Quest Journals
Journal of Research in Pharmaceutical Science
Volume 9 ~ Issue 3 (2023) pp: 22-30
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Ocular Anti-Inflammatory Activity of *Linum Usitatissimum*

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ABSTRACT
The aim of present work was to evaluate the effect of ocular anti-inflammatory activity via aqueous extract of Linum usitatissimum (AELU) seeds.The inflammation induced by 0.25M sodium chloride Sol[n] was used. Guinea pigs were divided into five groups, the first group received vehicle topically, the second, third and fourth groups received 0.5%, 1% and 2% w/v of aqueous extract of Linum usitatissimum, topically, while the fifth group was administered flurbiprofen at 0.3% topically, used as the standard drug. The AELU of seeds possess significant ocular anti-inflammatory properties in a dose dependent manner. AELU of seeds at a dose of (1%, topically) exhibited significantly control the level of physical appearance, leucocytes counts, protein level and GSH level by 73%, as compared to the control group. The extract probably reduced the arachidonic acid consequently promoting the inhibition of leucocytes count. Thus,the data suggest that aqueous extract of Linum usitatissimum (AELU) seeds, are able to significantly retard experimentally induced ocular anti-inflammation.
KEY WORDS; *AELU, GSH, Flurbiprofen, Leucocytes count, Arachidonic acids*

***R**eceived 14 Mar., 2023; **R**evised 27 Mar., 2023; **A**ccepted 29 Mar., 2023 © The author(s) 2023.*
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I. Introduction

An inflammation of the eye generally occurs in response to viral or bacterial infection, allergies, environmental irritants, surgery or trauma. While most cases of eye inflammation are not too serious, it is important to control the severity of the inflammation as well as the duration to be sure to avoid any scarring and permanent damage. Because the eyes are such delicate organs, even a small amount of scar tissue can cause irreversible visual impairment.

Different kinds of ocular inflammation

There are many different types of eye inflammations depending on what area of the eye becomes inflamed, each condition differing in its symptoms and severity.

Conjunctivitis - commonly known as pinkeye, conjunctivitis is an inflammation of the conjunctiva which is the clear membrane that covers the outermost layer of the eye and the inner surface of the eyelids. Many causes are associated with this condition including bacterial and viral infections, allergies and eye irritants.

Episcleritis - an inflammatory condition of the episclera which is the connective tissue between the conjunctiva and sclera. The cause of episcleritis is uncertain.

Blepharitis – an inflammation of the eyelids, often as the result of poor hygiene, chronically dry eyes or oily skin.

Keratitis – an inflammation of the cornea region of the eye. This is often caused by bacterial or fungal infections and is increasingly prevalent in those with poor contact lens hygiene.

Uveitis – an inflammation of the eyeball which is generally considered to be one of the more serious forms of eye inflammation. There are also a number of types of Uveitis depending on what area of the eye ball is infected and these may include: Iritis, Cyclitis,
Retinitis and Choroiditis.

Scleritis - an inflammation of the sclera or white of the eye. When the uvea is inflamed near the front of the eye in the iris, it is called iritis. Iritis usually has a sudden onset and may last six to eight weeks; usually the eye is red and

painful.

The plant selected for the proposed study is *Linum usitatissimum.* **Flax** (also known as **common flax** or **linseed**) (binomial name: ***Linum usitatissimum***) is a member of the genus *Linum* in the family Linaceae.

Taxonomy (Kokate et al. 2007, Sirisha et al. 2010)

Kingdom : Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Rosidae
Order : Linales
Family : Linaceae
Genus : *Linum*
Species : *usitatissimum*

Components of flaxseed

Alpha-linolenic acid (ALA), cyanogenic glycosides (linamarin, linustatin, neolinustin), unsaturated fatty acids (linolenic acid, linoleic acid, oleic acid), soluble flaxseed fiber mucilage (d-Xylose, L-Galactose, L-Rhamnose, d-galacturonic acid), lignans (secoisolariciresinol diglycoside (SDG)), monoglycerides, triglycerides, free sterols, sterol esters, hydrocarbons (protein), balast, phenylpropane derivatives.

Components of flaxseed oil: Alpha-linolenic acid (ALA), unsaturated fatty acids (linolenic acid, linoleic acid, and oleic acid).

