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ORIGINAL ARTICLE

In-Vitro Antioxidant and Anti-Catarctogenic activity of Finger Millet, Kodo Millet and Proso Millet

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ABSTRACT
The anti-oxidant activity of selected millets Finger millet (FM), Kodo millet (KM), Proso millet (PM) and their combined extract designated as FKP is evaluated using nitric oxide radical scavenging method using Griess reagent. The anticataract activity is done using isolated chick lens in four groups. Group I: Lens incubated in artificial aqueous humor (normal control). Group II: Lens incubated with glucose 55mM (toxic control). Group III and IV: Lens incubated with glucose and FKP (50µg and100µg) and subjected to evaluation for opacity using reflectometer. Lenses were homogenized using Tris-phosphate buffer and sodium, potassium, total protein content was determined. The results indicate that all the extracts posses anti-oxidant activity equivalent to that of ascorbic acid. Among all extracts, combined extract (FKP) is having more antioxidant activity. The results of anticataract activity revealed that, the grades of opacity were 0, 3, 1, and 1 in group I, II, III and IV respectively. An increase in total proteins, potassium levels, and decrease in sodium levels were observed in lens treated with FKP indicating that the combined extract of these millets can reduce cataract.
KEYWORDS: *Cataract, Anti-oxidant, Chick lens, Glucose, Finger millet, Kodo millet, Proso millet.*

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INTRODUCTION

Plants being the beauty of nature, also plays a significant role in possessing pharmacological activity apart from survivalance of other creatures on earth. Of all the plants, millets are a group of highly variable small seeded grasses grown widely around the world as cereal crops. Belonging to poaceae family, millets of small grained, annual, warm weather crops are highly tolerant of drought and other extreme weather conditions. The term millet is employed for several related genera, some used to produce grain, or forage or both. Millets are mostly used as food for diabetic people due to their low glycemic index. The most widely cultivated millets are Finger millet (*Eleusine coracana*) Proso millet (Panicum miliaceum), Barnyard millet *(Echinochloa colona*), Kodo millet (*Paspalum scrobiculatum*) etc.[1].

Oxidative stress comprises major role in formation of chronic and degenerative diseases like cancer, autoimmune disorders, arthritis, cardiovascular and neurodegenerative diseases. The human body itself uses several mechanisms to combat the oxidative stress by producing antioxidants, *in situ*, or externally supplied through diet and/or supplements. These antioxidants act as "free radical scavengers" both by preventing and repairing damages caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), and therefore enhances the immune defense and lower the risk of cancer and degenerative diseases. Herbal plants are considered as good antioxidants since ancient times. The phytochemicals like phenols, poly phenols and flavonoids can scavenge free radicals and thus inhibit oxidative mechanisms [2].

Cataract (lens opacification) is a major contributing factor of blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for ocusing and producing a clear sharp image. It is called as a "peril of sight" because cataracts have blinded more people throughout the ages than any other affliction of the eye. It is also called as "Senile cataract." Cataract is derived from the Latin word "cataracta" meaning waterfall. Age-related nuclear cataract is the most common form of cataract which is found in ages more than

45 years old and opacity forms in the center of the lens [3]. Cataract is nothing but visual impairment as a result of a disturbance of lens transparency. It is one of the leading causes of blindness worldwide; it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28,000 new cases are reported daily worldwide approximately, 25% of the populations over 65 and about 50% over 80 have a serious loss of vision because of cataract [4-5]. Cataractogenesisis is associated with diverse risk factors such as aging, trauma, diabetes, toxins, smoking, genetics, and other ocular diseases. Various mechanisms like protein aggregates, osmotic graduation, oxidative stress, post-translational protein changes, and phase separation are proposed for cataract formation. Presently, surgery is the only approach for the treatment of cataract, and while favorable outcomes are quite predictable, the limited number of surgeons is underdeveloped countries, and the high cost of surgery has made cataract a major health problem. Drugs developed to delay or prevent lens opacification have failed to give convincing positive results in clinical trials. This stimulates the research toward the experimental work on cataract to understand the all possible pathway and mechanism which is responsible for the generation of cataract [6].

