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EVALUATION OF FLUOXETINE AS AN ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTICATARACT

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ABSTRACT

Fluoxetine can also protect against environmental causes of free radicals such as smoking. Cigarette tar is a source of free radicals which has been found to damage erythrocyte membranes. It was also found that Fluoxetine and its conjugate metabolites could protect erythrocytes from the membranous damage that is caused by smoking. The ability of Fluoxetine is claimed to exert many beneficial effects on health, including protection against various diseases such as osteoporosis, lung cancer, and cardiovascular disease. The studies showed that there has been a reduction in the risk of cardiovascular disease in subjects, who had a high intake of flavonoids. Progressive disorder of the lung parenchyma and airways or also known as chronic obstructive pulmonary disease (COPD) which happens to be the third-leading cause of death in the USA. Therapies thus far for COPD, unfortunately, is said to be partially effective with possibilities of side effects.

KEYWORD: Anti-cataract, Anti-oxidant, Anti-inflammatory and Fluoxetine.

1. INTRODUCTION

Cataract is a clouding of the lens in the eye which leads to decrease in vision. Cataracts often develop slowly; symptoms may include blurry vision and trouble seeing at night. Cataracts are most commonly due to aging but May also occur due to trauma or radiation exposure, be present from birth, or occur following eye surgery for other problems. Prevention includes wearing sunglasses and not smoking. Early on the symptoms may be improved with glasses. If this does not help, surgery to remove the cloudy lens and replace it with an artificial lens is the only effective treatment. Surgery is needed only if the cataracts are causing problems and generally results in an improved quality of life. Cataract surgery is not readily available in many countries, which are especially true for women, those living in rural areas, and those who do not know how to read.

About 20 million people are blind due to cataracts. It is the cause of approximately 5% of blindness in the United States and nearly 60% of blindness in parts of Africa and South America. Blindness from cataracts occurs in about 10 to 40 per 100,000 children in the developing world, and 1 to 4 per 100,000 children in the developed world. Cataracts become more common with age. More than half the people in the United States had cataracts by the age of 80. Risk factors include diabetes, smoking tobacco, prolonged exposure to sunlight, and alcohol. The underlying mechanism involves accumulation of clumps of protein or yellow-brown pigment in the lens that reduces transmission of light to the retina at the back of the eye. Diagnosis is by an eye examination.[1]

1.1 Signs and symptoms

Signs and symptoms vary depending on the type of cataract, though considerable overlap occurs. People with nuclear sclerotic or brunescent cataracts often notice a reduction of vision. Those with posterior subscapular cataracts usually complain of glare as their major symptom. The severity of cataract formation, assuming no other eye disease is present, is judged primarily by a visual acuity test. Other symptoms include frequent changes of glasses and colored halos due to hydration of lens.

Oxidation is the process whereby an atom increases the number of bonds it has to oxygen, decreases the number of bonds it has to hydrogen, or loses electrons. The types of drugs that are affected include phenols (such as morphine), catechol amines (for example, adrenaline (epinephrine) and noradrenaline (norepinephrine)) as well as polyunsaturated compounds such as oils, fats and fat-soluble vitamins (e.g. vitamins A and E)[1]. Oxidation occurs when the oxidation state of a molecule, atom or ion is increased. The opposite process is called reduction, which occurs when there is a gain of electrons or the oxidation state of an atom, molecule, or ion decreases.[2]

Oxidation occurs when an atom, molecule, or ion loses one or more electrons in a chemical reaction.[3] Oxidation is complementary to reduction, i.e. oxidation and

reduction involve electron release and uptake processes, respectively. These electron transfer processes take place only to some extent with regard to redox processes involving covalent bonds such as in alcohols. Although covalently bound carbon is encompassed by the same numbers of electrons before and after its oxidation process, the oxidation state may change because electrons are regarded as belonging to the most electronegative atom involved in the bond.[4] At its most basic level, oxidation is the loss of electrons. It happens when an atom or compound loses one or more electrons. Some elements lose electrons more easily than others. These elements are said to be *easily oxidized*. Generally speaking, metals including sodium, magnesium, and iron are easily oxidized.[5]

