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Piperine: A Potent Antioxidant from Black Pepper in Treatment of Glucose-Induced Cataract

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ABSTARCT

Cataract is an opacity within the clear natural crystalline lens of the eye that causes eyesight impairment over time. Ageing, free radical production, diabetes, and oxidative stress-induced protein denaturation are all factors that contribute to cataract formation. Antioxidants protect the eyes by minimizing the effects of free radicals. In this study, we tested the anti-cataract effect of antioxidants such as piperine and ascorbic acid using an in vitro glucose induced cataract model. The current work used goat lenses to test the anti-cataract efficacy piperine in vitro against glucose-induced cataractogenesis. Clear isolated goat lenses are separated into six experimental groups and cultured in produced aqueous humor. For a period of 24 hours, 15µg/ml, 30µg/ml, and 60µg/ml of piperine are incubated simultaneously with glucose (55mM) and glucose (5.5mM). Higher levels of Na+ and MDA (P0.001) were seen in cataract-inducing lenses, as well as lower sodium-potassium ATPase activity and water-soluble protein content. The typical medication is ascorbic acid (40µg/ml). The opacity of the lens is measured at the end of the incubation period by photographic evaluation. The piperine shows significant prevention of cataractogenesis of eye lenses by piperineat 60µg/ml.

KEYWORDS: Anti-cataract, piperine, glucose, in-vitro, ascorbic acid, antioxidants.

I. INTRODUCTION

"Because increasing levels of glycosylated hemoglobin are involved in an increased risk of cataract formation, the disease cataract, or lens opacification, occurs commonly in diabetic patients of advanced age".[1]"According to varied experimental data, numerous cataract-inducing variables have been identified; nonetheless, the molecular cause of cataractogenesis remains unknown. Cataracts are caused by a variety of reasons, the most common of which is the aggregation of proteins on the eye lenses. The activity of the lens's Na+ — K+ -ATPase plays a critical role in maintaining the lens's transparency, and its imbalance causes Na+ deposition and K+ loss, as well as water absorption through the lens fibers, resulting in cataract formation". [2] "A variety of medicines have been tried for the treatment of cataracts, but none of them have proven to be effective". [3]

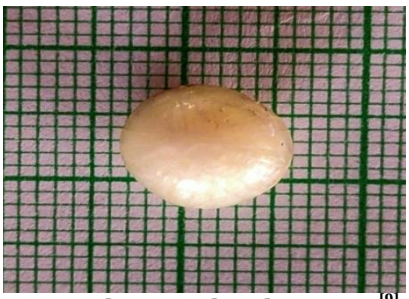

Fig. 1: Glucose induced cataract [9]

"The enzyme aldose reductase is thought to play a role in the development of eye problems such as cataracts". [4]"The aldose reductase converts sugar molecules such as glucose, galactose, and xylose into their corresponding alcohols via metabolic pathways. These alcohols, known as polyols, accumulate within the lens, causing osmotic consequences. As a result, polyols do not easily diffuse out and do not metabolize

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quickly, causing hyper tonicity, which leads to cataract formation". [5]"The oxidative pathway is also important in biological processes such as cataract development. The production of superoxide radicals in the aqueous humor and in the lens, as well as their derivatization to other strong oxidants, may be responsible for triggering a variety of metabolic damaging processes that contribute to cataract formation". [6]"In various experimental techniques, angiotensin converting enzyme inhibitors have been shown to protect against free radical damage".[7]

"Due to antioxidant and free radical scavenging properties, ascorbic acid (ACE Inhibitor) was proven to have substantial anticataract activity in vitro. Ascorbic acid is used as a reference, and we assess several parameters in vitro on goat lenses, such as (Na + & K+) estimate, Na+ -K + ATPase activity, Proteins (total proteins and water-soluble proteins), and malondialdehyde (MDA)". [7]