Components of flax root: The roots of *L. usitatissimum* contain measurable concentrations of lignans and isoflavones.It also includes acetic acid, chlorides of calcium, magnesium and potassium, fixed oil, linamarin, glucoside, mucilage, phosphate, resins, sugar, sulphates, wax etc.

Traditional and clinical uses

- Flaxseed is most commonly used as a laxative.
- Flaxseed is also used for hot flashes and breast pain.
- Flaxseed oil is used for different conditions than flaxseed, including arthritis.
- Both flaxseed and flaxseed oil have been used for high cholesterol levels and in an effort to prevent cancer.
- The below uses are based on tradition or scientific theories. They often have not been thoroughly tested in humans, and safety and effectiveness have not always been proven. Some of these conditions are potentially serious, and should be evaluated by a qualified healthcare provider.

Abdominal pain, acute respiratory distress syndrome (ARDS), allergic reactions, antioxidant, benign prostatic hypertrophy (BPH), bipolar disorder, bladder inflammation, blood thinner, boils, bowel irritation, bronchial irritation, burns (poultice), catarrh (inflammation of mucous membrane), colon cancer, cough (suppression or loosening of mucus), cystitis, depression, diarrhea, diabetic nephropathy, diverticulitis, dry skin, dysentery, eczema, emollient, enlarged prostate, enteritis, eye cleansing (debris in the eye), gastritis, gonorrhea, headache infections, inflammation, irritable bowel syndrome, liver protection, malaria, melanoma, menstrual disorders, ovarian disorders, pimples, psoriasis, rheumatoid arthritis, skin infections, skin inflammation, sore throat, stomach upset, stroke, ulcerative colitis, upper respiratory tract infection, urinary tract infection, vaginitis, vision improvement.

Pharmacological screening

(A) Ocular inflammation induced by alkali 0.25M Sodium Chloride(Vogel et al., 2007)

Guinea pigs were divided into five groups and three animals in each group. The first group received vehicle topically, the second, third and fourth groups received 0.5%, 1% and 2% w/v of aqueous extract of *Linum usitatissimum*, topically. while the fifth group was administered Flurbiprofen topically.

- Group-I (control) administered vehicle
- Group-II(Test group) aqueous extract of *Linum usitatissimum* in isotonic buffer solution administered topically 0.5% w/v
- Group-III(Test group) aqueous extract of *Linum usitatissimum* in isotonic buffer solution administered topically 1% w/v
- Group-IV(Test group) aqueous extract of *Linum usitatissimum* in isotonic buffer solution administered topically 2%w/v
- Group-V (Standard) administered topically Flurbiprofen eye drop

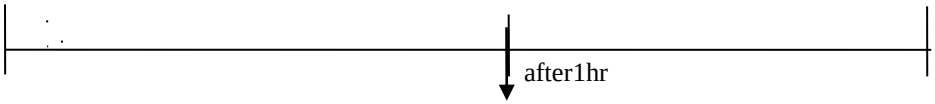
After 2 hours, drug and vehicle were administred to respective group. The aqueous humor was taken from each eye of the animals and marked. After 4 hours again aqueous humor was withdrawn from each eye of the animals and sample was collected in apendop tubes and marked. The sample of aqueous humor were obtained at various time points using a 27 gauge needle on a microlitre syringe. The physical appearance of the eye was considered according to the parameters and it was rated on a scale of 1-3, 1=clear(normal),2=opaque(protein accumulation), 3=very cloudy (protein accumulation in clumps).The total leucocyte count was determined from aqueous humor by using the Neubauer's chamber. The effect of the aqueous preparations on the leucocyte count was determined at 2 h and 4 h post administration.
The diagrammatical representation of experimental protocol procedure is given below.

(B) Protocol For Ocular Inflammation Induced in Guinea pig by Alkali (0.25M Sodium Chloride)

Group1: Control Vehicle Administered Topically (3 drops in each eye)

Group2,3,4: Test Drug Administered Topically (3drops in each eye)

Group5: standard Standard drug Administered Topically (3drops in each eye)



after1hr

Animals were anaesthised with Pentobarbitone 60mg/kg

0.25M Sodium Hydroxide alkali administered topically to each eye of the animals after 2hr

Aqueous Humor taken from each eye of the animals and marked After 4hr

Aqueous Humor taken from each eye of the animals and marked

Appearance of the eye considered according to parameters

Leucocytes count from aqueous humor done by Neubaur's Chambers

Results complied and statistics applied

II. Results and discussion

Percentage yield of plant extracts

Percentage yield of the various extracts of *Linum usitatissimum* seeds (Petroleum ether, methanol and aqueous extract) was found to be 45.21, 9.5 and 13.82 respectively.(Table 1.)