The hydrolysis involves a two-electron transfer reaction, oxidation proceeds through one-electron transfer, i.e. free radical transfer reactions. Many drug substances and candidates exist in a reduced form, e.g. alcohols, alkyl benzenes, aldehydes, alkenes, amines, so that the presence of oxygen in the atmosphere may create oxidized degradation products.[6]

1.2 Physiology of cataract disease

Cataracts are changes in clarity of the natural lens inside the eye that gradually degrade visual quality. The natural lens sits behind the colored part of the eye (iris) in the area of the pupil, and cannot be directly seen with the naked eye unless it becomes extremely cloudy. The lens plays a crucial role in focusing unimpeded light on the retina at the back of the eye. The retina transforms light to a neurologic signal that the brain interprets as vision. Significant cataracts block and distort light passing through the lens, causing visual symptoms and complaints.

The term cataract is derived from the Greek word cataractos, which describes rapidly running water. When water is turbulent, it is transformed from a clear medium to white and cloudy. Keen ancient Greek observers noticed similar-appearing changes in the eye and attributed visual loss from "cataracts" as an accumulation of this turbulent fluid, having no knowledge of the anatomy of the eye or the status or importance of the lens. Cataract development is usually a gradual process of normal aging, but can occasionally occur rapidly.

Many people are in fact unaware that they have cataracts because the changes in their vision have been so gradual. Cataracts commonly affect both eyes, but it is not uncommon for cataracts in one eye to advance more rapidly. Cataracts are very common. Experts have estimated that visual disability associated with cataracts accounts for over 8 million physician office visits a year in the United States. This number will likely continue to increase as the proportion of people over the age of 60 rises.

When people develop cataracts, they begin to have difficulty doing activities they need to do for daily living or for enjoyment. Some of the most common complaints include difficulty driving at night, reading, participating in sports such as golfing, or traveling to unfamiliar areas.

1.3 Cataract Causes

The lens is made mostly of water and protein. Specific proteins within the lens are responsible for maintaining its clarity. Over many years, the structures of these lens proteins are altered, ultimately leading to a gradual clouding of the lens. Rarely, cataracts can present at birth or in early childhood as a result of hereditary enzyme defects, and severe trauma to the eye, eye surgery, or intraocular inflammation can also cause cataracts to occur earlier in life. Other factors that may lead to development of cataracts at an earlier age include excessive ultraviolet-light exposure, diabetes, smoking, or the use of certain medications, such as oral, topical, or inhaled steroids. Other medications that are more weakly associated with cataracts include the long-term use of statins and phenothiazine.

1.3.1 Types of Cataracts

All cataracts are fundamentally a change in the clarity of the overall lens structure; however, cataracts may result either early in life or as a result and different portions of the lens may be more affected than others.[7] Cataracts that occur at birth or present very early in life (during the first year of life) are termed congenital or infantile cataracts. These cataracts require prompt surgical correction or they may prevent the vision in the affected eye from developing normally. When the central portion of the lens is most affected, which is the most common situation, these are termed nuclear cataracts. The outside of the lens is called the lens cortex, and when opacities are most visible in this region, the cataracts are called cortical cataracts. There is an even more specific change that occasionally happens, when the opacity develops immediately next to the lens capsule, either by the anterior, or more commonly the posterior, portion of the capsule; these are called subcapsular cataracts. Unlike most cataracts, posterior subcapsular cataracts can develop rather quickly and affect vision more suddenly than either nuclear or cortical cataracts.

1.3.2 Cataract Symptoms

Having cataracts is often compared to looking through a foggy windshield of a car or through the dirty lens of a camera. Cataracts may cause a variety of complaints and visual changes, including blurred vision, difficulty with glare (often with bright sun or automobile headlights while driving at night), dulled color vision, increased near sightedness accompanied by frequent changes in eyeglass prescription, and occasionally double vision in one eye. Some people notice a phenomenon called "second sight" in which one's reading vision improves as a result of their increased nearsightedness from swelling of the cataract. A change in glasses may help initially once vision begins to change

from cataracts; however, as cataracts continue to progress and opacify, vision becomes cloudy and stronger glasses or contact lenses will no longer improve sight.