"Diabetes was discovered to be one of the primary risk factors for cataract progression in an animal investigation. Extracellular glucose diffuses into the lens during hyperglycemia, causing post-translational changes. The synthesis and aggregation of additional sorbitol in the lens fibers, as well as subsequent osmotic stress, are the primary causes of cataract formation. Sorbitol is made by aldose reductase using NADPH and cannot easily pass cell membranes; it can build up in cells and disrupt osmotic balance, causing injury. Deficient glutathione (GSH) levels play a role in the pathophysiology of cataract formation by retaining lens proteins in their reduced state. However, when compared to normal, GSH levels in cataracts were found to be considerably lower". [8]"Cataract is associated with a reduction in the activity of protective antioxidant enzymes including as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione (GSH). One of the main causes of diabetic cataract advancement is a high blood glucose level. Glycation of sugars and their binding with amino groups on proteins to form adducts causes proteins to lose their biological properties. This induces structural alterations in enzymes, which leads to their deactivation". [10]

Oxidative stress

"Another factor involved in cataract development is oxidative stress, which promotes oxidation of lens protein and affects the lens. Reduced levels of glutathione, ascorbate, and antioxidant enzymes like catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase, as well as increased age in the human eye, were the key causes in cataract formation".[8]

"Life's complexity Fortunately, most other compounds react slowly with oxygen in the air around us. The superoxide radical (O2-) is formed when an additional, unpaired electron (reduction) is added to the oxygen molecule, weakening the oxygen-oxygen link and making it somewhat more reactive. Free radicals are species that possess one or more unpaired electrons and are capable of autonomous existence. Free radical reactivity varies greatly, and some non-radical oxygen generated chemicals are actually more reactive than oxygen free radicals. The term "reactive oxygen species" (ROS) is more commonly used because it encompasses both oxygen free radicals and non-radical O2 derivatives".[8]

II. MATERIALS AND METHODS

Extraction of Piperine from Black Pepper

Fig. 2: Black pepper [12]

"10 gm of black pepper powder was taken and extracted it with 150ml 95% ethanol in Soxhlet extractor for 2 hours. The solution was then filtered and concentrated on the water bath at 60^{o}C. 10 ml 10% of alcoholic potassium hydroxide was added to the filtrate with continuous stirring. After this the insoluble residue was filtered and alcoholic solution was left overnight and again filtered through a membrane filter".[11]

Fig. 3: SoxhletExtraction and Evaporation of Piperine

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[8]Requirements

Test Drug: Piperine

Chemicals:

Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium chloride (MgCl2),
Sodium bicarbonate (NaHCO3), Calcium Chloride (CaCl2), Glucose, Penicillin,
Streptomycin, Ascorbic acid.

Instruments:

Incubator, Wired mesh, Petri dish.

Dose selection: Piperine - 15, 30 and 60µg/ml, Standard Ascorbic Acid:40µg/ml.

Collection of Eyeballs:

In this experiment, goat eyeballs were used. They were taken straight from the slaughterhouse and transferred to the lab at a temperature of 0-4°C.

Procedure

Lens Culture:

"A Fresh goat eyeballs were received from the slaughterhouse and delivered to the laboratory at a temperature of 0-4°C almost immediately. Extra capsular extraction was used to remove the lens, which was then incubated at room temperature in unreal aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaHPO_4$ 0.5 mM, $CaCl_2$ 0.4 mM, and glucose 5.5 mM) with pH 7.8 maintained by the injection of $NaHCO_3$). To prevent bacterial contamination, penicillin G 32 percent and streptomycin 250 mg percent were added to the culture media. The lens was metabolized by the sorbitol pathway at high concentrations, resulting in polyol buildup and overhydration, as well as oxidative stress. Cataractogenesis is the result of this".[8]

Induction of in-vitro cataract:

"Cataracts were induced using glucose at a concentration of 55mM. The sorbitol pathway metabolizes glucose in the lens at high quantities. Polyols (sugar alcohols) build up in the body, causing overhydration and oxidative stress. Cataractogenesis is the result of this. These lenses were cultured for 72 hours in artificial aqueous humor with various concentrations of glucose (5.5 mM as a normal control and 55 mM as a hazardous control)".[8]