Table 1. Nature and percentage yield of various extracts of *Linum usitatissimum*

Solvent	Extraction period	Colour of the extract	Weight of extract	% yield
Petroleum ether (60-80 ^{o} C)	30(Hrs)	Dark yellowish oil	25.06 gm	45.21
Methanol	30(Hrs)	Semi solid yellowish	5.17gm	9.51
Aqueous	7 days	Semi solid yellowish brown	6.91gm	13.82

Result of phytochemical test

The results of phytochemical tests are tabulated in Table 2.

Table 2. Phytochemical test of various extracts of *Linum usitatissimum*.

Chemical tests	**Extracts**		
	Petroleum ether (60-80°)	**Methanol**	**Aqueous**
Test for Alkaloids			
Dragendorff's test	–	–	–
Mayer's test	–	–	–
Wagner's test	–	–	–
Hager's test	–	–	–
Test for amino acids			
Million's test	–	–	–
Ninhydrin test	–	+ive	+ive
Test for carbohydrates			
Molisch test	–	+ive	–
Selivanoff's test	–	–	–
Fehling's test	–	–	+ive
Test for fats and fixed oils	+ive	+ive	+ive
Test for flavanoids			
Shinoda test	–	–	–
Alkaline reagent test	+ive	+ive	+ive
Zinc hydrochloride test	+ive	+ive	–
Test for glycosides			
Test for antharaquinone glycoside			
Borntrager,s test	+ive	–	–
Test for cardiac glycosides			
Keller-Killiani test (performed on powdered drug)	———		
Legal's test	–	–	–
Baljet's test	–	–	–
Test for coumarin glycosides	–	+ive	+ive
Test for cynogenetic glycoside	+ive	+ive	+ive
Test for saponin glycosides			
Froath formation test	+ive	+ive	+ive
Test for tannins			
Ferric chloride test	–	–	–
Gelatin test	+ive	–	–
Test for Proteins			
Heat test	+ive	+ive	+ive
Hydrolysis test	–	+ive	+ive
Test for steroids and triterpenoids			
Salkowski test	+ive	–	–
Sulphur powder test	+ive	–	+ive

Keys: (+) = Present, (-) = Absent.

Preliminary phytochemical screening revealed that Fat and fixed oils, proteins, saponins,flavanoids and cynogenetic glycosides were present in each extract while carbohydrate,amino acid were present in methanolic and aqueous extract. Petroleum ether extract contains steroids, alkaloids,Tannins and anthraquinone glycoside whereas aqueous extract also showed the presence of steroids.

Animal study

Physical appearence of the eye is rated on a scale 1-3.

The appearance of the eye in different groups were rated on the scale referred from model ocular inflammation induced by alkali (Vogel.*et al* 2007) .These are as follow:

1=clear (normal),

2=opaque (protein accumulation),

3= very cloudy (protein accumulation in clumps)

In control group appearance was very cloudy due to accumulation of protein in clumps , whereas test groups showed both appearance clear as well as opaque due to normal and protein accumulation respectively. Standard showed clear appearance of the eyes presented in Table 3.

Table 3. Physical Appearence of the eye is rated on a scale 1-3.

S.No	Group	Body Weight(gms)	Appearence of Eye
1.	Control	250	3
		232	3
		245	3
2.	Test		
	Test 1 (0.5%)	242	2
		236	2
		247	2
	Test 2 (1%)	248	1
		238	2
		241	1
	Test 3 (2%)	236	2
		248	1
		245	1
3.	Standard	236	1
		242	1
		252	1

1=clear (normal), 2=opaque (protein accumulation), 3= very cloudy (protein accumulation in clumps)

Leucocytes count in aqueous humor of Guinea pig.

The leucocytes count were done from the aqueous humor withdrawn from animals after 2 hours and 4 hours of administration of inducer 0.25M Sodium hydroxide.The aqueous humor collected from the eyes of different groups of animals and diluted with White blood cell diluting fluid or turks solution.It contains following ingredients:

3ml glacial acetic acid

1ml gentian violet 1% aqueous solution

100ml distilled water

The function of the gentian violet is to stain the nuclei of white blood cells.It also acts as preservative.

