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Pharmacological study on the possible involvement of a Nrf2 -Heme oxygenase pathways in anti-cataract activity of fisetin

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ABSTRACT

DM otherwise diabetes is now a days an epidemic with the percentage of patient population rising to almost 10% of the world population. Out of all the DM complications, cataract leads the way contributing to disabilities to about 60% of diabetic population. But the pathogenesis of DM cataract is still a half-understood area of medicine there by posing a problem in the therapy. The data that we have till now gives us enough evidence to advocate the oxidative stress has a major role for the pathogenesis of DM complications like DMnephropathy, DMneuropathy, and cardiac hypertrophy, which suggests the oxidative stress is a central feature of diabetes. In the current research, the pharmacological evaluation of Fisetin for its DM based anti-cataract property was performed. This research concentrates to estimate the possible involvement of Nrf-2 / heme oxygenase (HO)-pathway in the observed therapeutic effect, if any. The data obtained in this study also indicate that the observed beneficial effects mainly due to activation of Nrf2/HO-1 pathway. These effects probably result in increased tissue anti-oxidant status as well as decreased free radical production, which ultimately responsible for the observed beneficial effects of Fisetin against hyperglycemia-induced cataract.

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INTRODUCTION

DM otherwise diabetes is now a days an epidemic with the percentage of patient population rising to almost 10% of the world population. It is fore casted to a death of over 15lakhs patients only because of DM. out of these more than 75% of the deaths that occur are in the low-income classes or middle classes only (WHO, 2014). Out of all the DM complications, cataract leads the way contributing to disabilities to about 60% of diabetic population. But the etiology of DM cataract is yet a half understandable area of medicine there by posing a problem in the therapy. A major study has proved the role of various molecular pathways, which includes, Polyol pathway that result in accumulation of sorbital and elevation of osmotic pressure that result in apoptosis in lens epithelial cells.

Increased formation of advanced glycation endproducts (AGE). Stress is viewed as a critical attribute to cause DM and play a vital part in the causing of DM complications. The factor nuclear 2 (Nrf2) factor catalyses the antioxidant induction and cyto protective genes. This is the important factor of the inter-

nal prevention of oxidation and removal of toxins in the system (Cheung et al., 2005). The endogenous system in the body that works as antioxidants works for bilirubin and biliverdin is provided by a Heme-oxygenase-1 (HO-1). This reduces the lipid peroxidation and by antioxidant activity and anti-complement activities [1]. Besides this, the anti-inflammatory activity of the CO was also suggested by some reaserches. This may be responsible to inhibit the pro inflammatory expression of the cytokines [2] .

The biliverdin was oxidatively degraded from heme by Heme oxygenase (HO-1) which is rate limiting step in the process and CO and iron are biproducts. Importantly, the transcription factor Nrf2 by binding at the promoter site of antioxidant response elements (ARE), regulate the expression of a set of genes code for electrophile-conjugating enzymes and antioxidation enzymes like HO-1, NADPH, glutathione S transferase, quinone oxido reductase-1 & glutathione peroxidise [3]. Other researches proved that Nrf2 and HO-1 gene expressions & activity is decreased in subjects with T2DM (WHO, 2014). The data that we have till now gives us enough evidence to advocate the oxidative stress has a major role for the pathogenesis of DM complications like DMnephropathy, DMneuropathy, and cardiac hypertrophy, which suggests the oxidative stress is a central feature of diabetes.

Fisetin is a 3, 7, 3′, 4′ - tetrahydroxyflavone which is present in many fruits like mangoes and strawberries and vegetables. In previous researches it was found that falvonoids could avoid the stress caused by oxidation on the death of neuronal cells due to oxidative stress. It was also proved to show Fisetin has been shown to possess direct and indirect intrinsic activity of anti oxidation properties due to which it helps in raising the lowered glutathione levels in the neural cells invitro [4] . It also exhibits other activites which includes RA, and anti cancer too.

In the current research, the pharmacological evaluation of Fisetin for its DM based anti-cataract property was performed. This research concentrates to estimate the possible involvement of Nrf-2 / heme oxygenase (HO)-pathway in the observed therapeutic effect, if any.

MATERIALS AND METHODS

Isolation and culture of goat Lens

The eye balls of a goat are obtained from the fresh slaughter of the goat from a slaughter house nearby. They are transported to the laboratory and stored at 0-4^{o}C. the lens from the eye are removed by lateral incision of the eye. A fresh solution of aqueous solution of humour was prepared from NaCl-140mM, KCl-5mM, MgCl-2.2mM, NaHCO3-0.5mM, NaHPO4-20.5mM, CaCl2-0.4mM and Glucose-5.5mM and the fresh lens was stored in this solution at room temperature. The pH of the solution was approximately 7.8 and the lens is stored for 72hr. Penicillin 32mg % & streptomycin 250 mg % were mixed with the culture media to prevent the contamination from any bacteria and viruses [5].