MATERIAL AND METHODS

Collection & Extraction of Millets:

A pack of selected millets of Manna brand were procured commercially, 25gm of selected millets FM, KM, PM were taken separately macerated with alcohol and water (50:50) for 7-14 days, with occasional shaking for every 2 days. After 14 days the macerated products are separated by filtration technique. The obtained filtrates are then evaporated to a concentrate and evaluated using preliminary phytochemical screening methods.

Preliminary Phytochemical Screening:

The extracts were subjected to chemical evaluation for identification of various phytoconstituents [7].

Test for Carbohydrates:

Molisch Test:

The hydro-alcoholic extracts of FM, KM,PM were treated with 2 ml of molisch reagent and few drops of concentrated sulphuric acid is added along the sides of test tubes.

Test for Alkaloids:

Hagers Test:

Small amounts of hydro-alcoholic extracts of FM, KM, PM were taken into test tubes and picric acid is added in small amounts to all the 3 test tubes.

Dragendroffs Test:

Small amounts of hydro-alcoholic extracts of FM, KM, PM were taken into test tubes and dragendroffs reagent is added in small amounts to all 3 test tubes.

Test for Steriods:

All the 3 hydro-alcoholic extracts of FM, KM, PM were taken individually into 3 test tubes and to each test tube a pinch of sulphur powder is added.

Test for Flavanoids:

All the 3 hydro-alcoholic extracts of FM, KM, PM were taken individually into 3 test tubes and add few drops of sodiumhydroxide solution individually into all 3 test tubes.

Test for Glycosides:

All the 3 hydro-alcoholic extracts of FM, KM, PM were taken individually into 3 test tubes and add 1ml of sulphuric acid to all the 3 test tubes and heat. Filter and add chloroform to the obtained filterate from all the 3 test tubes. Separate the lower layers from all the test tubes and treat them with ammonia individually.

Evaluation of Anti-Oxidant Activity by Nitricoxide radical scavenging method:

The antioxidant activity of the hydro-alcoholic extracts of FM, KM and PM were determined using Nitric oxide radical method. Nitric oxide scavenging activity can be estimated by the use of Griess Illosvoy reaction [8]. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing nitric oxide (NO). Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

Sodium nitroprusside (10mM) in phosphate buffered saline (pH 6.4) was mixed with different concentrations (5 - 40µg/ml) of alcoholic extracts of each FM, KM, PM and combined extract of these three millets designated as FKP were dissolved in alcohol and incubated at 30°C for 2 hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% $H3PO4$ and 0.1% N-(1-naphthyl)

ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthyl ethylenediamine dihydrochloride was immediately read at 550nm. Inhibition of nitrite formation by the extracts of millets and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) i.e inhibitory concentration of each extract required to reduce 50% of the nitric oxide (IC 50) was linearized against the concentrations of test extracts and standard antioxidant Ascorbic acid.

Evaluation of Anti-Cataract Activity:

In this study, chick lens were used as they are easily available. Fresh eye balls were collected from slaughter house from Guntur.

Lens Culture:

Fresh eye balls were obtained from slaughterhouse were immediately transported to the laboratory at 0-5^{o}C. The lens were removed by extracapsular extraction and incubated in artificial aqueous humor (sodium chloride: 150 mM, hydrochloric acid: 5 mM, magnesium chloride: 2 mM, sodium bicarbonate: 0.5 mM, sodium dihydrogen phosphate: 0.5mM, calcium chloride:0.4mM, and glucose:5.5mM) at room temperature and pH of 7.8. Ceftriaxone at a dose of 5mg/ml was added to the culture media to prevent bacterial contamination [9].

Induction of cataract by in-vitro method:

A total of 16 lenses were used for the study. These lenses were incubated in artificial aqueous humor with various concentration of glucose (5.5 mM served as normal control and 55 mM served as cataract control) for 72 hrs. Glucose at a concentration of 55 mM was used to induce cataract in lenses. Effect of hydro-alcoholic extract of combination of the selected millets FM, KM, PM (designated as FKP) on isolated chick lenses was evaluated. Chick lens were divided into four groups with 4 lenses in each group and incubated as follows

Experimental design:

Group I: Normal control (Glucose 5.5 mM)
Group II: Cataract control (Glucose 55 mM)
Group III: Test 1 (Glucose 55 mM + FKP at a dose of 50 µg/mL)
Group IV: Test 2 (Glucose 55 mM + FKP at a dose of 100 µg/mL)

After 72 hrs of incubations, lenses were placed on a wired mesh with the posterior surface touching the mesh (the pattern of mesh number of squares clearly visible through the lens) was observed and determined the lens opacity. The degree of opacity was graded as follows: 0 - Absence of opacity, 1 - Slight degree of opacity, 2 - Presence of diffuse opacity and 3-Presence of extensive thick opacity.