Study Design and Groups:

Goat lenses were divided into six groups of six lenses each following table:

Group No.	Group Name	Treatment	Drug Dose
I	Normal control	Aqueous Humor + 5.5 mM Glucose	1:1
II	Negative control	Aqueous Humor + 55 mM Glucose	1:1
III	Standard (Positive control)	Aqueous Humor +55 mM Glucose + Standard (Ascorbic Acid)	40 µg/ml
IV	Test 1	Aqueous Humor + 55 mM Glucose +Piperine	15µg/ml
V	Test 2	Aqueous Humor + 55mM Glucose +Piperine	30 µg/ml
VI	Test 3	Aqueous Humor + 55mM Glucose + Piperine	60 µg/ml

Table No. 1: Study design and Groups [7]

Photographic Evaluation

"To test lens opacity, lenses were placed on a wired mesh with the posterior surface touching the mesh, and the pattern of mesh number of squares clearly visible through the lens was measured. The following is how the degree of opacity was graded":[8]

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Grade	Degree of Opacity
0	absence of opacity
1	slight degree of opacity
2	presence of diffuse opacity
3	presence of extensive thick opacity

Table No. 2: Degree of Opacity [8]

Preparation of lens homogenate

"After 72 hours of incubation, lenses were homogenized in tris buffer (0.23 M, pH 7.8) containing 0.25 10-3 M EDTA, and the homogenate was adjusted to 10% w/v, centrifuged at 10,000 G for 1 hour at 4°C, and the supernatant was used to estimate biochemical parameters".[8] "For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH-7.4)". [7]

Biochemical Analysis

"Flame photometry was used to quantify the electrolytes sodium and potassium (Na+ and K+). Unakar and Tsui's method"[13] was used to measure sodium potassium ATPase activity, and Lowry's approach was used to estimate protein. [14]"Wilbur's approach was used to determine the level of oxidative stress". [15]

Estimation of total protein content:

"4.0 mL alkaline copper solution was added to 0.1 mL lens homogenate and let to stand for 10 minutes. Then, 0.4 mL of phenol reagent was quickly added and stirred, and the mixture was incubated for 30 minutes at room temperature for color development. In a UV-visible spectrophotometer, readings were collected against a blank produced with distilled water at 610 nm. The protein content was determined using a bovine serum albumin standard curve and represented as g/mg lens tissue". [8]

Statistical Analysis

"All data was presented as mean standard deviation. SPSS/10 student program was used to examine all of the data. The done-way analysis of variance (ANOVA) and LSD are two approaches for evaluating hypotheses. The results were regarded substantially different if P0.05, and the values were reported as mean S.D. Normal Goat lens vs Goat lens + Glucose 55mM, Goat lens + Glucose55mM vs Goat lens + Glucose55mM + piperineare the statistical variations compared".[8]

III. RESULT:

In- vitro anti-cataract activity:

After 8 hours of incubation, lenses with glucose 55mM demonstrate opacification on the lens's periphery and posterior surface. Complete opacification is gradually increased to words the center at the end of 72 hours.

Photographic evaluation:

The transparency of the Group I (normal control group) was preserved after 72 hours of incubation, whereas the transparency of the Group II (negative control group) was completely lost, showing complete cataractogenesis. Squares of graph paper were visible through the lenses in Group III (Standard/Positive control), which contained lenses treated with conventional ascorbic acid. The squares of the graph paper were visible through the lenses of goat lenses in groups with increased amounts of piperine (Group IV, V, VI), showing that cataract formation was suppressed. Group VI (60g/ml) [Test III] was more efficient than Group IV [Test I] and Group V [Test II] in preventing cataract formation.