The aqueous humor samples were spread on counting chamber slide and counting of leucocytes were done in different groups of animals. The results are shown in the Table 4.

Table 4. Leucocytes count in aqueous humor of Guinea pig.

S. No	Groups	Body Weight (gm)	No. of Leucocytes after 2hrs of dose	No. of Leucocytes after 4hrs of dose
1.	Control	252	40	44
		230	41	42
		245	39	41
2.	Test			
	Test 1 (0.5%)	242	24	27
		236	26	30
		247	22	26
	Test 2 (1%)	248	20	24
		238	21	22
		241	17	19
	Test 3 (2%)	236	20	23
		248	18	21
		245	22	23

3.	**Standard**	**237**	**16**	**18**
		243	**18**	**21**
		251	**16**	**17**

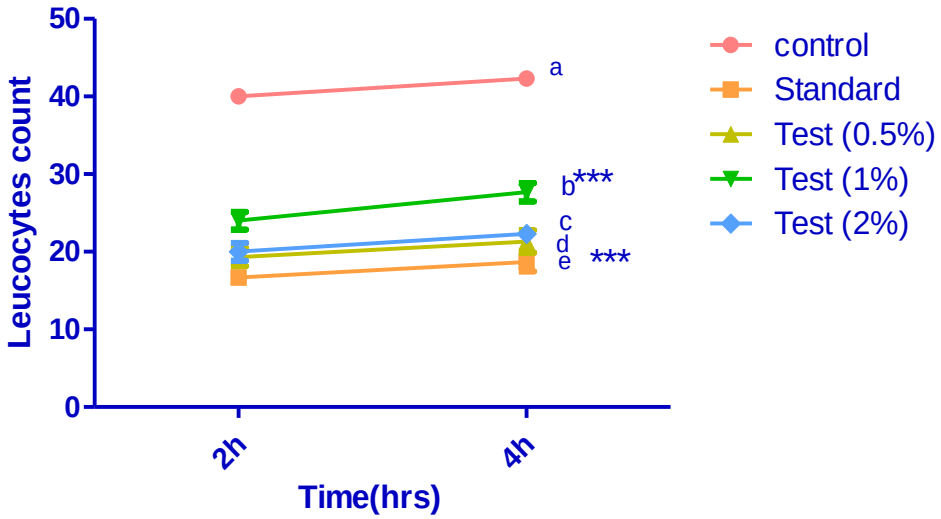


Figure 4a. Leucocytes Count in different Groups

Protein estimation from aqueous humor of Guinea pig.

The sample of aqueous humor withdrawn from the eyes of the animals were preserved at -4^{0}c. These samples were given in pathology lab for estimation of the protein level. The results are shown in Table 11.

Table 5.Protein estimation in aqueous humor of guinea pig.

S. No	Groups	Body Weight (gm)	Level of Protein in aqueous humor (mg/ml)
1.	**Control**	**252**	**17**
		230	**16**
		245	**18**
2.	**Test**		
	Test 1 (0.5%)	**242**	**1.8**
		236	**1.6**
		247	**1.4**
	Test 2 (1%)	**248**	**2.6**
		238	**2.3**
		241	**2.2**
	Test 3 (2%)	**236**	**1.9**
		248	**2.0**
		245	**2.1**
3.	**Standard**	**237**	**1.4**
		243	**1.2**
		251	**1.5**

GSH estimation from aqueous humor of guinea pig.

The sample of aqueous humor withdrawn from the eyes of the the animals of were preserved at -4^{0}c. These samples were given in pathology lab for estimation of the GSH level. Table 12 shows the results.

Table 6. Glutathione estimation in aqueous humor of guinea pig.

S. No	Groups	Body Weight (gm)	Level of GSH in aqueous humor (µM/L)
1.	Control	252	13
		230	12
		245	15
2.	Test		
	Test 1 (0.5%)	242	1.1
		236	0.8
		247	0.6
	Test 2 (1%)	248	1.9
		238	1.6
		241	1.2
	Test 3 (2%)	236	1.0
		248	1.3
		245	1.4
3.	Standard	237	1.1
		243	1.0
		251	0.9

