JOURNAL OF NATURAL REMEDIES

Antiglaucoma Activity of Aqueous Methanolic *Zingiber officinale* Extract on Carbomer Induced Glaucoma in Rabbits.

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Abstract

The present study was aimed to explore the anti-glaucoma activity of aqueous methanolic ginger extract (*Zingiber officinale*) against carbomer induced experimental glaucoma in rabbits. Aqueous methanolic extract of *Zingiber officinale* was orally administered to carbomer induced glaucomatous rabbits. Pilocarpine 2% eye drop was used as a standard drug. Intraocular Pressure (IOP) levels were determined after oral administration of a dose of *Zingiber officinale* (200 mg/kg, p.o.) in glaucomatous rabbits. IOP were determined for four weeks after oral administration of aqueous methanolic extract of *Zingiber officinale* (200 mg/kg, p.o). An aqueous methanolic extract of *Zingiber officinale* was found to reduce intra ocular pressure in carbomer induced experimental glaucoma in rabbits. Sufficient reduction in IOP was observed from second week of administration of ginger extract. A significant decrease in IOP ($p<0.01$) was observed in animals treated with standard pilocarpine and aqueous methanolic ginger extract. The effect of extracts of *Zingiber officinale* on serum pseudocholineterase was also measured. A significant decrease in the level of pseudocholinestrase ($p<0.01$) was observed in the rabbit serum treated with aqueous methanolic extract of ginger. Ginger is cheap, readily available, has beneficial effect on all the parts of body. Ginger lowers IOP in rabbits so can be used in human in the management of glaucoma. Based on the results obtained we suggest that after the complete phytochemical analysis and the individual pharmacological evaluation of phytoconstituents, ginger may be useful in improving the glaucoma condition.

Keywords: Aqueous methanolic extract, carbomer, intraocular pressure, *Zingiber officinale*

1. Introduction

Glaucoma is a disease where fluid pressure inside the eye increases causing irreversible damage to the optic nerve and loss of vision. It is often, but not always, associated with increased pressure of the fluid in the eye [1]. In most cases, this optic nerve damage is caused as a result of increased pressure within the eye although the damage may also be caused by poor blood supply to the vital optic nerve fibers, a weakness in the structure of the nerve, and/or a problem in the health of the nerve fibers themselves. While glaucoma is serious, if recognized and treated early, it can be controlled [2].

Glaucoma continues to be a major cause of irreversible visual disability all over the world. At present, there are approximately 7.5 million diagnosed cases of blindness, of which glaucoma accounts for 10-20%. It is estimated that there are about 2.47 million cases of primary open angle glaucoma in United States alone, the commonest type of glaucoma in the West [3].

The high intraocular pressure originates from an increased resistance to drainage of aqueous humor through the trabecular meshwork [4]. A sustained increase in aqueous humor may be due to an increase in the formation of aqueous humor, a difficulty in its exits, or a raised pressure in the episcleral vein. Several study

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showed that introduction of carbomer into the anterior chamber of eye produced best chronic glaucoma model in the rabbits [5].

Anterior chamber of eye is the best site of injection of carbomer for producing chronic glaucoma model [5]. Carbomer polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol [6].

Ginger (*Zingiber officinale*) belongs to Zingiberaceae family. The part of the plant used is rhizome. Ginger has been used as medicine from vedic period and is called "maha aushadhi", means the great medicine. The ginger plant is an erect perennial growing from one to three feet in height [7–9]. Fractured surface shows a narrow cortex, a well marked endodermis and a wide stele [10]. The oleoresin fraction of ginger rhizomes contains both volatile oils and nonvolatile pungent compounds. The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%), with lesser amounts of bisabolene and b-sesquiphellandrene.

Non-volatile components consist of pungent principles. The pungency of ginger is due to gingerol, an oily liquid consisting of homologous phenols [9]. In addition to the extractable oleoresins, ginger contains many fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain [11].

The purpose of this study is to find out the role of aqueous methanolic extract of ginger which exhibited prokinetic activity in rats via activation of post-synaptic muscarinic M3 receptor in rat stomach fundus. The study has postulated that ginger, is having a direct cholinergic agonistic effect on the post-synaptic M3 receptors [12]. Taking into consideration this observation we have hypothesized that the aqueous methanolic extract of ginger may act on the muscarinic receptors of the eye to produce miosis and subsequent drainage of aqueous humour leading to reduction in intraocular pressure.

2. Materials and Methods

2.1 Plant Material

The rhizomes of *Zingiber officinale* (Ginger) were collected from the local market of Aurangabad, Maharashtra and were authenticated from department of botany, Dr. Babasaheb Marathwada University, Aurangabad. Accession No. 0535.