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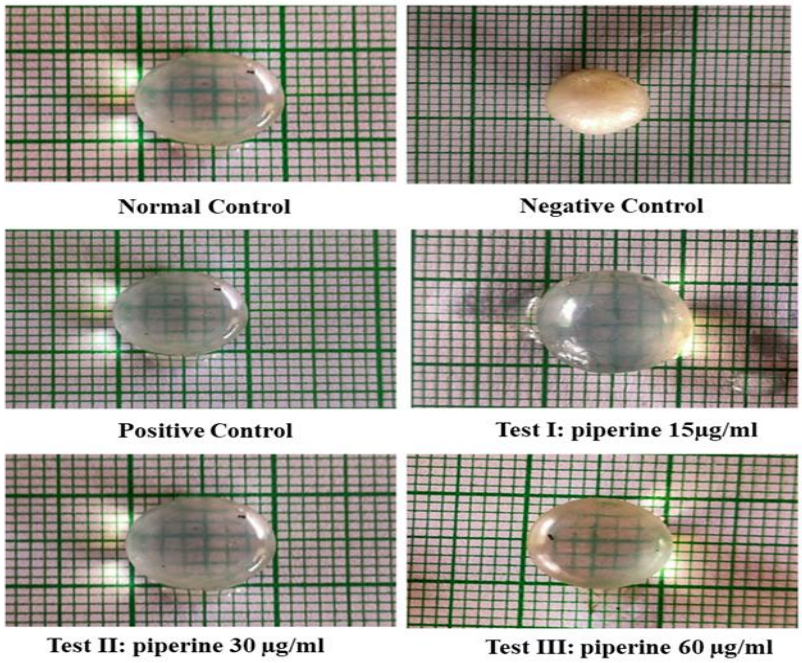
Fig. 4: Anti-cataract activity results



Sr. No.	Compound	Degree of Opacity
1	Normal	0
2	Negative control (Glucose 55 mM)	3
3	Positive control (Ascorbic acid 40µg/ml)	1
4	Test I (Piperine 15µg/ml)	2
5	Test II (Piperine 30µg/ml)	1
6	Test III (Piperine 60µg/ml)	0

Table No. 3: Effect of piperine on degree of opacity on lens by glucose induced cataract

Normal Group	0-degree opacity occurred; clear lens isobtained.
Negative Control (Glucose 55 mM)	The presence of extensive thick opacity, because of higher concentration of glucose-induced cataractogenesis.
Positive Control (Ascorbic acid 40 µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test I (Piperine 15µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test II (Piperine 30µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test III (Piperine 60µg/ml)	0-degree opacity is occurred, the clear lens is obtained. Test drug inhibits cataractogenesis.

Table No. 4: Effect of piperine on goat lenses by glucose induced cataract

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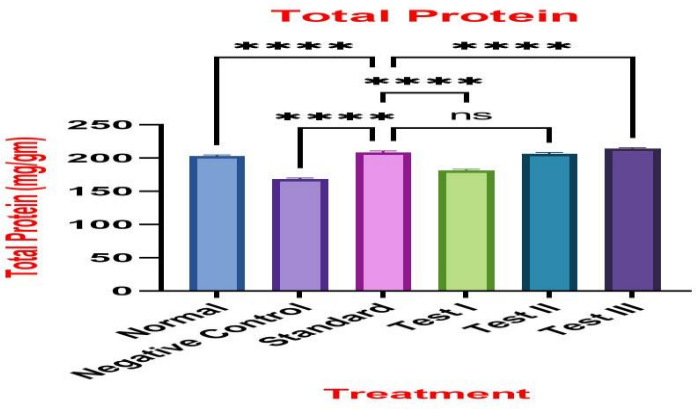
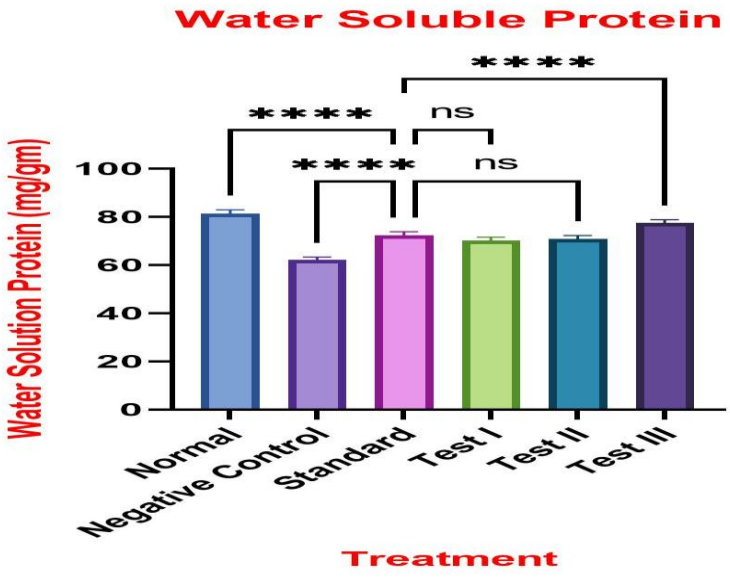
Group No.	Treatment	Total proteins [mg/gm]	Water-Soluble proteins [mg/gm]
I	Normal control (Glucose 5.5mM)	203	81.33
II	Negative control (Glucose 55mM)	168.5 ##	62.16##
III	Standard (Glucose 55mM+Ascorbic Acid 40µg/ml)	208.5	72.33
IV	Test I (Glucose 55mM+piperine 15 µg/ml)	181.66	70.16
V	Test II (Glucose 55mM + piperine 30 µg/ml)	206.5	70.83
VI	Test III (Glucose 55mM + piperine 60µg/ml)	214.5****	77.5****