Preparation of lens homogenate

After 72 hrs of incubation, homogenate of lenses was prepared in 0.23 M of Tris buffer (pH 7.8) containing EDTA and homogenate was adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 rpm at 5°C for 1 hour and the supernatant was used for estimation of biochemical parameters such as proteins as per Lowry OH et al., 1951, sodium and potassium ions [10].

Biochemical parameters

Electrolyte (Na^+) and potassium (K^+) estimation was done by flame photometry method, and protein estimation was done by modified biuret end point assay method [10].

Statistical analysis: Results were expressed as Mean ± SEM. The statistical significance of the difference between groups for the various treatments was determined by one-way analysis of variance followed by Dunnett's test. $p<0.05$ was considered statistically significant.

RESULTS

The results of phytoconstituents present in hydro-alcoholic extract of FM, KM, PM were given in Table 2.

The results of antioxidant activity revealed that the hydro-alcoholic extracts of FM, KM, PM and FKP posses anti-oxidant activity similar to that of Ascorbic acid. The combined extract (FKP) showed higher activity comparatively (Table 3).

The grades of opacity were 0, 3, 1, and 1 in Group I, II, III, and IV, respectively, and results were shown in Table 3. Incubation of lenses with FKP at (50 µg/ml, 100 µg/ml) concentrations seems to retard the progression of lens opacification (Fig.2). The results of present study revealed that treatment with FKP resulted in an increase in total proteins ($p<0.05$), K^+ ions ($p<0.05$), and reduction in concentrations of Na^+ions ($p<0.05$). The graphs of the obtained results were depicted in figures 3-6.

Table 1: Phytochemical and Pharmacological Review of Selected Millets [16, 17]:

Botanical Name	*Eleusine coracana*	*Paspalum scrobiculatum*	*Panicum miliaceum*
Telugu Name	Ragulu	Arikelu	Varigelu
Phytochemical Review	Aminoacids, Vitamins, Polyphenols, enzymes, Sinapic acid, Gentisic acid and Chlorogenic acid.	Phenolic Compounds, Flavanoids, Tannins, Proteins And Aminoacids Such As Lysine, Other Oils	Carotenoids such as Xanthophylls and cryptoxanthins, Polyphenols, Caffeic acid, Chlorogenic acid, Syringic acid and Falvanoids
Pharmacological Review	Anti diabetic and used in treatment of osteoporosis, anaemia, anxiety, insomnia, depression.	Antidiabetic, used to treat hemorrhages, and inflammation, reduces B.P	Antidiabetic, anti inflammatory, prevents pellagra, and possess skin whitening property.

Table 2: Phytochemical analysis of hydro-alcoholic extract of FM, KM and PM

S.No.	Phytoconstituents	FM	KM	PM
1.	Carbohydrates	+ve	+ve	+ve
2.	Alkaloids	+ve	+ve	+ve
3.	Phenolic compounds	+ve	+ve	+ve
4.	Terpenoids	+ve	+ve	+ve
5.	Flavanoids	+ve	+ve	+ve
6.	Glycosides	+ve	+ve	+ve
7.	Steroids	+ve	+ve	+ve

Table 3: Antioxidant activity of hydro-alcoholic extracts of FM, KM, PM and FKP

Concentration	Finger Millet	Kodo Millet	Proso Millet	Combined extract (FKP)	Standard (Ascorbic acid)
µg/ml	Absorbance at 550 nm				
5	0.013±0.0005	0.012±0.0003	0.015±0.0004	0.015±0.0004	0.021±0.001
10	0.016±0.0003	0.019±0.0003	0.028±0.0005	0.0315±0.0003	0.038±0.0001
20	0.138±0.0005	0.130±0.001	0.148±0.0004	0.164±0.0002	0.184±0.0001
40	0.159±0.0004	0.153±0.0002	0.179±0.0004	0.1981±0.0003	0.224±0.003
IC50 values	15.039±1.508	14.822±1.261	15.027±1.315	14.785±1.135	14.59±1.36