The rhizomes were dried under shade at room temperature, coarsely powdered and packed in airtight container.

2.2 Preparation of Extract

The coarsely powdered ginger rhizomes were defatted with petroleum ether followed by extraction with 80% methanol and 20% water. The dried aqueous methanolic extract was formulated as suspension using distilled water and the strength of the suspension adjusted according to the dose administered (i.e. 200 mg/kg). The aqueous methanolic ginger extract was administered once daily for 4 weeks, in a dose of 200 mg/kg body weight, with the help of oral gavage.

2.3 Animals

Adult rabbits of either sex weighing between 1 to 2 kg were used. They were acclimatized for a period of 7 days at room temperature (25 ± 2°C) and 50 ± 15% relative humidity. They were housed in cages and maintained on standard pellet diet and water *ad libitum*. The study was carried out in the pharmacology laboratory of college. The study was carried out with the approval of Institutional Animal Ethics Committee (Proposal No. CPCSEA/IAEC/ P'col-15/2011-12/39).

2.3.1 Toxicity Studies

Acute toxicity study was carried using Rabbits by up and down staircase method as per OECD guidelines. The aqueous methanolic extract of ginger was orally administered to different groups of rabbits at the doses of 50, 300, 1000, 2000 and 3000 mg/kg body weight, respectively. The animals were observed for 48 hr to study the general behavior and for any sign of discomfort to the animals. There was no mortality found upto dose 3000 mg/kg.

2.3.2 Induction of Glaucoma

0.3% carbomer solution was prepared in the distilled water and solution was kept for some time in order to dissolve the carbomer particles uniformly. A single dose of 0.1 ml of 0.3% of carbomer was administered by intracameral injection into the anterior chamber of

right eye of each rabbit. The animals were having free access to standard pellet diet and water *ad libitum*. After 10 days all 25 animals showed elevated levels of IOP and were divided into 5 groups each comprising of five animals.

3. Experimental Design

Group I: Normal animals: received only normal saline (2ml/kg, p.o).

Group II: Toxic Control animals: Received carbomer + Normal Saline. (2 ml/kg, p.o.).

Group III: Standard Group animals: Received carbomer + Pilocarpine 2% Eye Drop topically.

Group IV: Received carbomer + Standard Pilocarpine 2% Eye Drop and 200 mg/kg, p.o. aqueous methanolic ginger extract.

Group V: Received carbomer + 200 mg/kg, p.o. aqueous methanolic ginger extract. In all the group the left eye was used as control for baseline.

3.1 Intraocular Pressure Measurement

The IOP was measured after 10 days of intracameral injection of carbomer using the Schiotz indentation tonometer (Eichtabelle, Germany) after instillation of the local anesthetic lignocaine hydrochloride 2% eye drops (Xylocaine 2% from AtraZeneca, India). In each eye, IOP was measured by using two weights (7.5 and 10 gms) and the average IOP was calculated for each measuring.

3.2 Biochemical Estimation of Cholinesterase Enzyme

It was done by a modified method of Elmann et al [13]. The rate of formation of thiocholine from acetylthiocholine iodide in the presence blood cholinesterase (true and pseudo) was measured by first treating with 5-5'bis-dithionitrobenzoic acid and then measuring the optical density of yellow coloured compound formed, during the reaction at 412nm, every min. for a period of 3 minutes.

After 10 days of intracameral injection of 0.1 ml of 0.3% carbomer, the drug treatment was carried out every day for a period of four weeks and IOP and pseudocholinetrase level were determined.

3.3 Statistical Analysis

All the values are expressed as mean ± SEM. The data of IOP obtained through careful observation were analyzed using Tukey test. Wherever required ANOVA followed by Dunnet's test was used. ** $p<0.01$ was considered statistically significant, respective to toxic control.

4. Results

Single intracameral injection of 0.1 ml of 0.3% of carbomer induced glaucoma and the antiglaucoma effect of aqueous methanolic extract of *Zingiber officinale* was established by assessing the intraocular pressure and level of pseudocholinetrase in rabbit serum.

4.1 Intra Ocular Pressure (IOP)

Measurment of IOP with 7.5 gm and 10 gm indentation was carried out using Schoitz tonometer before and after induction of glaucoma.

The IOP of the control group I have not changed throughout the experiment. Induction of glaucoma model in group II produced significant elevation of IOP to 34.4 ± 0.808 mm Hg and 35.85 ± 1.184 mm Hg after 2 and 3 weeks respectively. Animals of group III, IV and V treated with pilocarpine, pilocarpine + aqueous methanolic ginger extract, and aqueous methanolic ginger extract respectively, showed IOP values significantly lower than those of animals in the toxic group treated with carbomer only after 2 and 3 weeks. After 3 weeks, IOP values of groups III, IV and V were about same as that of normal saline treated group. The results were found to be similar with both the indentations 7.5 gm and 10 gm weight (Table 1 and 2).

