RADIOPROTECTION BY CURCUMIN ON DNA DAMAGE OF BLOOD CELLS IN DUCKS (*Anas platyrhyncos domesticus*)

***Ramnath V.[1], Aziz Zarina[2], Beena V.[3] and Sureshkumar P.[4]**
Department of Veterinary Physiology
College of Veterinary and Animal Sciencess, Mannuthy, Thrissur

ABSTRACT

Curcumin (Diferuloyl methane), a principal curcuminoid of the Indian spice turmeric (family:Zingiberaceae) was examined for its radioprotective effect in Kuttanad ducks.Forty two Kuttanad adult female ducks were utilized. An intravenous injection of curcumin (40mg/kg b.w.) in Dimethyl sulfoxide, as well as Dimethyl sulfoxide (DMSO) alone was given, 20 min prior to blood withdrawal. Whole blood from curcumin treated, DMSO alone administered and untreated ducks was exposed to 0.5 and 1 Gy gamma irradiation. Single cell gel electrophoresis under alkaline condition was performed to assess the comet parameters yielded by cellular DNA. Curcumin treated ducks showed a significant (P< 0.005) decreased DNA damage, induced by both 0.5 and 1 Gy exposure. Results were suggestive of radioprotective property of curcumin in blood cells of ducks.

INTRODUCTION

Water fowl such as ducks and geese are included in the diets of human. Water fowl that eat fish are higher on the food chain strata than those that eat plants or insects like geese. Contaminants like organic and inorganic pollutants (insecticides, herbicides and radioactive metals) ultimately reach water resources and build up in the fat tissue of aquatic fauna, are a concern to the health of water fowl. Contaminants become more concentrated when predators eat prey leading to biomagnification within the body system.

At high levels, radiations can cause cell damage and even cancer. Ionizing radiation inflicts deleterious effects by damaging cellular DNA and membrane (Weiss and Landauer, 2003). Little information is available about effects of low levels of radiations in health aspects of water fowl.

Several plant compounds are reported to have radioprotection properties (Arora *et al.,* 2005). Curcumin (Diferuloyl methane) is the principle curcuminoid of popular Indian spice turmeric; member of ginger family (Zingiberaceae). Curcuminoids are polyphenols, which impart yellow colour to rhizomes of *Curcuma longa*. Curcumin has been extensively studied for its anti-oxidant, anti-inflammatory (Menon and Sudheer, 2007) and anti-cancer (Jagetia and Aggarwal, 2007) properties. However its radio protective potential has not yet been exploited in the veterinary field.

Alkaline comet assay is a sensitive technique to monitor strand breaks and alkali labile DNA lesions and rightly used to study genotoxicity, cellular DNA lesions, apoptosis and DNA repair (Olive, 1999). The present study is focused to find on the DNA protecting ability of curcumin in Kuttanad ducks by adopting comet assay, which is considered to be a rapid and sensitive method for detection of primary DNA damage at the single cell level.

MATERIALS AND METHODS

The study was performed on healthy adult female Kuttanad ducks; six months age; procured from University Poultry Farm, KAU, Mannuthy. These ducks were grouped (6 / group) into G I: untreated and non irradiated, G II: Untreated and irradiated, G III: treated with curcumin @40mg/kg

[1]Associate Professor, [2&3] Assistant Professor,
Department of Veterinary Physiology
[4]Professor and Safety Officer, College of Horticulture
* Corresponding author Email : drvrnath@gmail.com. Ph: 9446579057

body wt. and irradiated and G IV: administered with Dimethyl sulfoxide (DMSO) intravenously and irradiated.

Required quantity of curcumin (40 mg/kg body weight) was dissolved in 500µL of DMSO and given by slow intravenous route through saphenous vein to G III and 500µL of DMSO alone to G IV birds. Blood was collected (3ml) in anticoagulant, dipotassium EDTA, from all birds of G I and G II, but after 20 min of intravenous administration of curcumin from G III and of DMSO from G IV. Whole blood samples from untreated (G II), curcumin treated (G III) and DMSO alone administered (G IV) which were exposed to 0.5Gy Gamma radiation, were named as G IIA, G IIIA and G IVA respectively. Those blood samples from untreated, curcumin treated and DMSO alone administered exposed to one Gy Gamma radiation were considered as G IIB, G IIIB and G IVB respectively.

The alkaline comet assay (Singh, 2000) was used to access the effect of irradiation on cellular DNA and for any protective effect of curcumin on DNA damage. In short, the comet assay was conducted in alkaline medium on frosted slides coated with agarose. Precoating of slides was done with normal melting point agarose (1% in PBS: pH 7.4). Immediately coversliped and kept at 4^0C for 10 min to get the agarose solidified. After removal of coverslip, 200 µL of 0.8% low melting point agarose containing 5 µL of whole blood, was added to the slide. Cover slips were placed immediately and slides were kept at 4^oC for 10 min. After solidification cover slips were removed and slides were immersed in prechilled lysing solution containing 2.5M NaCl, 100mM Na_2EDTA, 10mM Tris-HCl; pH-10, 1% DMSO, 1% Triton X and kept for 1 hour at 4^oC. After lysis , slides were drained properly and placed in a horizontal electrophoresis apparatus filled with freshly prepared electrophoresis buffer containing 300mM NaOH, 1mM EDTA and 0.2% DMSO; pH $\geq$ 13. The slides were equilibrated in buffer for 20 min and electrophoresis was carried out for 30 min at 25 V. The slides were washed gently with 0.4mM Tris-HCl buffer, pH-7.4 to remove alkali. The slides were again washed with distilled water. One per cent propidium iodide was used for staining the gel and comets were visualized under fluorescent microscope with 40X magnification. The images were captured and analyzed using software 'CASP' which gives % DNA in tail, tail DNA length, tail DNA moment and olive tail DNA moment directly. The tail moment (TM) was an index calculated from tail length and % DNA in tail and olive tail moment (OTM) as the product of the distance between the centre of gravity of the head and the centre of gravity of the tail and % DNA in tail.

