hydrogen peroxide scavenging activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD). Standard compound, ascorbic acid was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

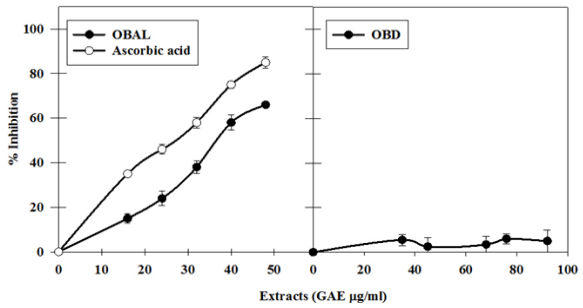

Fig. 3: Hydroxyl radical scavenging activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD). Standard compound, ascorbic acid was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

Nitric oxide (NO) scavenging

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell-mediated toxicity. It is a diffusible free radical which plays many roles as an effect or molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. It has been suggested that NO play important role in the pathogenesis of inflammation and bowel disease. Thus, the present study offers scientific evidence for the use of the plant in the indigenous system in inflammatory conditions. The alcoholic extract shown significantly more efficient inhibition on the production of nitric oxide in the *in vitro* system when to compare to decoction with IC_{50} of 43±1 and 82±1 µg/ml EAA respectively (table 1& fig. 4).

Anti-lipid peroxidation assay

Protection against free radical-induced lipid peroxidation of membranes by the extracts might be found application in the use against inflammatory diseases. The alcoholic extract and decoction of *Ochna obtusata DC.* inhibited the $FeCl_3$ induced lipid peroxidation. The hydroxyl radicals generated in this system is highly effective and can damage biological molecules when it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and results into lipid hydroperoxides.

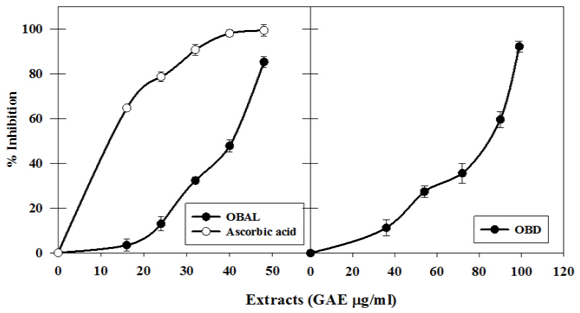

Fig. 4: Nitric oxide scavenging activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD). Standard compound ascorbic acid was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

Lipid hydroperoxide further decomposed into alkoxy and peroxy radicals, eventually triggers formation of carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases. In the present investigation the extracts were potentially prevented the formation of MDA which was detected by the formation of TBARS (thiobarbituric acid reactive substances). Thus the decrease in the MDA level with the increase in the concentration of the extracts indicates the role of the extract as an antioxidant (fig. 5). Interestingly the decoction has shown good lipid peroxidation inhibition with IC_{50} of 1.68±0.2µg GAE, rather than alcohol extract (IC_{50} of 4.37±0.4 µg/ml GAE) (table 1).

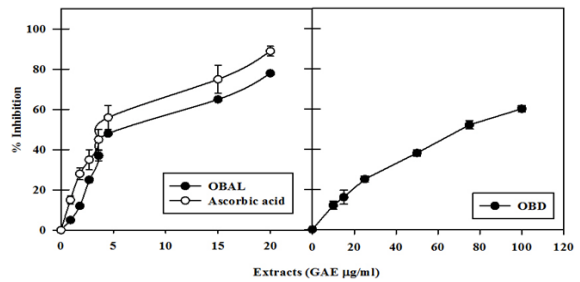

Fig. 5: Anti lipid peroxidation activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD) in rat liver tissue homogenates. Standard compound ascorbic acid was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

Acetyl cholinesterase inhibition assay

Acetyl cholinesterase inhibition activity

Alzheimer's disease (AD) is associated with significant losses in cholinergic neurons and decreased concentrations of the neurotransmitter, acetylcholine, which is involved in learning and memory processes. Acetyl cholinesterase inhibitors (AchEI) exert pharmacologic effects by increasing availability of intra synaptic

acetylcholine in the presence of intact cholinergic neurons by inhibiting the cholinesterase enzyme from breaking down acetylcholine, increasing both the level and duration of action of the neurotransmitter acetylcholine. They are used medicinally to treat myasthenia gravis (increase neuromuscular transmission), Dementia and as an antidote to anticholinergic poisoning in addition to AD. Their role in cognitive enhancement appears to be mediated through an anti-inflammatory effect, independent of their cholesterol-lowering properties. However, the 'cholinergic anti-inflammatory pathway' provides a physiological mechanism linking acetylcholine with inhibition of inflammation. Increasing evidence now points towards an anti-inflammatory role for AChEIs through action against free radicals or through decreasing release of cytokines from activated microglia in the brain and blood.