Table No.5: Effect of Piperine on Protein levels (total proteins and water-soluble proteins) in Goat lens homogenate after 72 hours of incubation in glucose 55 mM induced cataract



Graph No. 1 Effect of piperine on Total protein levels in Goat lens in glucose induced cataract



Graph no: 02: Effect of piperine on water soluble protein contained in goat eye lenses

A goat eye lens model was used to test piperine'santicataract effects in vitro. Piperine has a variety of biological roles in this area, including anti-cataract action. Piperine dramatically improved lens morphology, activity, and clarity: 50% of the eyes had nearly clear lenses, while

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100% of the negative control eyes acquired dense nuclear opacity. Piperine appears to protect the lens from oxidative stress in the current investigation.

These findings in in vitro investigations of glucose-induced cataracts not only show that Piperine has a protective effect, but also that it prevents cataractogenesis due to its antioxidant capabilities. As a result, piperine may be effective for cataract prevention or treatment. The lens becomes entirely opaque after 72 hours of incubation in glucose 55 mM, as opposed to normal control lenses. Lenses were incubated with Piperineand ascorbic acid at both doses, which appeared to slow the progression of opacification as compared to lenses treated with glucose 55 mM. (Negative Control). When compared to the negative control group, the effect of Piperine demonstrated a significant delay in the advancement of lens opacification and was close to normal.

The values are expressed as Mean SEM for N=6. When compared to a normal control, the following comparisons were made: # p <0.05, ## p< 0.01. When compared to the negative control, * p<0.05 and **** p< 0.01 were found. (Values are compared using a one-way ANOVA Dunnett t test after 72 hours) It's insignificant.

When compared to normal lenses, the treated lenses (Group-II) with glucose 55 mM solution had significantly lower protein concentrations (total and water-soluble proteins) in the lens homogenate (P<0.01) (Group-I). When compared to Glucose 55 mM treated lenses, lenses treated with ascorbic acid (Group-III) and lenses treated with piperine (Group-IV, V, VI) had greater protein concentrations (total and water-soluble proteins) (P<0.01) (Group-II).

IV. DISCUSSION

Anti-cataract activity:

Electrolytes sodium and potassium, malondialdehyde (MDA), and proteins are some of the parameters that are usually considered in cataractogenesis (total proteins including water soluble proteins). The total protein content of goat lens homogenate was depleted in general and water-soluble protein in particular after incubation in medium containing 55 mM glucose concentration. Hydration and inflammation of the lens, as well as changes in the protein composition, are caused by changes in the Na+ /K+ ratio caused by reduced Na+ /K+ ATPase activity in the lens.Both of these factors contribute to cataract development. In this study, ascorbic acid and piperine treated groups had higher total and water-soluble proteins. Ascorbic acid and piperine treatment groups appear to reduce cataract development pathogenesis. This could be owing to their ability to scavenge free radicals.After 72 hours of incubation in aqueous humor with 55 mM glucose, the lens was clear due to the low concentration of glucose, which had no effect on the lens, and the number of squares could be seen through the lens. The lens was completely transparent.