Cataracts are usually gradual and usually not painful or associated with any eye redness or other symptoms unless they become extremely advanced. Rapid and/or painful changes in vision are suspicious for other eye diseases and should be evaluated by an eye-care professional.

2. MATERIALS AND METHODS

2.1 In vitro Antioxidant activity

2.1.1 DPPH radical scavenging activity[8]

Procedure

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extract. The reaction mixture contained ml of different conc of ascorbic acid and 5 ml of 0.04%(w/v) solution of DPPH I n 80% methanol. After 30 min at room temperature, the absorbance was recorded at 517 nm using spectrophotometer (HITACHI U-1900 spectrophotometer 200V). The commercial known antioxidant, ascorbic acid was used as a positive control. The experiment was performed in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

DPPH scavenging effect (%) = [(A0-A1)] × 100

2.1.2 Scavenging of Hydrogen Peroxide[9]

Procedure

Scavenging of hydrogen peroxide. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentration of plant extract and standard ascorbic acid solution viz. 10,20,40.60,80 and 100 mg/ml in methanol (1ml) were added to hydrogen peroxide solution (2ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

The percentage inhibition activity was calculated using the following equation:

Scavenging activity (%) =[(A0-A1)/A0]×100

Where A0 is the absorbance of the control and A1 is the absorbance of extract/standard.

2.2 In vitro anti cataract activity

Procedure

1. Lens culture

A fresh goat lens were obtained from the slaughter house and immediately transported to the laboratory at 0-4˚C. The lens were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl 140mM, KCl mM, MgCl2 2mM, NaHCO3 0.5mM, NaHPO4 0.5mM , CaCl2 0.4mM and glucose 5.5mM) at room temperature and maintain pH 7.8 by addition of NaHCO3). Peniciline G 32% and streptomycin 250 mg% added to the culture media to prevent bacterial contamination. At high concentration glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols causing over hydration and oxidative stress. This lead to carctogenesis.

2. Induction of in vitro cataract[10]

Glucose at a concentration of 55mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataractogenesis. These lens were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55mM served as toxic control) for 72 hours.

3. Photographic Evaluation[11]

Lenses are placed on a wire mesh with the posterior surface touching the mesh, the pattern of mesh number of squares clearly visible through the lens was observed to measure lens opacity. The degree of opacity was graded as follows:

"0"– absence of opacity.
"1"– slight degree of opacity.
"2"– Presence of diffuse opacity.
"3"– Presence of extensive thick opacity

2.3 Anti-inflammatory models used

2.3.1 Rate paws edema Model.

Procedure

1. Standard drugs and test compounds were dissolved in minimum amount of dimethyl sulfoxide (DMSO) and diluted with phosphate buffer (0.2M, pH 7.4).
2. Final Concentration of DMSO in all solution was less than 2.5%.
3. Test solution (1ml) containing different concentrations of drug was mixed with 1ml of 1%mM egg albumin solution in phosphate buffer and incubated at 27o C+- 1o C in BOD incubator for 15min.
4. Denaturation was induced by keeping the reaction mixture at 60o C+- 10 o C in water bath for 10min.
5. After cooling the absorbance of turbidity was measure at 660nm on UV-visible spectrophotometer.
6. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The Fluoxetine was used as standard drug.

% inhibition of denaturation = 100 *(1- A2/A1)

Where,

A1= Absorption of control sample

A2 = Absorption of test sample

3. RESULT AND DISCUSSION

3.1 Antioxidant activity

In vitro antioxidant activity by DPPH radical scavenging activity and scavenging of hydrogen peroxide and reducing power assay, Fluoxetine as lasted at various concentration and the IC_{50} values had been determined

for each compound and compared with standard antioxidant. Ascorbic acid (AA) was used as the standard antioxidant.

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

Table no 3.1: Antioxidant activity by DPPH radical scavenging activity.