4.2 Estimation of Pseudocholinetrase

Inhibition of pseudocholinesterase due to 200 µg/ml dose of aqueous methanolic ginger extract was found to be non significant. The inhibition of pseudocholinesterase by neostigmine and 400 µg/ml dose of aqueous methanolic ginger extract was about same, and 800 µg/ml of aqueous methanolic ginger extract was found to be more significantly inhibiting the enzyme pseudocholinesterase than by neostigmine. It indicates that inhibition of pseudocholinesterase by aqueous methanolic ginger extract was dose dependent Table 3.

Table 1: Effect of aqueous methanolic *Zingiber officinale* extract (200 mg/kg, p.o.) and Pilocarpine on intraocular pressure with 7.5 gm weight indentation in rabbits eyes in three weeks

Group	Treatment	Intra Ocular Pressure (IOP) (mmHg)			
		Initial	1st Week	2nd Week	3rd Week
I	Normal (Vehicle treated)	16.35 ± 0.904	16.67 ± 0.692	15.62 ± 0.551	15.95 ± 0.350
II	Control (Toxic)	34.62 ± .566#	34.72 ± 2.940#	34.4 ± 0.808#	35.85 ± 1.184†
III	Pilocarpine (2% eye drops)	33.75 ± 1.297	25.97 ± 1.738†	21.52 ± 1.103#	18.62 ± 1.288#
IV	Pilocarpine + Aqueous Methanolic Ginger Extract (200 mg/kg, p.o.)	30.00 ± 2.149	22.97 ± 1.564†	18.62 ± 1.288#	16.67 ± 0.692#
V	Aqueous methanolic ginger Extract (200mg/kg, p.o.)	32.40 ± 1.288	23.82 ± 0.796†	19.95 ± 1.818#	17.02 ± 0.592#

Variation of Intraocular Pressure (IOP) with time in right eye in different groups of animals. All values are expressed as mean ± SEM, n = 5. Data were analyzed by one way ANOVA followed by Tukey test. Comparisons were made with control toxic group vs. all groups # represents p<0.01 and † represents p<0.05.

Table 2: Effect of aqueous methanolic *Zingiber officinale* extract (200 mg/kg, p.o.) and Pilocarpine on intraocular pressure with 10 gm weight indentation in rabbits eyes in three weeks

Group	Treatment	Intra Ocular Pressure (IOP) (mmHg)			
		Initial	1st Week	2nd Week	3rd Week
I	Normal (Vehicle treated)	13.50 ± 1.222	13.4 ± 1.449	14.32 ± 1.425	14.27 ± 2.306
II	Control (Toxic)	37.40 ± 2.371#	33.0 ± 1.288#	31.3 ± 1.498#	32.0 ± 2.045†
III	Pilocarpine (2% eye drops)	40.75 ± 4.049	27.65 ± 3.327†	15.35 ± 1.747#	14.57 ± 1.206#
IV	Pilocarpine + Aqueous Methanolic Ginger Extract (200 mg/kg, p.o.)	43.65 ± 2.739	27.92 ± 4.017†	18.55 ± 2.818#	13.9 ± 1.022#
V	Aqueous methanolic ginger Extract (200mg/kg, p.o.)	43.65 ± 2.739	26.77 ± 3.806†	16.6 ± 1.185#	13.9 ± 1.022#

Variation of Intraocular Pressure (IOP) with time in right eye in different groups of animals. All values are expressed as mean ± SEM, n = 5. Data were analyzed by one way ANOVA followed by Tukey test. Comparisons were made with control toxic group vs. all groups # represents p<0.01and † represents p<0.05.

Table 3: Invitro pseudocholinesterase inhibitory activity of aqueous methanolic *Zingiber officinale* extract and neostigmine in rabbit's serum

Groups	Treatment	Pseudocholinesterase nmoles/min/mg of protein
I	Normal Control	9.78 ± 0.153
II	Standard (Neostigmine)	6.42 ± 0.168**
III	Aq. methanolic ginger extract (200 µg/ml)	9.24 ± 0.250
IV	Aq. methanolic ginger extract (400 µg/ml)	6.58 ± 0.128**
V	Aq. methanolic ginger extract (800 µg/ml)	5.60 ± 0.209**

Variation in levels of Pseudocholinesterases with different concentration of aqueous methanolic ginger extract and standard neostigmine solutions. All the results were expressed as Mean ± S.E.M, n = 5. All data were analyzed using one-way ANOVA followed by Dunnett's test.
** p<0.01 was considered statistically significant, respective to control.