All values are expressed as Mean±SEM, n=3

Table 4: Effect of hydro-alcoholic extract of FKP on Opacity of lens

Study Groups	Grade
Group I (Normal control)	0
Group II(Toxic control)	3
Group III(Test1)	1
Group IV(Test 2)	1

Table 5: Effect of hydro-alcoholic extract of FKP on Total protein content of lens

Groups	Total protein content gm/dl
Group I (Normal control)	7.14±2.10**
Group II(Toxic control)	1.96±3.30
Group III(Test 1)	8.37±1.93**
Group IV(Test 2)	10.62 ±1.29***

Values are expressed as Mean±S.E.M, n=3,
P<0.05,**P<0.01,P<0.001 Vs Toxic control**

Table 6: Effect of hydro-alcoholic extract of FKP on Potassium levels

Groups	Potassium levels µg/ml
Group I (Normal control)	11.01±0.44**
Group II (Toxic control)	6.17±0.11
Group III(FKP 50µg/ml)	8.95±0.12
Group IV(FKP 100µg/ml))	9.08±0.15*

Values are expressed as Mean±S.D, n=3
*P<0.05,**P<0.01,***P<0.001 Vs Toxic control

Table 7: Effect of hydro-alcoholic extract of FKP on Sodium levels

Groups	Sodium levels µg/ml
Group I (Normal control)	80±+2.10***
Group II(Toxic control)	200±3.30
Group III(Test-1)	140±1.93**
Group IV(Test-2)	100±1.29***

Values are expressed as Mean±S.E.M, n=3
P<0.05, **P<0.01,P<0.001 Vs Toxic control**

FM
Finger millet
Eleusine coracana

KM
Kodo millet
Paspalum scrobiculatum

PM
Proso millet
Panicum miliaceum

Figure 1: Image of show grains of Finger millet, Kodo millet and Proso millet

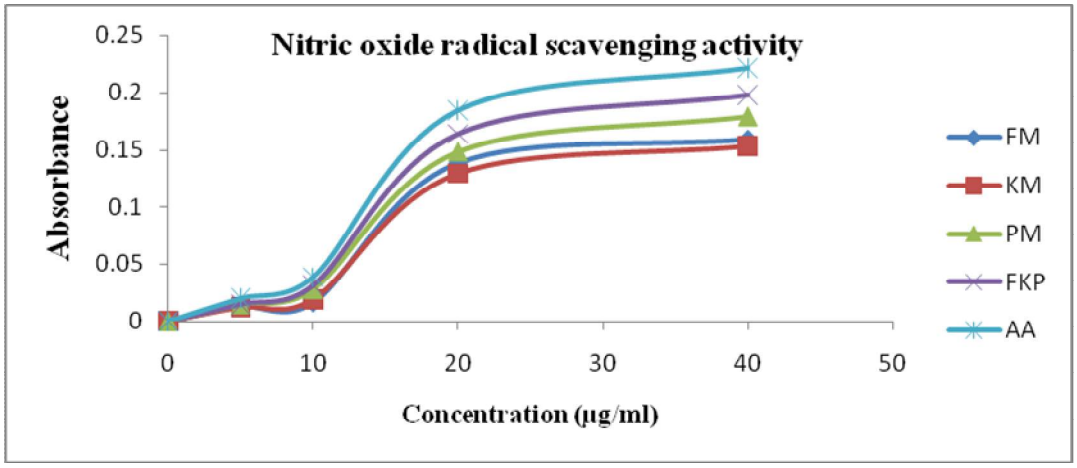
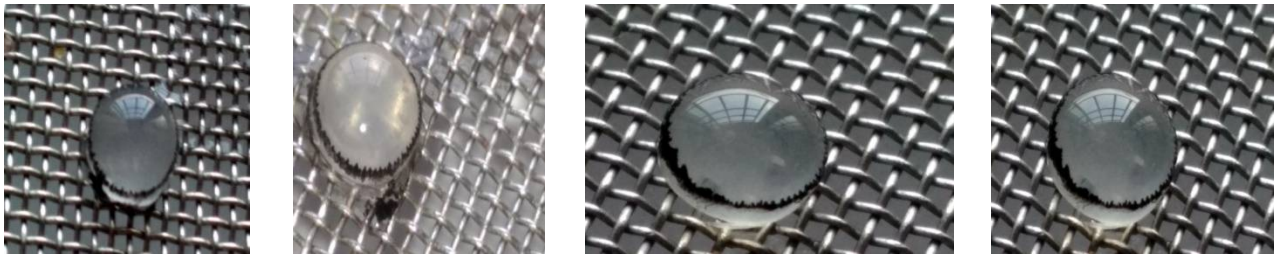


