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# Evaluation of anticataract potential of Waltheria indica in albino rats

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## ABSTRACT

Waltheria indica L., a member of Sterculiaceae family, is widely used as an Antioxidant traditionally to treat a variety of infections in humans. The present study was carried out to evaluate the Anticataract activity of Waltheria indica. The ability of Waltheria indica to tweak the biochemical parameters were explored in this study. Naphthalene cataract was actuated in rats by sustaining with naphthalene 1g/kg for 28 days. Rats in the test gathering were sustained orally with 100mg/kg and 200mg/kg of Waltheria indica every day for 28 days and rats in the control gathering just received the vehicle. In this study, morphological assessment demonstrated that Naphthalene treated gathering demonstrates increased opacities when contrasted with normal gathering. A fall in the glutathione level and a climb in the Malondialdehyde levels were seen in control rather than normal lenses. Administration of Waltheria indica significantly restored the glutathione and Malondialdehyde levels. SOD, Catalase and Glutathione S transferase levels were significantly restored to normal levels (p<0.05 and p<0.01 respectively). Oral administration of Waltheria indica significantly delayed the onset and progression of cataract in Naphthalene induced cataract. The Anticataract potential is evident from the slit lamp microscopic images. It can be said that the leaves Waltheria indica protected the lens against naphthalene damage which may be due its antioxidant activity.

Keywords: Naphthalene, Lipid peroxidation, Waltheria indica, Oxidative stress.

## INTRODUCTION

Visual debilitation because of the formation of visual lens cataract speaks to a huge wellbeing issue to the elderly worldwide<sup>1</sup>. Cataract is still a real reason for difficulty in seeing, representing 13 - 27% of the visually impaired individuals and medications that may have prophylactic or healing impacts for Cataract are lacking<sup>2</sup>. Oxidative stress has been ensnared in numerous age-related ailments of the eye, including cataractogenesis<sup>3</sup>.

Naphthalene is a white solid that is discovered characteristically in fossil energizes. Blazing tobacco or wood produce naphthalene. It is additionally utilized for making colors, saps, cowhide tanning specialists, explosives, greases and insect poisons. It is likewise utilized as the beginning material for the blend of different mixes, as moth repellent, soil fumigant and latrine antiperspirant. Most exposure happens through low dosage inhalation, skin contact or ingestion through the sustenance chain<sup>4</sup>. Ingestion of naphthalene can result in cataract in man and also in exploratory animals<sup>5</sup>. Also, cataracts affected by administration of naphthalene have been ascribed to oxidative stretch in ocular tissue<sup>6</sup>.

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It was recommended that naphthodiquinone, an oxidation result of ingested naphthalene, is the cataractogenic substance. The morphology of these cataracts has provoked some to recommend that trial naphthalene cataracts may be a decent model for human age-related cataracts<sup>7</sup>. The capacity to secure against the dangerous impacts of naphthalene by utilizing different antioxidants and free radical scroungers has been exhibited.

In light of the sufficient proof that oxidative stress assumes a part in the instruments of cataractogenesis, there is an expanding enthusiasm toward creating suitable antioxidant prevention agent supplements, both of engineered and plant origin that can be compelling in deferring or preventing the formation of cataracts<sup>8</sup>.

Lately, an extraordinary attention has been laid on investigating the likelihood of utilizing our natural assets to defer the onset and progression of cataract. A number of medicinal plants and their formulations are accounted for to have antioxidant properties and offer protection against cataract.

The present study was attempted to assess the Anticataract capability of *Waltheria indica* ethanolic concentrate in Naphthalene affected test model of cataract.