5. Discussion

As carbapol is polymer, it affect outflow of aqueous humor facility by affecting trabecular meshwork. Xu et al [5] showed that the drug-induced changes of anterior chamber angle consisted of early inflammatory response and late fibrous changes. Inflammatory response occurred in early stage and reduced or disappeared after 3 weeks. Fibrous degeneration and adhesion obstruction occurred in the anterior chamber angle after 4 weeks. Under the electron microscope, the trabecular was expanded and deformed, with hyperplasia of collagen and elastic fibers. Endothelial cells were separated from the trabecular, and showed the morphology of lymphocytes, with the function similar to the macrophages. Phagocytized carbomer particles were transported through the vacuoles of Schlemm's canal endothelial cells. Large vacuoles gradually reduced. Excessive carbomer particles were accumulated in the

endothelial cells and obstructs the Schlemm's canal. This induces the fibrous proliferation and the destruction of anterior chamber angle structures [5].

Ginger (*Zingiber officinale*) is a universally known food plant reputed for its medicinal use in gastrointestinal disorders as a prokinetic and laxative [14]. Ghayur et al recently showed that 70% aqueous-methanolic extract of ginger exhibits prokinetic activity in rats via activation of post-synaptic muscarinic M3 receptor in rat stomach fundus. There results showed that ginger, is having a direct cholinergic agonistic effect on the post-synaptic M3 receptors [12]. Many studies showed that ginger stimulates muscarinic receptors. These are the receptors over which acetylcholine acts from the parasympathetic nervous system [15–17].

In the eye, multiple muscarinic receptor subtypes have been reported in the ocular surface, ciliary body, lens, retina, and sclera [18]. Studies have suggested that muscarinic receptors are involved in the regulation of eye development [19], corneal epithelial wound healing [20, 21], tear fluid and aqueous humor production [22, 23] iris and ciliary muscle contraction [24], lens cell signaling [25], and the regulation of scleral growth [26]. The ocular cholinergic system is also a pharmacologic target in the treatment of myopia [26].

In this study we confirm what it was hypothesized that the aqueous methanolic *Zingiber officinale* mimics the action of acetlycholine by binding to the muscarinic receptors located on the effector organs. As ginger is having stimulatory effect on muscarinic receptor in the GIT, it should also produce stimulatory effect on muscarinic receptors in the eyes similar to that of pilocarpine which is used in the treatment of glaucoma.

Our hypothesis was found to be proved that aqueous methanolic extract of *Zingiber officinale* may decrease intraocular pressure by causing contraction of the iris sphincter muscle causing miosis via M3 receptor. When the iris muscle of eye contracts, it helps in the drainage of aqueous humor from the anterior chamber of eyes. This may decrease intraocular pressure. Neostigmine increase acetylcholine levels by inhibiting Acetylcholinestrase (AChE) and Butyrlcholineestrase (BuChE). Thus, in the present study neostigmine was used to confirm the probable mechanism of action of aqueous methanolic extract of *Zingiber officinale*. The increased levels of acetylcholine facilitate neuromuscular transmission in skeletal muscle, decrease pupillary size, and increase aqueous humor outflow in the eye and may help in the reduction of raised intraocular pressure.

6. Conclusion

Several studies showed that ginger is having anti inflammatory activity, prostaglandin inhibitory activity, it also contains vitamin C, which are also contributing factors in the improvement of glaucoma condition. Hence on the basis of our study results we strongly suggest that after the complete phytochemical analysis and the individual pharmacological evaluation of phytoconstituents, ginger may be useful in improving the glaucoma condition. The probable mechanism of action may be its cholinergic activity through stimulation of muscarinic receptors of eyes.

7. Acknowledgement

We thank Hon'ble Padmashree Mrs. Fatma Rafiq Zakaria, Chairman, Maulana Azad Educational Trust and Society for providing the research facility. We thank to Dr. Thakur, Shivkala Netrarugnalaya, Aurangabad and Dr. Mundada, Optech Eye Care Hospital, Aurangabad for giving valuable guidance on glaucoma. We thank Mr. Bhikan Pathan for assisting in the experimental work.