Conc.(mg/ml)	%free radical scavenging effect	
	STD(AA)	L-Carnitine
10	27.13+ 1.46	49.69+ 1.54
20	29.15+ 1.67	52.6+1.27
30	34.1+1.72	54.25+0.64
40	39.89+2.58	58.66+2.41
50	45.8+0.43	73.88+0.93
IC50	62.5	59.5

Values in parenthesis are expressed as mean +- S.D (n=3).

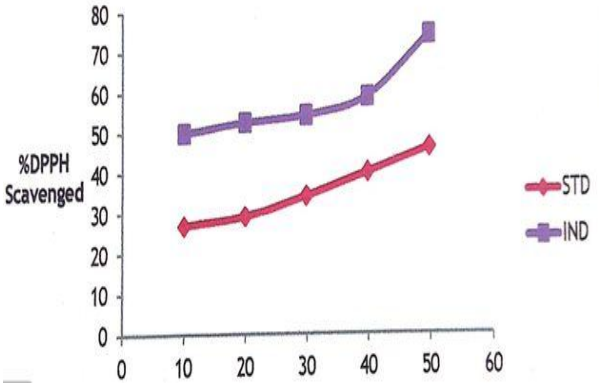


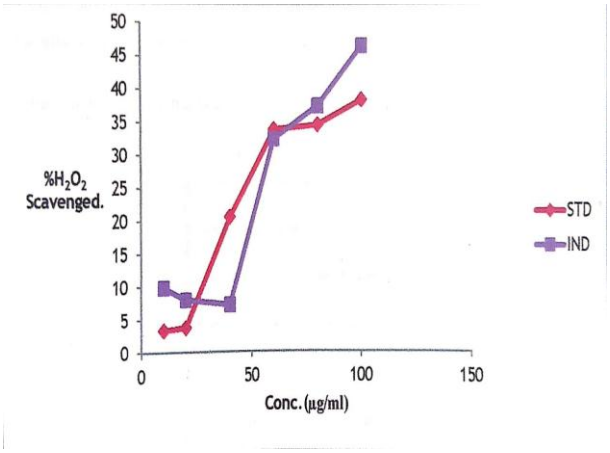
Fig. 3.1: Scavenging effect of test compound on DPPH radicals compared with Ascorbic acid.

3.1.2 Scavenging of hydrogen peroxide

Table no 3.2: Anti-oxidant activity by Hydrogen peroxide radical scavenging.

Conc.(g/ml)	% free radical scavenging effect	
	Std (Ascorbic acid)	Fluoxetine
10	3.36+1.61	9.84+1.12
20	3.77+1.27	7.98+1.26
40	20.6+1.17	27.21+1.28
60	31.97+1.23	32.51+1.46
80	34.57+1.92	37.51+1.67
100	38.44+0.70	46.58+1.41
IC_{50}	110	103

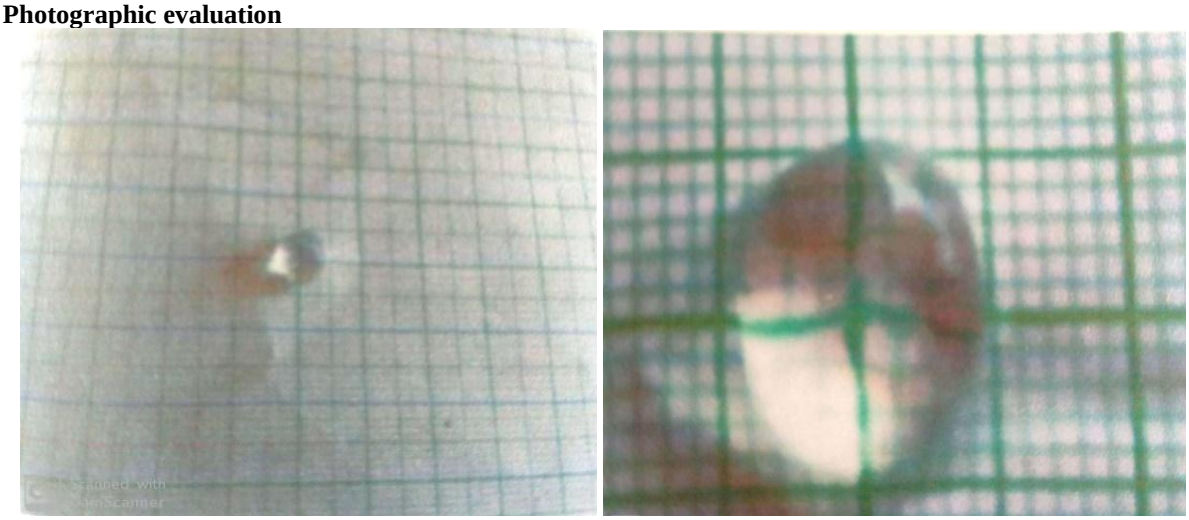
Activity. Values in parenthesis are expressed as mean +S.D (n=3).



