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Research Article

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Inhibition of UVB induced oxidative stress in *Catla catla* & Glutamate pyruvate transaminase (GPT) activities by embelin using molecular docking tool

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ABSTRACT

The present study aimed to evaluate the inhibitory effect of embelin against UVB induced oxidative stress using in vivo (Catla catla) fish model. Larve [Catla catla (1.2+0.01 mg)] were fed with three types of diets control diet (Group I & Group II with and without UVB exposure) and diet containing 0.1 & 0.5% embelin (Group III & IV) respectively. After 40 days of feeding, embelin fed fishes (Group III & IV) were exposed to UVB radiation (145 μW/cm^2) for six days for 10 minutes. Survival rate of fishes after UVB exposure was recorded. Tissue samples were collected from the whole body and used for biochemical and enzyme analyses. In additional to these, we also docked embelin, 5-O-methyl embelin and vilangin with glutamate pyruvate transaminase (GPT) chain-A using Discovery Studio Version 3.1. Results revealed that statistically significant difference in the survival rate was observed between the control verses embelin fed fishes. Similarly, significant reduction in Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) enzymes were observed in the embelin fed fish groups. With reference to docking studies, vilangin fails to dock, whereas embelin and 5-O-methyl embelin showed interaction energy of -38.0 & -38.3 kcal/mol respectively. Thus, the present study suggested that embelin protects Catla catla (fish) against UVB induced oxidative stress, which could also substantiate our earlier in vitro study.

INTRODUCTION

Gradual deterioration of the ozone layer in recent years are due to extensive release of man-made ozone depleting chemicals into the atmosphere which has led to concern over the exposure of terrestrial organisms as well as aquatic organisms to the increased level of ambient UVB radiation. Many vertebrate and invertebrate species have been protected from UVB by natural self defense mechanism (pigments and/or extensions of the integument such as feathers, hair, shells or scales), however, these defenses are inadequate, and an increased risk of UVB mediated effects (including DNA damage, melanogenesis, skin erythema, non-melanoma skin cancer, immune-suppression and oxidative stress) were realized at higher level. To date, biological effects of UVB radiation have been documented in aquatic organisms such as Daphnia [1], sea urchin [2], northern pike [3], crab [4], red sea bream [5], Indian crap [6-7], zebrafish [8], Caspian sea salmon [9] and fresh invertebrates [10].

Catla catla (Catla), an Indian carp is a commercial edible fish species cultured in the South Asian region. It has been used for stocking reservoirs and in polyculture systems [11]. *Catla catla* is one of the exotic fish species currently used in culture based fishery in inland reservoirs of Sri Lanka. *Catla catla* has a high market demand and as well as emerging as a useful vertebrate model organism in recent years, similar to that of zebrafish (*Danio rerio*). Ishaq Ahmed and co-workers [12] reported a new fibroblastic-like cell line from heart muscle of the Indian major carp (*Catla catla*). Dash and co-workers [13] reported embryonic stem-like cells of Indian major carp (*Catla catla*). Swain and co-workers [14] reported molecular characterization, inductive expression and mechanism of interleukin-10 gene induction in the Indian major carp (*Catla catla*). Moreover, 3D modeling and molecular dynamics simulation of an immune-regulatory cytokine, interleukin-10, from the Indian major carp (*Catla catla*) was reported by Sahoo and co-workers [15].

In our earlier studies, we reported the antimicrobial [16] and UVB inhibitory activity (*in vitro*-lymphocytes model) of embelin [17]. Similarly, embelin has been reported to bind with collagen [18], tyrosinase [19] and human neutrophil elastase [20] using molecular docking studies. Therefore, in the present study, UVB protective effect of embelin was assessed using *Catla catla* larvae for the period of 52 days and examined for the survival rate before and after UVB exposure. In addition, to the above biochemical parameters [lipid peroxidation, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)] were studied. Furthermore, we also docked embelin, 5-O-methyl embelin and vilangin with glutamate pyruvate transaminase (GPT) using Discovery Studio Version 3.1.