Figure 2: Nitric oxide radical scavenging activity of selected millets



GROUP-I GROUP- II GROUP- III GROUP-IV

FIGURE 3: Effect of Hydro-Alcoholic Extract of FKP On Opacity Of Lens

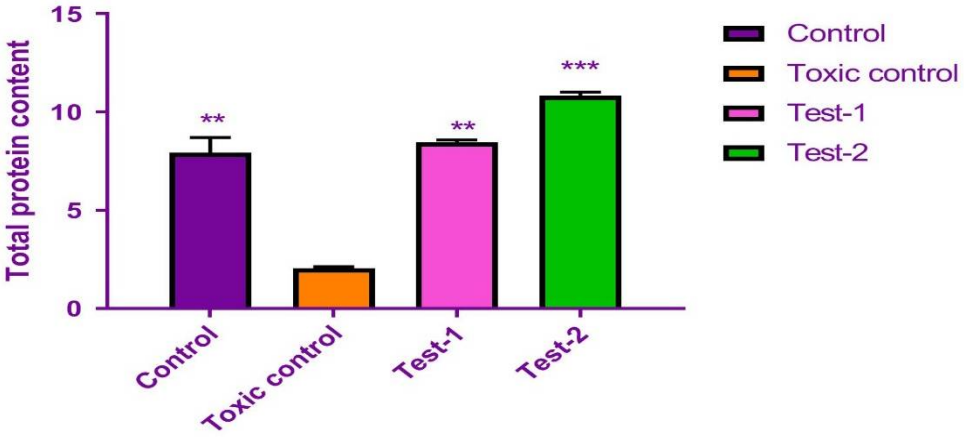


Figure 4: Effect of hydro-alcoholic extract of FKP on Total protein content

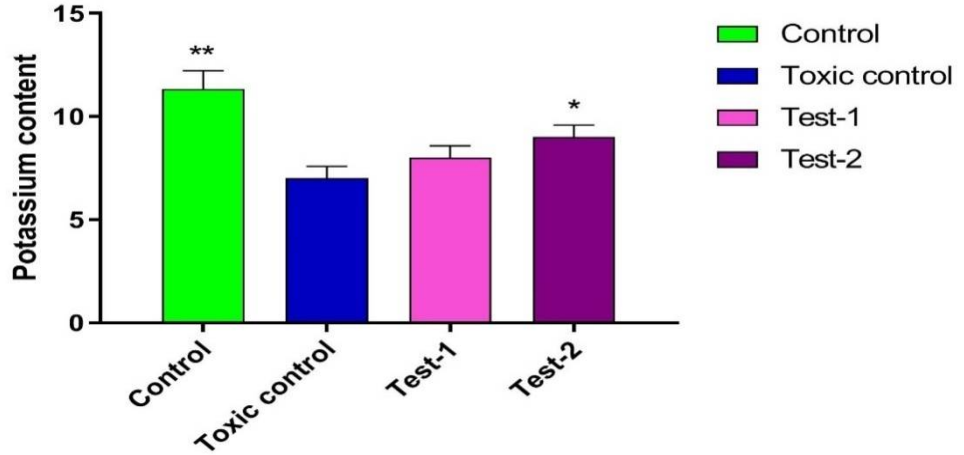


Figure 5: Effect of alcoholic extract of FKP on potassium content

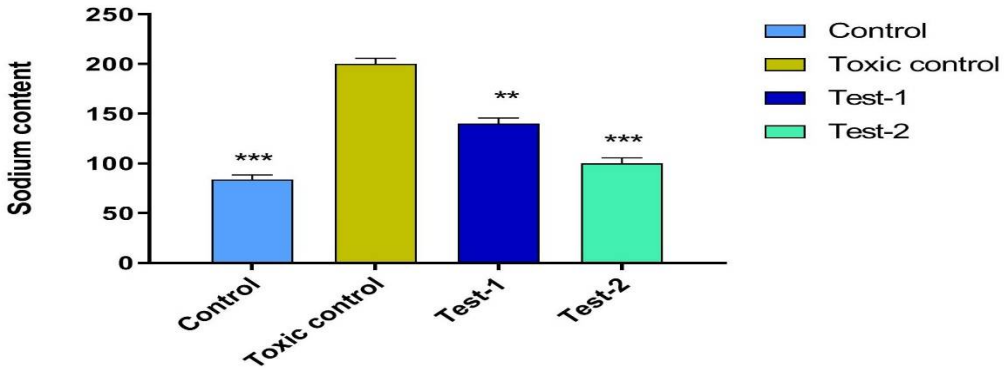


Figure 6: Effect of hydro-alcoholic extract of FKP on sodium content

DISCUSSION

The present study was undertaken to evaluate the antioxidant and anticatract activity of hydro-alocholic extracts of FM, KM, PM. The phytochemical constituents present in the hydro-alocholic extracts of FM, KM, PM were observed by performing preliminary phytochemical screening. The phytochemical constituents present in alcoholic extract of FM,KM,PM includes carbohydrates, alkaloids, phenolic compounds, terpenoids, flavanoids and anthraquinone glycosides.

Oxidative stress of a cell is dependent on both enzymatic and non-enzymatic antioxidants produced by cells. A balance of these antioxidants with free-radical production determines the status of oxidative stress of a cell [11]. The antioxidant activity was evaluated in hydro-alcoholic extracts of FM, KM, PM and FKP by nitric oxide radical method. It is found that the extracts posses anti-oxidant activity equivalent to that of ascorbic acid. Among all extracts, combined extract (FKP) is having more antioxidant activity comparatively. Earlier studies indicate that the antioxidant activity is due to composition of different phytochemical constituents in all the three extracts.

Cataract is one of the universal processes of ageing and is consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species play an important role in the process leading to lens opacification [12]. Studies have shown that substitution with vitamin C and vitamin E can prevent experimental cataract [13].