Conc (µg/ml)

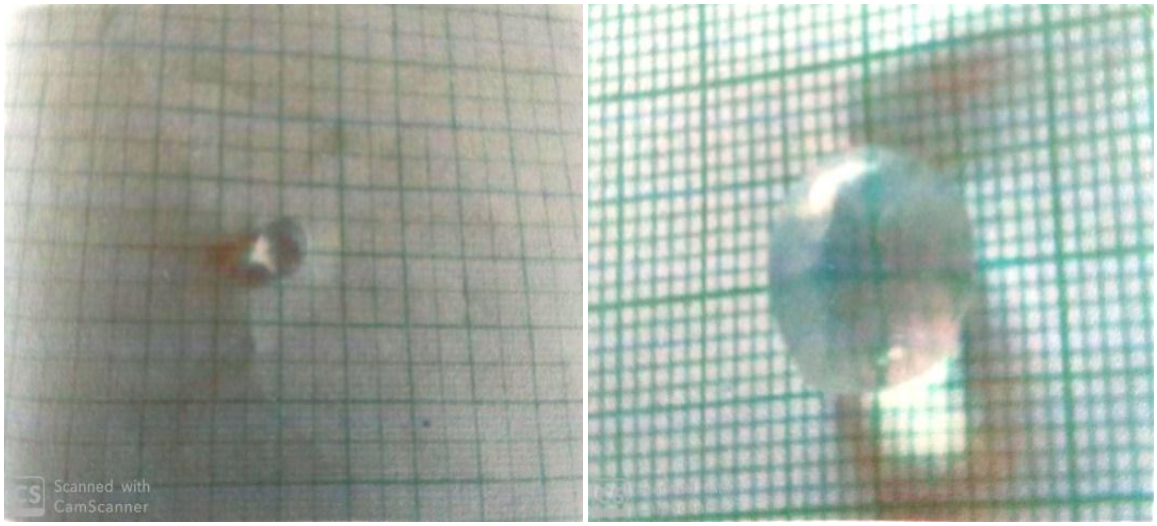
Fig. 3.2: Scavenging effect of the test compound on hydrogen peroxide radical compared with Ascorbic acid.