## MATERIALS AND METHODS

#### Collection and Authentication of the plant material

Leaves of *Waltheria indica* commonly known as Shengalipoondu (Tamil), Waltheria americana (Synonym: English) <sup>9</sup>, the plant was checked for data in www.plantlist.org with the following statement (This name is accepted name of a species in the genus Waltheria (family Malvaceae {sub family: Sterculiaceae}). The record derives from WCSP (in review) which reports it as an accepted name with original publication details: *Sp. Pl. 636 1753*<sup>10</sup>. The plant parts like leaves are used as an Antioxidant<sup>11</sup>, Anti-inflammatory<sup>12</sup>. The leaves of *Waltheria indica* were procured from Tirupati, Andhra Pradesh, India supplied and authenticated by Dr. K. Madhava Chetti, Assistant Professor, Department of Botany, Sri Venkateshwara University during the month of March 2013.

#### **Experimental Animals**

Wistar Albino rats weighing about (120-160gm) of either sex were obtained from animal house. The animals were maintain under standard condition i.e., housed in polypropylene cages and maintained at a temperature  $27 \pm 2^{0}$  C, relative humidity  $65 \pm 10\%$  under 12 hour light and dark cycle. The animals were acclimatized for 10 days under laboratory condition before carrying out the experiments. The animal house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number – (1330/AC/10/CPCSEA). The study was carried out after the approval by the institutional animal ethical committee (IACE)<sup>13</sup>.

## Chemicals

All the chemicals were Analytical grade. Naphthalene, Vitamin E and Ethanol were obtained from SD chemicals, Hyderabad.

Naphthalene (99%) was purchased commercially in the form of whitish water-insoluble crystals. It was broken down in warm corn oil in a 10% arrangement and offered orally to the rats for the entire span of the experiment<sup>14</sup>.

## Method of Preparation of Extract

The collected leaves were washed thoroughly under running water, cut into smaller pieces and air dried for eight days. Then the dried leaves were coarsely powdered using grinder and were continuous extracted in a soxhlet apparatus at  $30^{0}$ C with 2500 ml ethanol. The extract was filtered through a fine muslin cloth and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in amber colored glass bottle for further processing<sup>15</sup>.

#### Preliminary Phytochemical Screening

The solution of the methanolic extract was prepared using distilled water and subjected to preliminary phytochemical screening. Test for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Kokate, Khandelwal and Trease and Evans<sup>16-18</sup>.

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#### **Determination of Acute toxicity (OECD guideline 423)**

The acute toxicity for ethanolic extract of leaves of *Waltheria indica* was determined in albino rats following OECD guideline 423, maintained under standard conditions<sup>13</sup>.

#### **Incitement of Cataract:**

Cataract actuation was accomplished by oral organization of naphthalene solution (10%), which was prepared in warm corn oil by warming at  $60^{\circ}$  C for 30 minutes. This arrangement was directed orally by gavage at a measurement of 1g/kg to all rats aside from the control<sup>19</sup>.

#### **Evaluation of Anticataract Potential**

#### Naphthalene induced Cataract<sup>20</sup>

Wistar rats of either sex weighing 100 to 150 Gms were used, divided into 5 groups each comprising of 6 rats each.

**Group I**: Normal saline 5 ml/kg body weight.

Group II: Normal saline + Naphthalene 1g/kg (Negative control).

**Group III**: Vitamin E 50 mg/kg body weight, p.o. + Naphthalene 1g/kg.

**Group IV**: *Waltheria indica* ethanolic extract Test dose 1 + Naphthalene 1g/kg.

Group V: Waltheria indica ethanolic extract Test dose 2 + Naphthalene 1g/kg.

All the groups were treated for 28 days. Cataract was examined on the 28<sup>th</sup> day under slit lamp. On the next day lenses were removed from the eyes of all the rodents for estimation of Total protein, Catalase, Malondialdehyde (MDA), Glutathione, Glutathione peroxidase and Superoxide dismutase (SOD).