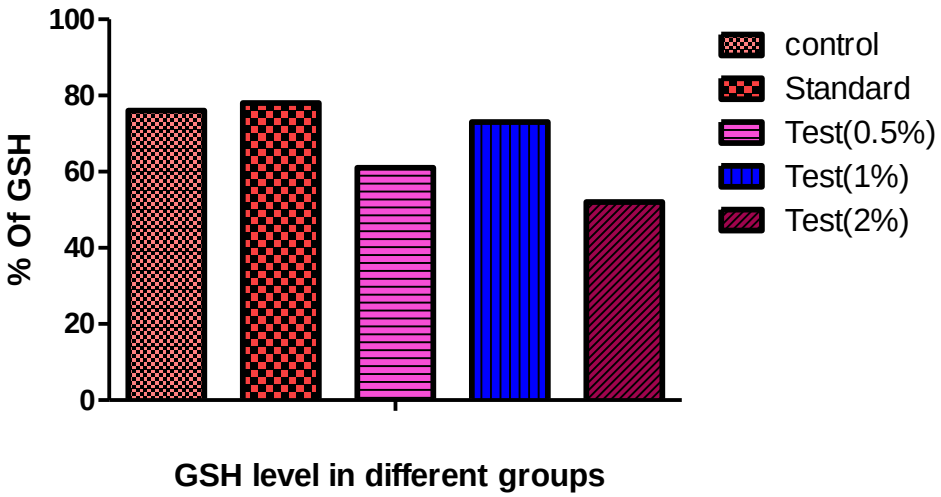


Figure 6a. Percentage of GSH in different Groups

III. Discussion

The present study was carried out to investigate the ocular anti-inflammatory activity of aqueous extract of *Linum usitatissimum* (AELU) seeds in alkali induced ocular inflammation in the guinea pig. Effect of vehicle in ocular inflammation has caused the increase in accumulation of protein and GSH level. The physical appearance, leucocytes counts, protein level and GSH Level were varied (Vogel, 2007), (Ellam *et .al*., 1956), (Lowry, *et al*., 1951). Flurbiprofen and aqueous extract of seeds of *Linum usitatissimum* significantly control the level of physical appearance, leucocytes counts, protein level and GSH Level . Aqueous extract of *Linum usitatissimum* at a dose of (1% topical) showed significant inhibition of ocular inflammation as compared to the control value. The AELU of seeds possess significant ocular anti-inflammatory properties in a dose dependent manner. The activities exhibited by this extract are probably responsible for the reduced arachidonic acid consequently promoting the inhibition of leucocytes count. Alkali induced inflammation are due to excessive secretion of leucocytes and prostaglandin via arachidonic acid pathway. These factors are associated with the development of ocular inflammation including precipitation of protein, rise in glutathione level, prostaglandin level and protein level in the aqueous humor. Prostaglandin E2 and I2 are predominantly synthesized by the arachidonic acid and are known to be responsible for ocular inflammation.

The AELU produced significant (p< o.oo1) reduction in inflammation due to decrease in leucocyte count , GSH and protein level as compared with standard result. The AELU at a dose of 1% topically, showed

significant inhibition of GSH level by 73% respectively, as compared to the control group. These results of *L.usitatissimum* extracts probably have an anti-inflammatory effect, as the extract represented significant results in the alkali model.

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents like fat, fixed oils, proteins, saponins, flavanoids and cynogenetic glycosides were present in each extract while carbohydrate, amino acid were present in methanolic and aqueous extract. Among these compounds the flavanoid possibly shows ocular anti-inflammatory activity. Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce anti-inflammatory effect. Although preliminary biological study has revealed that AELU possesses significant anti-inflammatory activity. The fixed oil of linseed contains relatively large amount of gammalinolenic acid (GLA) which is rapidly converted to dihomogammalinolenic acid (DGLA) a precursor of prostaglandin E_1 which has anti-inflammatory and immunomodulatory properties (Singh,*et al.*,2008).GLA suppresses acute and chronic inflammation as well as joint tissue injure in several experimental animal models.

IV. Conclusion

The following conclusion can be drawn from the results obtained from animal experimentation conducted in our research lab.

- The 1% topical dose of AELU showed anti-inflammatory activity as indicate by the normal physical appearance of the eyes according the scale 1-3.
- The anti-ocular inflammatory study was confirmed by leucocytes count in aqueous humor which was reduced by the same dose.
- The anti-ocular inflammatory study was further confirmed by changes in GSH level and protein level.

So this is clear that the aqueous extract of seeds of *Linum usitatissimum* possesses protective effect against experimentally induced ocular inflammation.However, the exact mechanism and the active compound involved in these effects need to be clarified in future studies.

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