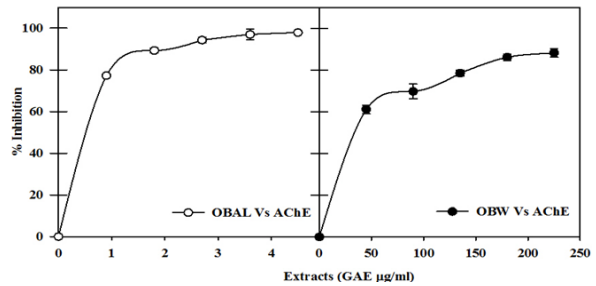

Fig. 6: Anti-acetyl cholinesterase activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD). Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

α-Glucosidase inhibition activity

Controlling blood sugar is a measure for the prevention of diabetes. Natural resources provide a huge and highly diversified chemical bank from which we can explore for potential therapeutic agents by bioactivity-targeted screening. α-glucosidase inhibitors inhibit the enzyme in the gut, which restrains the liberation of glucose from oligosaccharides and thereby reduces the postprandial glucose levels. In type II diabetic patients, it enhances the complexity of disease as it liberates glucose from carbohydrates [25, 46]. The present investigation revealed that the alcoholic extract *Ochna obtusata* DC. is strongly inhibited the activity of glucosidase with the IC_{50} of 3.5±0.41µg when to compare to decoction and standard α-glucosidase inhibitor (acarbose) with IC_{50} of 45±3.03 and IC_{50} of 17.5±1.9µg respectively (table 1 & Fig.7). Hence, the ethanol extract is highly beneficial as oral antidiabetic medicine.

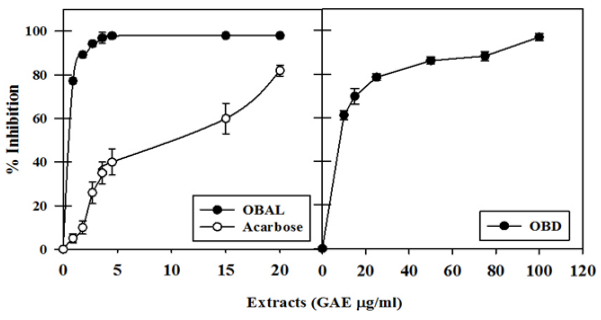

Fig. 7: α-Glucosidase inhibitory activity of *Ochna obtusata* DC. Alcoholic extract (OBAL) and *Ochna obtusata* DC.decoction (OBD) in rat liver tissue homogenates. Standard compound acarbose was used as drug control. Concentrations were mentioned as gallic acid equivalent (GAE) µg/ml. The experiments were conducted in triplicates and the differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with α≤ 0.05

The present study revealed that the extracts of *Ochna obtusata DC.* were highly significant in the inhibition of AChE (fig. 6) and proved its efficacy as a protective medicament. The alcoholic extract, as well as decoction, were efficient in inhibition of acetylcholinesterase with the IC_{50} of 0.5±0.2 and40±2.12 µg/ml of GAE (table 1) respectively.

CONCLUSION

The anti-oxidative property of *Ochna obtusata* DC.ethanol extract and decoction obtained from leaves were proved on various oxidizing radicals' viz., hydrogen peroxide, hydroxyl (site specific) and nitric oxide along with DPPH assay systems. Inhibitory activity of α-glucosidase plays key role in managing the type II diabetes and the leaf extract may help in the decreasing sugar level during postprandial condition. Acetylcholinesterase inhibition activity may help in controlling neurodegenerative diseases which might help in controlling Alzheimer's disease. Hence, the present investigation infers that the extracts can be used as a source of natural antioxidants with potential application to reduce oxidative stress with health benefits.

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CONFLICT OF INTERESTS

Declared none

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