Generation of cataract

The cataracts are induced by using Glucose 55mM directly into the lens. The injected glucose is metabolized via pathways of sorbitol and polyphenol accumilations which causeas a serious hydration and stress induced by oxidation. This led to cataract genesis. the experimental division was done into 9 groups and the grouping of lens is done as described in Table 1.

Photographic evaluation

Lenses incubated in glucose 5.5 μM remained transparent, whereas, the lens incubated in 55 μM glucose exhibited opacities which are dense and opaque. There was seen a total opacification in the center by the end of 72hrs. The Anti-cataract activity can be evaluated by assessments of whether treatment of drugs can retard the development of opacity.

Estimation of stress due to oxidation / and bilirubin

Estimation of lipid peroxidation is performed by estimating the concentration of thio barbituric acid reactive substance which is shortly called as TBARS which was spectrophotometrically evaluated at 532 nm. Estimation of nitrite was performed by estimating the concentration of the nitrites in the sciatic nerve & spinal cord was also evaluated in spectrophotometric method. Super oxide dismutase activity was evaluated in UV at 480 nm. Catalase was assayed by estimating the decomposition rate of hydrogen peroxide was evaluated in UV at 620 nm [6].

Bilirubin determination wtihin tissue homogenate was done by separating aliquots of 500 μl of occular tissue was mixed to 250mg of Barium chloride and mixed thoroughly.

Statistical Analysis

All the results obtained are expressed as mean $\pm$ S.E.M. The values obtained for parameters, except formalin flinching behavior, were analyzed using two-way variance in ANOVA by following Bonferroni multiple comparison test.

Table 1: Treatment group

S.No	Group	Treatment
1	GP-I: Normal control	Normal lens [Control (Glucose 5.5 mM)];
2	GP-II: Disease Control	Glucose55 mM exposed
3	GP-III: ZnPP per se	Glucose55 mM + ZnPP (10 μg/ml)
4	GP-IV: brusatol per se	Glucose55 mM + Brusatol ((5 μg/ml)
5	GP-V: 5 Fisetin treated	Glucose55 mM + Fisetin 5 μg/ml
6	GP-VI: 10 Fisetin treated	Glucose55 mM + Fisetin 10 μg/ml
7	GP-VII:: 10 Fisetin + ZnPP treated	Glucose55 mM + Fisetin 10 μg/ml + ZnPP (10 μg/ml)
8	GP-VIII:: 10 Fisetin + brusatol treated	Glucose55 mM + Brusatol (5 μg/ml) + Fisetin 20 μg/ml
9	GP-IX: 400 Vit.C treated	Glucose55 mM + Vit. C 400 μg/ml

Table 2: Effect of various doses of Fisetin and Vitamin C on tissue homogenate TBARS and nitrite/nitrate levels.

Treatment /group	MDA (μmoles/mg protein)	Nitrite/nitrate (μmoles/mg protein)
NC	12.29 $\pm$1.33	0.43 $\pm$0.14
DC	19.98 $\pm$2.15a	0.65 $\pm$0.17a
ZnPP per se	20.12 $\pm$1.85	0.67 $\pm$0.13
Brusatol per se	21.12 $\pm$ 1.94	0.69 $\pm$0.19
Fisetin (5 μg/ml treated)	17.44 $\pm$0.62	0.61 $\pm$0.05
Fisetin (10 μg/ml treated)	12.93 $\pm$0.85b, c	0.44 $\pm$0.08 b, c
Vit.C (10 μg/ml treated)	14.28 $\pm$0.66b	0.51 $\pm$0.05 b
ZnPP+ Fisetin (10 μg/ml treated)	18.16 $\pm$0.77 d	0.59 $\pm$0.08 d
Brusatol + Fisetin (10 μg/ml treated)	20.86 $\pm$0.45d	0.63 $\pm$0.07d

Table 3: Effect of various doses of Fisetin and Vitamin C on tissue homogenate SOD, catalase and glutathione levels.

Treatment/ group	SOD (U/mg protein)	Catalase (μM of H2O2/mg protein)	Glutathione (μM of GSH/mg of protein)
NC	22.72 $\pm$2.07	14.26 $\pm$1.32	5.15 $\pm$0.33
DC	24.34 $\pm$1.65	16.46 $\pm$0.89	2.32 $\pm$0.12a
ZnPP per se	23.95 $\pm$2.75	15.80 $\pm$1.8	2.17 $\pm$0.15
Brusatol Per se	23.13 $\pm$2.64	15.18 $\pm$ 2.1	2.22 $\pm$0.13
Fisetin (5μg/ml treated)	30.45 $\pm$2.24 b	25.26 $\pm$0.86 b	3.87 $\pm$0.34 b
Fisetin (10 μg/ml treated)	38.81 $\pm$2.26 b,c	36.08 $\pm$1.23 b, c	5.25 $\pm$0.18 b, c
Vit.C (10μg/ml treated)	26.44 $\pm$1.83	21.64 $\pm$0.84	5.17 $\pm$0.12b
ZnPP+ Fisetin(10 μg/ml treated)	28.81 $\pm$2.26 d	21.08 $\pm$1.23 d	3.15 $\pm$0.18 d
Brusatol + Fisetin (10 μg/ml treated)	26.43 $\pm$1.88d	19.64 $\pm$1.15d	2.98 $\pm$0.15d