References

1. Satoskar RS, Bhandarkar SD, Nirmala NR. Ocular Pharmacology in Pharmacology and Therapeutics. Mumbai: Popular Prakashan; 2008.
2. Kim MH, Esther VS, Hans GL. Cost-effectiveness of monitoring glaucoma patients in shared care: an economic evaluation alongside a randomized controlled trial. BMC Health Serv Res. 2010; 10:1–11.
3. Quigley HA, Vitale S. Models of open angle glaucoma prevalence and incidence in the United States. J Invest Ophthalmol. 1997; 38:83–91.
4. Barany EH. Physiological and Pharmacological Factors Influencing Resistance to Aqueous Outflow in Transactions of the First Conference on Glaucoma. New York: JJ Macy Foundation; 1955.
5. Xu Y, Chen Z, Song J. A study of experimental carbomer glaucoma and other experimental glaucoma in rabbits. Zhonghua Yan Ke Za Zhi. 2002; 38:172–75.

6. Florence AT, Jani PU. Novel Oral-Drug Formulations-Their Potential in Modulating Adverse-Effects. Drug Saf. 1994; 410:233–66.
7. Narayan DP, Purohit SS, Sharma AK. Medicinal Plants A to Z in Handbook of Medicinal Plants. Jodhpur: Agrobios India; 2004.
8. Rangari VD. Resins and Resin combinations in Pharmacognosy and Phytochemistry. Nasik: Career Publication; 2009.
9. Evans WC. Volatile oil and Resins in Trease and Evans Pharmacognosy. Delhi: Elsevier; 2006.
10. Wallis TE. Rhizomes and Roots in Textbook of Pharmacognosy. New Delhi: CBS Publishers; 2005.
11. Mohammad A. Natural Allergens in Pharmacognosy and Plant Cultivation. New Delhi: CBS Publishers; 2002.
12. Ghayur MN, Gilani AH, Ahmed T, Khalid A, Nawaz SA, Agbedahunsi JM. Muscarinic Ca^{++} antagonist and specific butyryl cholinesterase inhibitory activity of dried ginger extract might explain its use in dementia. J Pharm Pharmacol. 2008; 60:1375–83.
13. Ellman GL, Courtney KD, Andress VJR, Feather-Stone RM. A new and rapid colourimetric determination of acetylcholinestrase activity. Biochem Pharmacol. 1961; 7:88–95.
14. Singletary K. Ginger: An Overview of Health Benefits. Nutr Today. 2010; 45:171–83.
15. Pertz HH, Lehmann J, Roth-Ehrang R, Elz S. Effects of Ginger Constituents on the Gastrointestinal Tract: Role of Cholinergic M3 and Serotonergic 5-HT3 and 5-HT4 Receptors. Planta Med. 2011; 77:973–8.
16. Al-Azhary DB. Ginger Enhances Antioxidant Activity and Attenuates Atherogenesis in Diabetic Cholesterol-Fed Rats. Aus J Basic and Appl Sci. 2011; 5:2150–8.
17. Nietgen GW, Schmidt J, Hesse L, Honemann CW, Durieux ME. Muscarinic receptor functioning and distribution in the eye: molecular basis and implications for clinical diagnosis and therapy. Eye. 1999; 13:285–300.
18. Salceda R. Muscarinic receptors binding in retinal pigment epithelium during rat development. Neurochem Res. 1994; 19:1207–10.
19. Oztürk F, Kurt E, Inan UU, Emiroğlu L, Ilker SS. The effects of acetylcholine and propolis extract on corneal epithelial wound healing in rats. Cornea. 1999; 18:466–71.
20. Er H. The effect of topical parasympathomimetics on corneal epithelial healing in rabbits. Doc Ophthalmol. 1997; 93:327–35.
21. Nilsson SF. The uveoscleral outflow routes. Eye. 1997; 11:149–54.
22. Jumblatt JE, North GT, Hackmiller RC. Muscarinic cholinergic inhibition of adenylate cyclase in the rabbit iris-ciliary body and ciliary epithelium. Invest Ophthalmol Vis Sci. 1990; 31:1103–8.
23. Pang IH, Matsumoto S, Tamm E, De Santis L. Characterization of muscarinic receptor involvement in human ciliary muscle cell function. J Ocul Pharmacol. 1994; 10:125–36.
24. Collison DJ, Coleman RA, James RS, Carey J, Duncan G. Characterization of muscarinic receptors in human lens cells by pharmacologic and molecular techniques. Invest Ophthalmol Vis Sci. 2000; 41:2633–41.
25. Chua WH, Balakrishnan V, Chan YH, Tong L, Ling Y, Quah BL. Atropine for the treatment of childhood myopia. Ophthalmol. 2006; 113:2285–91.
26. Luu CD, Lau AM, Koh AH, Tan D. Multifocal electroretinogram in children on atropine treatment for myopia. Br J Ophthalmol. 2005; 89:151–3.