EXPERIMENTAL SECTION

Animal source, housing conditions and maintenance

Catla catla larvae (weight: 0.8 ± 0.04 mg) were obtained from local aquarium and cultured in recirculating system. The stocking density was 50 fish/ aquarium (15 liters). Portable water was used and the depth of water in the aquarium was 20 cm. Water temperature and pH was ranges from 27–28° C and 7.25–7.5 respectively throughout the study period. Dissolved oxygen (DO) level was maintained as >5 mg/l. The photoperiod was set at 12:12 h. Fluorescent lamps (Philips 20 W) were used as ambient light source for both treated and control fish [6].

Exposure to UVB light

Based on the pilot study, two concentrations of embelin (0.1 & 0.5% (w/w) was prepared [5] and subsequently chosen for the following experimental studies. In brief, the experiments were designed as four different groups as described below with six animals in each group; Group I- larvae fed with normal diet for the period of 40 days without any exposure to UVB radiation, Group II, larvae fed with normal diet for the period of 40 days and exposure to UVB radiation as described by Sharma & Chakrabarti[6]; Group III, larvae fed with 0.1% embelin along with normal diet for the period of 40 days and exposed to UVB radiation; Group IV larvae fed with 0.5% embelin along with normal diet for the period of 40 days and exposed to UVB radiation..

Survival and biochemical studies

Followed by exposure of UVB radiation for six days larvae were collected for each group and the survival was checked by counting the live fishes and results were tabulated. After the experimental period, fishes were subjected to homogenization in the presence of homogenization buffer (50mM Tris-HCl mixed with 1.15 gram of Potassium chloride and pH adjusted to 7.4) and subjected to varied biochemical assays which includes lipid peroxidation, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT).

Lipid peroxidation assay

Lipid peroxidation was assayed by measuring malondialdehyde (MDA) formation as described by Sharma and Krishnamurthy [21]. The homogenate was centrifuged at 10,000 g for 20 min in a refrigerated centrifuge at 4ºC. The clear supernatant was collected and one ml of supernatant was taken as an aliquot in separate tube to which one ml of thiobarbituric acid reacting substances (TBA) solution was added. The tubes were kept in boiling water bath for 10 min. After cooling the tubes, the optical density was read at 535 nm using UV-Visible spectrophotometer (Shimadzu, Japan). Results were expressed in terms of TBARS formation in nmol/mg of protein.

Enzyme assays
For determination of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), the homogenate was centrifuged and the supernatant was used. Both GOT and GPT were determined by using diagnostic kits (Bayer Diagnostics, Baroda, India).

Docking studies
Docking studies were carried out on the crystal structure of glutamate pyruvate transaminase (GPT) retrieved from Protein Data Bank (pdb id: 3IHJ, with resolution 2.3̊ A) using the CDOCKER protocol under the protein-ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA). The docking protocol was followed as described by Radhakrishnan et al [20].

RESULTS AND DISCUSSION

The most critical stage for any living organism is exposure to harmful agents like UVB radiation, during the embryonic and larval developmental stages. The outcome of information on UVB exposure versus response (survival time) was highly required. The ecological consequence of UV radiation has to be well understood for the betterment of commercial fisheries and also for the betterment of human kind. Recently, Dieckol from *Ecklonia cava* demonstrated strong protective property against UVB irradiation in zebrafish as reported by Ko and co-workers [22]. Similarly, Grape seed proanthocyandin extract (GSE) injected into zebrafish showed protective activity against UVB irradiation has been reported by Luo and co-workers [23].

In the present study emphasizes inhibitory effect of embelin (natural antioxidant) against UVB induced oxidative stress in *Catla catla* (fish). Results on survival rate of fish larvae showed no significant difference between the control group larvae (Group I) and larvae fed with 0.1% embelin (Group III). However, an increase in embelin concentration to 0.5 % in the feed mixture, significant reduction in survival rate was observed as shown in Table 1.