In the negative control, the lens was incubated for 72 hours in aqueous humor and 55 mM glucose solution to induce high glucose concentrations in the lens, which are metabolized via the sorbitol route, inducing hydration and oxidative stress. Cataractogenesis is the result of this. The lens has a lot of thick opacity.When the number of squares visible through the lens in the Standard group was compared to Test-3 after 72 hours of incubation in Aqueous humor + 55 mM Glucose+ 40g/ml Ascorbic acid standard medication, the lens showed little degree of opacity.

After 72 hours of incubation in Aqueous humor + 55 mM Glucose + 15 g/ml and 30 g/ml piperine test drugs, the number of squares were not clearly visible through the lens in Test-1 and Test-2, however the lens in piperine 60 g/ml test-3 drug showed slight opacity. In Test-3, the number of squares visible through the lens after 72 hours of incubation in Aqueous Humor + 55 mM Glucose + 60 g/ml piperine medication because the test medication prevents cataractogenesis and oxidative stress, the lens was clear.

On isolated goat lens, an in-vitro model for developing cataract using 55 mM glucose solution provides an efficient model. Incubation of goat lenses in fluid with increased glucose (55 mM) concentration resulted in cataract formation and a significant decrease in Na+/K+ - ATPase activity as opacity progressed. With hydration and inflammation of the lens fibers, a defect in the Na+/K+ ATPase promotes sodium buildup and potassium loss, resulting to cataractogenesis. The protein content of the lens changes as the Na+/K+ ratio changes, resulting in a decrease in total proteins and lens opacification.

The action of piperine, which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentration, and intracellular glucose, inhibited the imbalance of Na+ and K+. This impact can be attributed to piperine'sadoptogeniccapability.The effects of

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piperine on a glucose-induced cataract in a goat eye were tested. The lens morphology and activity were greatly protected by piperine, and 50% of the eyes had practically clear lenses; in comparison, 100% of the negative control eyes exhibited dense nuclear opacity. According to the present research, piperine protects the lens from oxidative damage. These findings in glucose-induced cataracts in vitro not only demonstrate piperine's protective impact, but also suggest that its antioxidant properties inhibit cataractogenesis. As a result, piperine may be effective for cataract prevention or treatment. In contrast to normal lenses, lenses incubated in glucose 55 mM for 72 hours become completely opaque.

Lenses were incubated with piperine and ascorbic acid at both concentrations, which appeared to slow the progression of opacification as compared to lenses incubated in glucose 55 mM. (Negative Control). When compared to the negative control group, the effect of piperinerevealed a significant delay in the advancement of lens opacification, which was near normal.

V. CONCLUSION

According to the results of this investigation, piperine treatment increased the quantity of water-soluble proteins and slowed the course of cataractogenesis caused by high glucose levels. Because of its antioxidant qualities, piperine has anti-cataract action, which may be useful in avoiding cataract formation. The mechanism of action of piperine in anti-cataract activity could be due to the following factors:

- Because of the reduced oxidative stress of the lens and the end products of glycation such as protein, lipid, and nucleic acid produced during the development of cataract, piperine suppresses advanced glycation and glyco-oxidation.
- Piperine's anti-oxidant properties also reduce the creation of reactive oxygen species, which suppresses cell growth, apoptosis, and cell dysfunction, as well as producing an anti-cataract effect.

Piperinemay also help to prevent cataractogenesis by increasing the water content of lens proteins. More research into the mechanism of action of piperine is needed, and it could have a significant impact on future clinical treatments for cataract patient's illnesses.

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