#### **Grading Stages of Cataract**

At the end of experimental period the degree of lenticular opacification was graded and photographed. The degree of opacification was graded as follows,

- Stage I- Lenses similar to normal lenses
- Stage II- Lenses showing faint peripheral opacity
- Stage III- Nuclear cataract
- Stage IV- Mature opacity involving entire lens

#### **Statistical Analysis:**

Results were expressed as Mean  $\pm$  SEM. Statistical analysis were performed with Graph pad prism software using one way Analysis of Variance followed by Dunnett's *t*-test.

P values were considered significant when \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 when the test and standard were compared with the untreated groups<sup>13</sup>.

#### **RESULTS AND DISCUSSION**

#### **Preliminary Phytochemical Analysis:**

The phytochemical screening of ethanolic extract of *Waltheria indica* leaves revealed the presence of alkaloids, flavonoids, sterols, terpenes, cardiac glycosides, saponins, anthraquinones and carbohydrates.

#### Acute toxicity studies

The acute toxicity studies of *Waltheria indica* ethanolic leaves extract was carried out as per OECD guideline no. 423. There was no gross evidence of any abnormality observed up to a period of 4-6 hrs or mortality up to a period of 24hrs at the maximum tolerated dose level of 2000 mg/kg body weight p.o. Further pharmacological screening were carried out with two dose ranges i.e. 100 mg/kg b.w. p.o. and 200 mg/kg b.w. p.o.

# Effect of *Waltheria indica* ethanolic extract (WIEE) on Naphthalene induced cataract: Effect on GSH, MDA levels and on Enzyme Activity

Administration of naphthalene resulted in a significant decrease of GSH levels in negative control group (44%) in comparison to normal control group (11.36 $\pm$ 0.29). Supplementation with Vitamin E and test extract WIEE 200 mg/kg significantly restored the GSH levels (p<0.001 and p<0.01 respectively).

The mean concentration of Malondialdehyde (MDA) in lenses of group II rats demonstrated an uncommon ascent of 84.1% in negative control group in examination to normal gathering. Vitamin E and WIEE 200mg/kg b.w, p.o altogether turned around the expanded MDA levels.

Glutathione peroxidase, Superoxide dismutase, Catalase and Total protein levels were significantly lowered. A significant restoration of the activities was found as a result of treatment with Vitamin E and WIEE 200mg/kg b.w, p.o.

Table 1: Effect of Waltheria indica ethanolic extract on GSH, MDA levels and on Enzyme Activity in Naphthalene induced Cataract

Groups	GSH (µmol/mg of protein) Mean± SEM	MDA (nmol/gm) Mean± SEM	SOD (IU/g) Mean± SEM	GPx (µmol/mg of protein) Mean ± SEM	Catalase (µmol/mg) Mean± SEM	Total Protein (mg/ml)
Group I (Normal)	11.36±0.29	53.37±1.16	$4.97\pm0.49$	49.17±1.17	8.96±0.36	$842.3 \pm 14.3$
Group II (Negative control)	5.11±0.36 <sup>a</sup>	$98.29 \pm 0.97^{a}$	$1.99 \pm 0.21^{a}$	23.39±0.72 <sup>a</sup>	4.17±0.22 <sup>a</sup>	279.1±13.3 <sup>a</sup>
Group III (Vitamin E)	10.99±0.11***	56.67±0.7**	3.68 ±0.33**	45.36±1.21**	8.11±0.42**	797.5±15.21***
Group IV (WIEE 200mg/kg)	7.86±0.27*	80.19±0.36*	2.52± 0.41*	30.63±1.33*	5.29±092	432.6±7.86**
Group V (WIEE 400mg/kg)	9.36±0.33**	63.39±1.23**	3.11±0.27**	38.99±1.11**	6.33±0.77*	616.7±8.02**

All the values are expressed as Mean  $\pm$  SEM (n = 6); a compared to normal group (p<0.001), significances values are \*\*\*p<0.001, \*\*p<0.01, \*p<0.05 (versus Negative control group)