3.2 Photographic evaluation



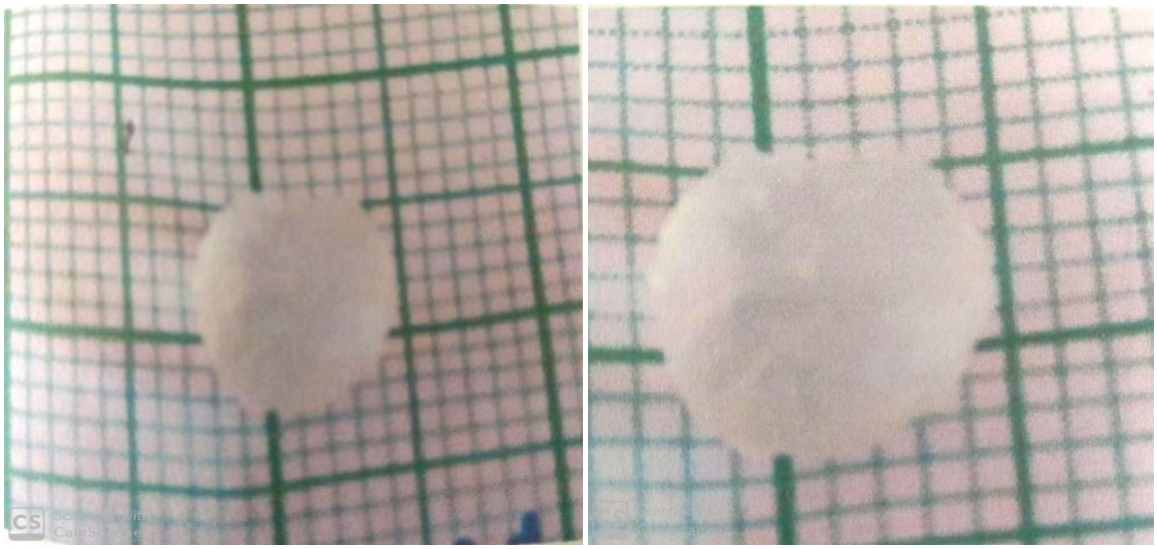
Normal view **Zoom view**

Fig. 3.3: (1) Aqueous humor only (Normal control).



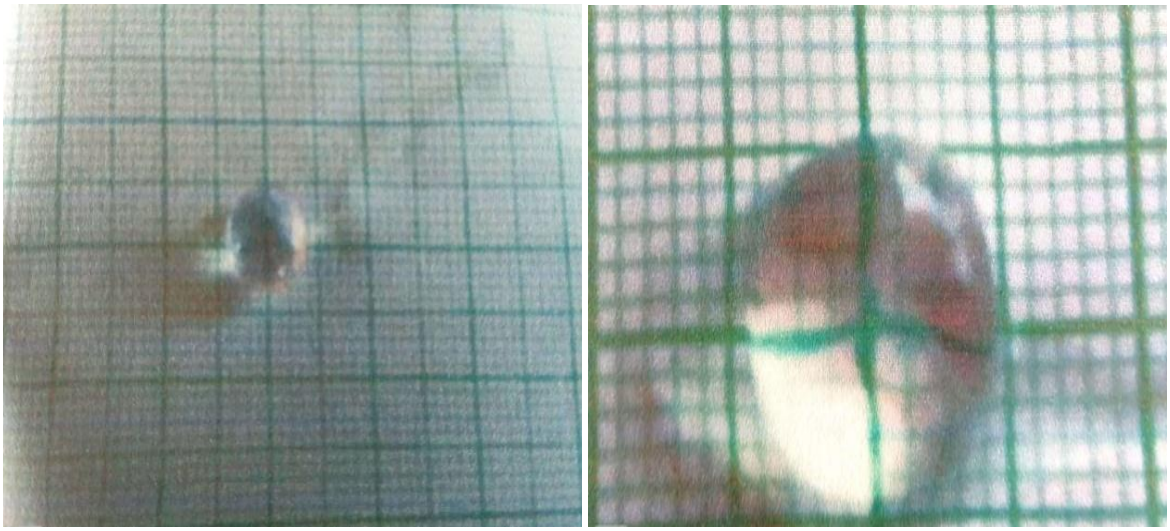
Normal view **Zoom view**

Fig 3.4: (2) Aqueous humor + 5.5 mm glucose (Negative control).