Drastic reduction in the levels of various lens antioxidants during cataractogenesis was reported earlier. These reduced levels persist even after cataract formation is complete. Therefore it was clearly understood that continuous oxidative stress in the eye lens leads to formation of cataract and the role of endogenous antioxidants in lens physiology became prominent [14] (Thiagarajan et al., 2013). Natural compounds that possess antioxidant and/or anti-inflammatory properties are the ideal choices for anticataract agents. These compounds by virtue of their pharmacological properties are thought to be important for preventing cataract. It is pertinent to note that though there are synthetic chemicals available with the above mentioned activities, extensive research is still being focused on natural compounds because of questions of efficacy, availability and occurrence of side effects with synthetic drugs [15]

The impairment of Na+/K+-ATPase causes accumulation of Na+ and loss of K+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na+, K+ ratio change the protein content of the lens, leading to a decrease in total proteins causing lens opacification. From the results of antioxidant activity, it was observed that FKP is having more antioxidant activitiy. Our results showed increase in the total proteins and K+ ions ($P<0.05$), and decrease in concentrations of Na+ ions ($P<0.05$) with FKP treated groups. The imbalance of Na+ and K+ is prevented by FKP, may be due to its antioxidant activity which corrects imbalances in the polyol pathway by decreasing aldose reductase activity.

CONCLUSION

The present investigation suggests that hydro-alcoholic extract of FKP effectively prevent the cataract, which was indicated by increase in the total protein content, potassium level, and decrease in the sodium. Due to toxicity and unwanted effects caused by synthetic medicines people are now fascinating towards the natural products for therapeutic purpose. Millets are easily grown grains and has less toxicity and no side effects. Hence they can be used as antioxidants to prevent the formation of free radical. In conclusion, all the above finding lends credence to hydroalcoholic extract of FKP in the treatment of cataract. Investigation into the potent pharmacological activities and the mechanism of action of natural anti-cataract agents would provide a better understanding of their therapeutic potential and future application as novel drugs.

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