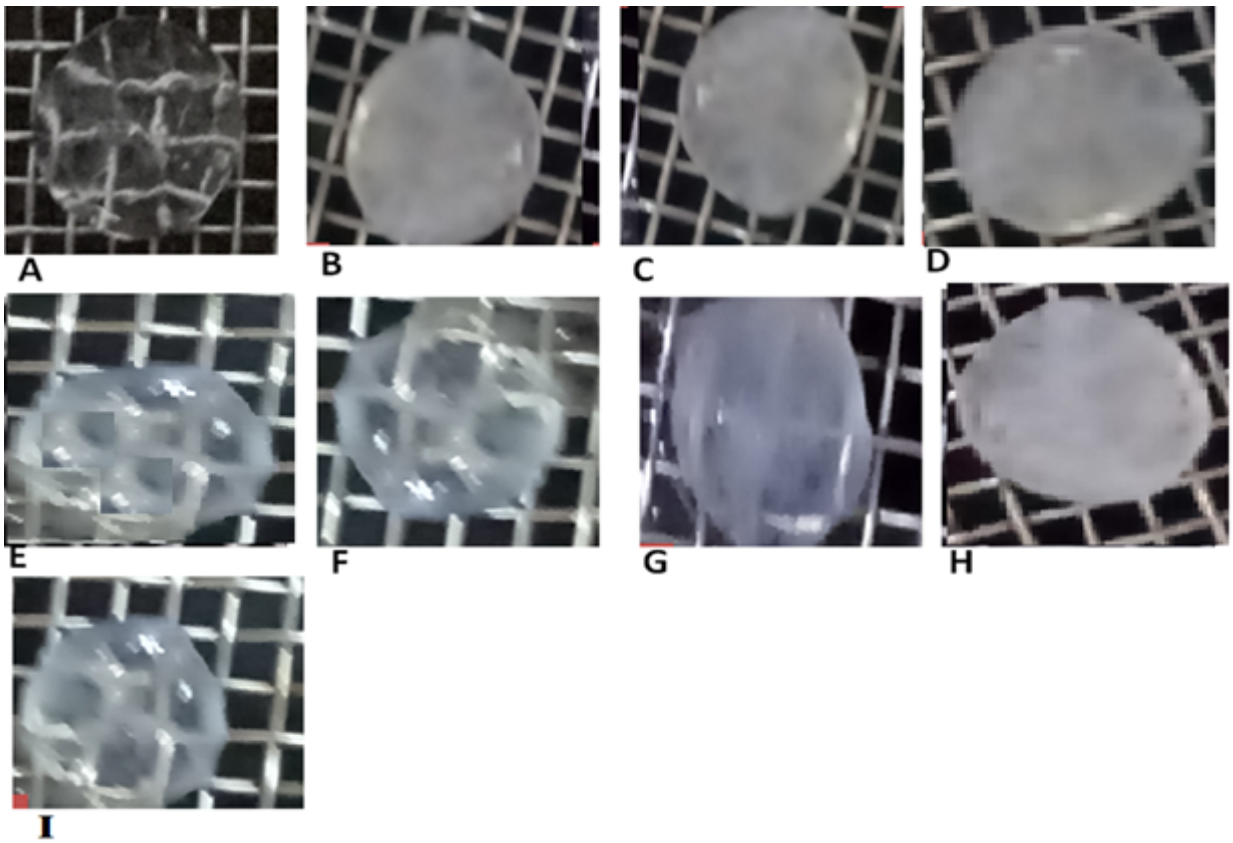

Figure 1: Photographic evaluation of effect of fisetin on glucose-induced opacity of lenses