Figure 1: Photographs showing the effect of Naphthalene on Lens Morphology



1. Normal Saline: Stage I clear lens



2. Control: Stage IV Mature opacity



3. Vitamin E: Stage II Faint opacity



4. WIEE 100mg/kg: Nuclear Opacity



5. WIEE 200mg/kg: Faint peripheral opacity

## Effect of Naphthalene on lens Morphology:

Cataract onset in Naphthalene fed rats in control group was observed on the 7<sup>th</sup> day. All eyes in the control group revealed cataractogenic changes. In WIEE 200mg/kg 35% exhibited normal eyes on day 7 whereas supplementation

in Vitamin E group rats had 77% clear lenses. By the end of experimental period i.e. on 30<sup>th</sup> day all the lenses in negative control group developed mature nuclear opacity, whereas Vitamin E treated group showed 45% clear lenses with 20% having stage II opacity. 18% lenses of WIEE 200mg/kg b.w, p.o exhibited clear nature, while 52% were stage I and the remaining 30% had fair nuclear opacity and faint peripheral opacity (Graph 2).

Cataract is a critical reason for visual weakness and of genuine visual impedance inciting two-sided trouble seeing in a normal 20 million people as far and wide as possible. In upcoming countries, the greater part of the visual debilitation is realized by Cataracts. Pharmacological treatment against human cataract has so far not been fulfilled. Hence, surgery to remove the opacified lens is the fundamental reasonable treatment for the cataract. The troubles are to expect or delay cataract formation moreover to treat over the long haul in case it happens. The accurate segment of cataract development is still not clear. There are studies to analyze the segment of Cataractogenesis using unique models of cataract and to target fundamental steps to stop this strategy. Among distinctive models, the selenite prompted and Naphthalene incited cataract model are a standout among the most by and large used test models.<sup>21</sup>

In the present study one of the mainly accessible plant *Waltheria indica* was chosen. Cataract was actuated by Naphthalene which is a normally utilized model as a part of screening of Anticataract medications. Glutathione (GSH), Malondialdehyde (MDA) levels and the antioxidant enzyme activity was assessed for deciding the Anticataract capability of the plant. Lens morphology was observed in the model to focus the adequacy of Vitamin E and the test concentrates.

The biochemical estimations showed decrease in the antioxidant enzyme levels such as SOD, Gpx, Catalase and total proteins and also a decrease in Glutathione levels in both models. But these enzyme levels were significantly restored to normal levels in Standard group (Vitamin E) and WIEE 200mg/kg b.w, p.o (p<0.001). Malondialdehyde levels showed a jump in negative control group. Treatment with standard and the test extracts reversed the levels of MDA in both the models.

Prevention or retarding oxidative damage to sulfhydryl groups in lens epithelium by WIEE 200mg/kg may be the mechanism behind the Anticataract potential of the plant. A decrease in the onset and a delay in progression of cataract in rats feeding on WIEE 400mg/kg were seen in Naphthalene fed rats.

Treatment with test extracts reduced the number of rats with mature nuclear opacity (stage IV) there by presenting a clear evidence of Anticataract potential of the plant. This effect may be associated with maintaining the antioxidant enzyme activities and decreased MDA levels. Our preliminary results are encouraging, but further molecular studies are needed to clarify the exact mechanism behind the anticataractogenic potential of the *Waltheria indica*.

## CONCLUSION

The phytochemical assessment indicated the presence of alkaloids, flavonoids, sterols, terpenes, cardiac glycosides, saponins, anthraquinones and carbohydrates. The acute toxicity studies of WIEE were carried out and no gross evidence of abnormalities or mortality were found in the rats even at a maximum tolerated dose level of 2000mg/kg b.w, p.o.

The study on the assessment of the Anticataract capability of *Waltheria indica* in exploratory animals showed that it tweaks the antioxidant parameters. It attenuates, defers the onset and progression of Naphthalene induced cataract, these biochemical changes reiterate the important role of oxidative stress in Cataractogenesis where the *Waltheria indica* ethanolic extract might be valuable for cataract treatment.

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