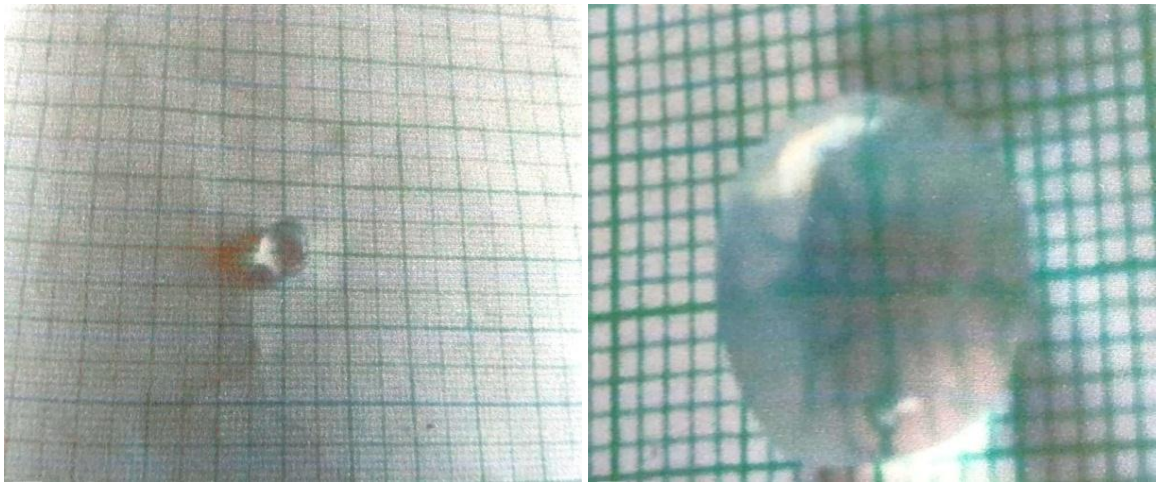
Normal view **Zoom view**

Fig 3.5: (3) Aqueous humor + 55mm glucose (Negative control.).



Normal view **Zoom view**

Fig 3.6: (4) Aqueous humor + 55mm glucose + 20 µg/ml Test compound.



Normal view **Zoom view**

Fig: 3.7: (5) Aqueous humor + 55mM glucose + 20 µg /ml std compound –(Positive control).

3.3 Degree of opacity shown by compound

Table 3.3: Degree of opacity.

Compound	Degree of opacity
Normal control	0
Negative control(a)	1
Negative control(b)	3
Positive control	1
Test (Fluoxetine)	0
Std(Ascorbic acid)	1

3.4 Estimation of total protein (TP)

Table No 3.4: Total Protein Content

Group	Dose	Total Protein Content
Normal control	------	103.6±8.89
Glucose control	55 mM	36.64±1.26
Std	20 µg/ml	86.22±3.12
Test 1	10 µg/ml	64.24±3.62
Test 2	20 µg/ml	82.22±3.72
Test 3	30 µg/ml	83.23±3.10

3.5 Anti-inflammatory activity

Table 3.5: Percentage Inhibition of Protein (%), IC50 ± SEM.

Conc of Drug[ppm]	5	10	15	20	25
Std Drug (Baicalein)	129 ± 0.24	140 ± 0.16	157 ± 0.45	194 ± 0.26	198 ± 0.14
test Drug (Fluoxetine)	110 ± 0.45	136 ± 0.63	151 ± 0.34	190 ± 0.18	191 ± 0.16

4. CONCLUSION

Fluoxetine is a flavonoid with antioxidant properties. The ability of Fluoxetine is claimed to exert many beneficial effects on health, including protection against various diseases such as osteoporosis, lung cancer, and cardiovascular disease. The studies showed that there has been a reduction in the risk of cardiovascular disease in subjects, who had a high intake of flavonoids. Flavonols is the most prominent flavonoids in fruits and vegetables and of these, Fluoxetine are the most commonly consumed in the human diet.

Although a wide number of drugs are available today for effective treatment of diabetes associated dyslipidemia, statins are gold standard drugs and the growing evidences of their pleiotropic effects establish their supremacy over other available lipid lowering agents, as they are most effective, best tolerated and can provide additional benefits like the antioxidant effect in diabetic cataract as evidenced in the present in vitro study.

Even though, in contrast to other workers' results, we did not find any activity of HMG-CoA-reductase in our experiments with goat lenses and also their incubation in a normal lens does not seem to be associated with an increased risk of cataract, the preventive role of statins in cataract was proved. With the increasing trend for initiating statin therapy among diabetics with or without hyperlipidemia, the need to assess the effect of long-term and high dose exposure on eye in various other animal models as well as clinical trials including post marketing surveillances remains.

5. ACKNOWLEDGEMENT

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