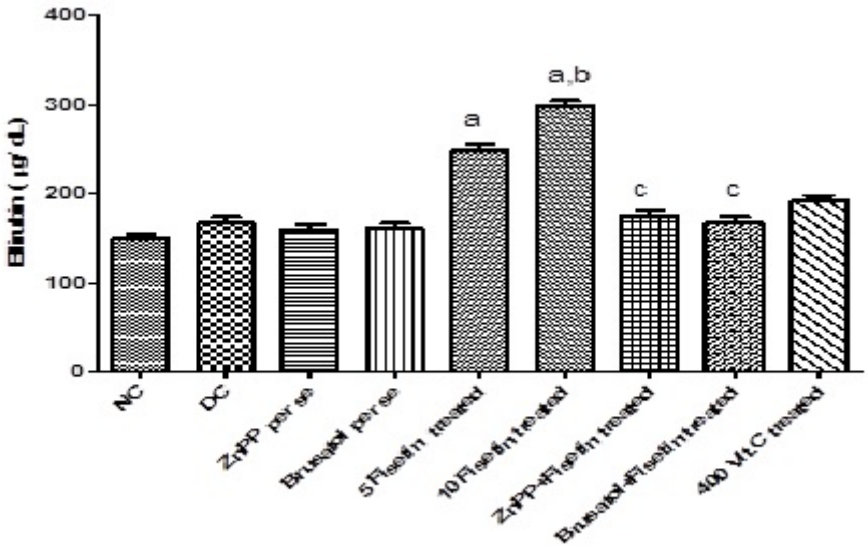

Figure 2: Effect of various doses of fisetin and Vitamin C on tissue homogenate bilirubin levels.

RESULT

An in-vitro hyperglycemia (55 mM glucose) has shown to induce cataract in cultured goat Lens, which is associated a considerable raise in TBARS values which is expressed as MDA and nitrite/nitrate levels were observed in disease control group, which is comparative to normal group, while, SOD, catalase & glutathione values were moderately, but not significantly increased in disease control group. Supplementation of Fisetin (5 and 10 μg/ml) showed a reduction depending on the dose on levels of TBAR and nitrates, comparable to vehicle group. Bilirubin, a product of the HO-1 system cleavage, is slightly, but not significantly increased in the hyperglycemia-induced in cataract lens tissue. However, Supplementation of Fisetin (5 and 10 μg/ml) has more significantly and dose dependently increased bilirubin, as compared to vehicle control as well as standard drug Vitamin C treatment group ($P \leq 0.05$). However, co-administration of both zinc protophorphyrin-IX (ZnPP; 10μg/ml), a selective HO-1 inhibitor and Brusatol (5μg/ml), a unique inhibitor of the Nrf2, were significantly reversed the observed beneficial effects of Fisetin (10 μg/ml). This indicates the observed beneficial effects may be secondary to increased expression of Nrf2/HO-1 pathway

DISCUSSION

In tune with previous studies, treatment with Fisetin associated with significant increasing bilirubin, an end product of HO-1 pathway. Similarity, treatment with fisetin associated with significant increasing bilirubin. On the other hand, co-administration of zinc protophorphyrin-IX (ZnPP; 10μg/ml), a selective HO-1 inhibitor, has significantly abolished the observed elevated levels of bilirubin associated with Fisetin (10 μg/ml). This may indicate that activation of Nrf2/HO-1pathway may be the reason for observed beneficial effect of Fisetin in this study. However, more data using enzyme-linked immunosorbant assay (ELISA) and Western blotting is needed to implicate the more clearly, whether increased expression and/or increased activity of HO-1.

Reduction in the levels of reduced glutathione is observed during cataract of any etiology. GSH plays a leading role in preserving lens clarity. It also acts as antioxidant and stabilizes proteins in reduced form [7]. The present studies proved that fisetin evidently reduces the raised values of nitrites/nitrates. This might be because of Fisetin decreases the iNOS & NO activities. Finally, it can be said that the results achieved from the present study shows the oxygen radicals plays a vital role in causing the DM cataract. Studies implicate that the liberation of oxygen free radicals and peroxidation of lipids are the reasons for the occurnace of DM cataract. Thus, fisetin exerts an activity which by counteracting the free radicals that cause DM cataract (Tables 2 and 3; Figures 1 and 2).

Values are denoted as mean$\pm$ SEM; n=6; a= P<0.05 compared to normal control, b= P < 0.05 compared to DC; c=P<0.05 vsfisetin5 μg/ml, d denotes for P<0.05 vs. fisetin10 μg/ml treated; Abbreviations: NC- normal control; DC- diseases control;

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Values are denoted as mean $\pm$ SEM; n=6; a=P<0.05 vs DC, b=P < 0.05 vs fisetin5 μg/ml; c=P<0.05 vs. fisetin10 μg/ml treated; Abbreviations: NC- normal control; DC- diseases control.

CONCLUSION

From the above work, it is clear that the Fisetin showed promising in-vitro potential in controlling DM cataract in extracted goat lens model. The data obtained in this study also indicate that the observed beneficial effects mainly due to activation of Nrf2/HO-1 pathway. These effects probably result in increased tissue anti-oxidant status as well as decreased free radical production, which ultimately responsible for the observed beneficial effects of Fisetin against hyperglycemia-induced cataract.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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