Table 1 Percentage of survival rates of *Catla catla* larvae when exposed to UVB radiation

Groups	Percentage of survival	Statistical significant
Group-I (Control)	83 ± 0.24	
Group-II (UVB Control)	62 ± 0.4	P< 0.05 vs Group-I
Group-III (0.1% embelin)*	90 ± 0.3	P< 0.05 vs Group-I & II
Group-III (0.5% embelin)*	95 ± 0.5	P< 0.05 vs Group-I & II

Note: % in w/w*

Similarly, earlier studies with artificial UV radiation (280 and 310 nm) demonstrated that mortality in sockeye salmon eggs varies with dose [24]. UVB exposure compromises antioxidant systems that could contribute to reduced survival of Zebra fish as reported by Charron et al. (2000). Even, minor increase in ambient UVB radiation coming to the earth's surface leads to cause of lethal effects to pike larval fish, as they are high sensitive to UVB [25].

With regard to TBARS formation of whole body of larvae enzymes (0.8 nmol/mg of protein were reduced significantly (P< 0.05) in the case of 0.1 % embelin fed experimental group (Group III), compared to control (Group I) (1.9 nmol/mg of protein) as well as compared to UVB control (Group II) (3.6 nmol/mg of protein). Similarly, in the case of 0.5% embelin fed experimental group (Group IV) TBARS formation was 0.4nmol/mg of protein. Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT) activities of whole body of larvae both enzymes (18.81 ± 0.51 & 44.04 ± 0.45 IU/L respectively) were reduced significantly (P<0.05) in the case of 0.1 % embelin fed experimental group (Group III), compared to control (Group I) (20.27 ± 0.43 & 46.34 ± 0.37 IU/L respectively) as well as compared to UVB control (Group II) (29.75 ± 0.41 & 52.56 ± 0.39). Similarly, in the case of 0.5% embelin fed experimental group (Group IV) GOT and GPT were 20.11 & 43.43 IU/L respectively. Both, GOT & GPT levels were drastically elevated in the groups of fish irradiated with UV-B light than the control group fish as reported by Sharma et al [26], which was par on the present findings. Whereas, in the present study elevated GOT & GPT levels were decreased due to pre-fed with embelin (0.1%) containing diet (Group II) and almost on par control group (Group I). These results indicate that pre-fed with embelin (0.1%) containing diet, prevents the UVB induced damage in the experimental fishes.

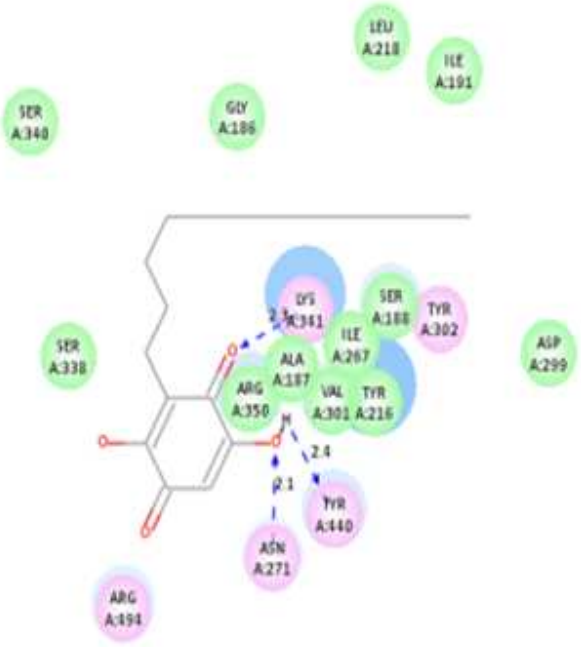
Docking studies was performed, in order to substantiate our *in vivo* results, where were docked with three ligands namely embelin (ID: 3218), 5 –O-methyl embelin (ID: 171489) & Vilangin (ID: 417182) with that human glutamate pyruvate transaminase (GPT) A-chain (PDB ID: 3IHJ). Glutamate pyruvate transaminase (GPT) is also known as alanine transaminase (ALT; EC: 2.6.1.2). This, enzyme was reported to elevate during UVB radiation, especially in fishes [26]. This enzyme also serves as biomarker of liver injuries, in human beings [27]. Interestingly, vilangin (dimeric form of embelin) fails to dock with glutamate pyruvate transaminase (GPT) A-chain, this might be generally due to poor binding phenomenon [28]. In contrast to the above, both embelin and 5-O- methyl embelin showed interaction energy of -38.0 kcal/mol & -38.3 kcal/mol respectively. In the present study, embelin showed interaction with Asn271, Lys341 and Tyr440 amino acid residues, whereas 5 methylembelin showed interaction only with Lys341 amino acid residue as shown in Table 2 and Figure1.

Table 2. The interaction energy analysis of three ligands (embelin , 5 –O-methyl embelin, & Vilangin) with that of human glutamate pyruvate transaminase (GPT) A-chain (PDB ID: 3IHJ) using Discovery Studio® 3.1

Ligand name	cDocker interaction energy* (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Embelin	38.0	Asn271 Lys341 Tyr440	2.1 2.3 2.4
5-O-methyl embelin	38.3	Lys341	2.3 & 0.8
Vilangin**	Nil	Nil	Nil

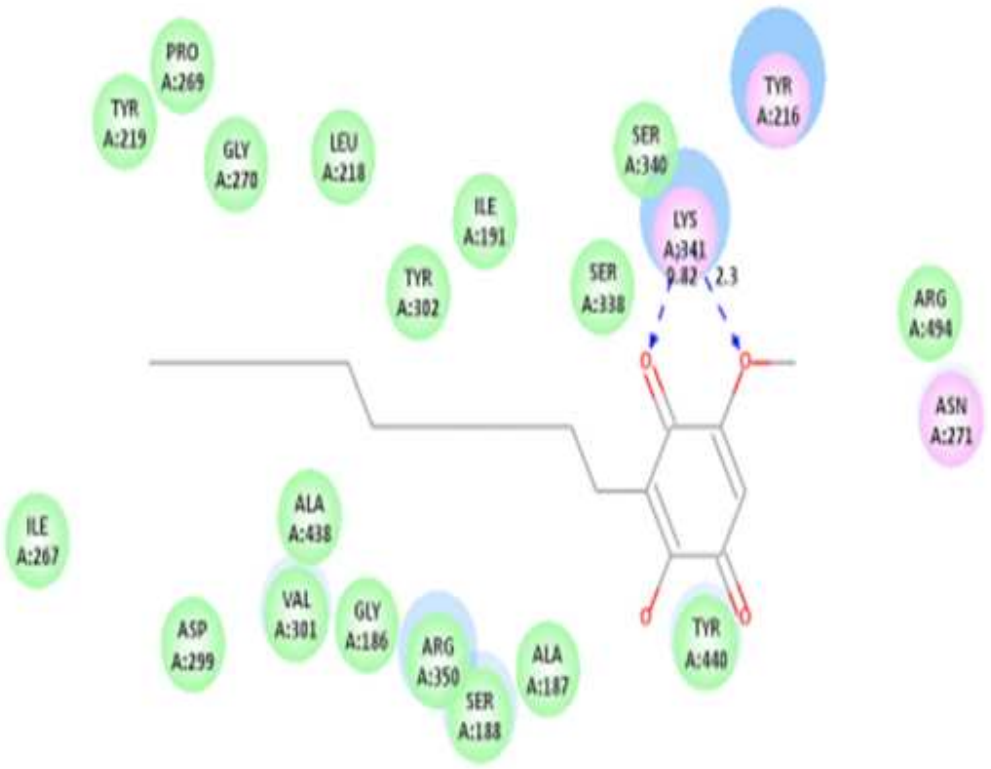
*(**Note:** *- Calculated interaction energy for the highest ranked, docking pose; **- Failed to dock with protein, may be due to poor binding).*

Figure 1a. The interaction analysis of the embelin, with that of human glutamate pyruvate transaminase (GPT) A-chain



*(**Note**: Hydrogen atoms have been omitted in the two dimensional diagram for better clarity. Pink line indicates the charge interaction. In addition to this, bond distances are indicated in angstroms (Å) unit)*

Figure 1b. The interaction analysis of the 5-O-methyl embelin, with that of human glutamate pyruvate transaminase (GPT) A-chain



*(**Note:** Hydrogen atoms have been omitted in the two dimensional diagram for better clarity. Pink line indicates the charge interaction. In addition to this, bond distances are indicated in angstroms (Å) unit)*

CONCLUSION

Thus, the present finds suggested that embelin protects *Catla catla* (fish) against UVB induced oxidative stress, which could also substantiate our earlier *in vitro* study.

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