Statistical analysis was carried out to find out any significant difference between untreated and treated groups when compared to normal group, using student 't'test.

RESULTS

Exposure of duck blood cells to Gamma radiation *ex-vivo* induced damage to cellular DNA as evident from the comet formation. Exposure of blood cells to one Gy resulted in a significant ($P<0.05$) increase in comet parameters such as % DNA in tail (from 11.0±1.2% to 30.8±4%) (Fig 1.), tail DNA length (from 19.4±2.5µm to 112.0±15.8µm)(Fig 2.), tail DNA moment (from 2.2±0.4 to 40.1±8.6) (Fig 3.) and olive tail DNA moment (from 5.4±0.9 to 27.9±4.7) (Fig 4.) in untreated (G IIB) when compared to normal (G I) blood samples. However, blood samples exposed to 0.5 Gy radiation (G IIA) exhibited significantly ($P<0.05$) lesser comet parameters such as % DNA in tail (24.9±4%), tail DNA length (79.4±4.4µm), tail DNA moment (20.4±3.4) and olive tail DNA moment (18.8±3.1) when compared to one Gy exposed samples. There was significantly ($P<0.05$) lesser damage of DNA, induced by 0.5 Gy (G IIIA) and one Gy (G IIIB) irradiated blood collected from curcumin treated ducks as evident by the comet parameters like % tail DNA 19.4±3.4% and 22.6±2.4%, tail DNA length 33.2±3.9 µm and 40.0±5.5 µm, tail DNA moment 6.2±0.9 and 9.2±2.1 and olive tail DNA moment 9.7±1.9 and 11.7±1.5 respectively.

Similarly blood samples exposed to 0.5Gy radiation of DMSO alone administered birds (G IVA) exhibited significantly ($P<0.05$) lesser comet parameters such as % DNA in tail (24.2±1.8%), tail DNA length (79.0±6.1µm), tail DNA moment (12.3±0.9) and olive tail DNA moment (14.7±2.0) when compared to one Gy

Fig. 1. Gamma radiation induced changes in Tail DNA% of duck blood cells

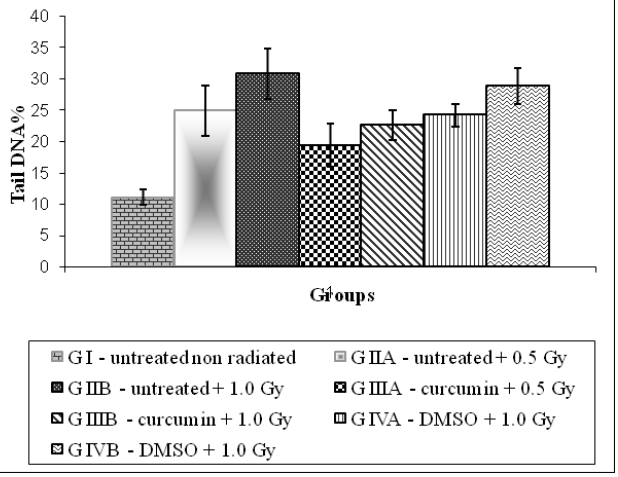


Fig. 2. Gamma radiation induced changes in Tail Length (micrometer) of duck blood cells

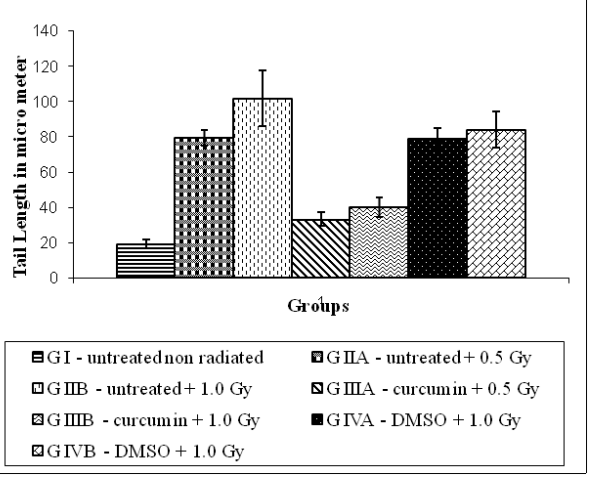


Fig. 3. Gamma radiation induced changes in Tail moment of duck blood cells

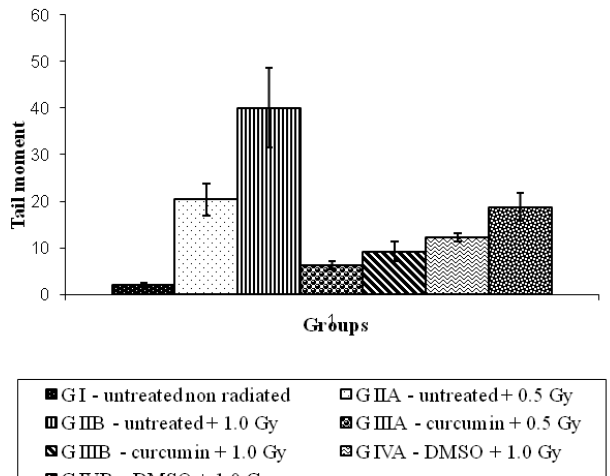
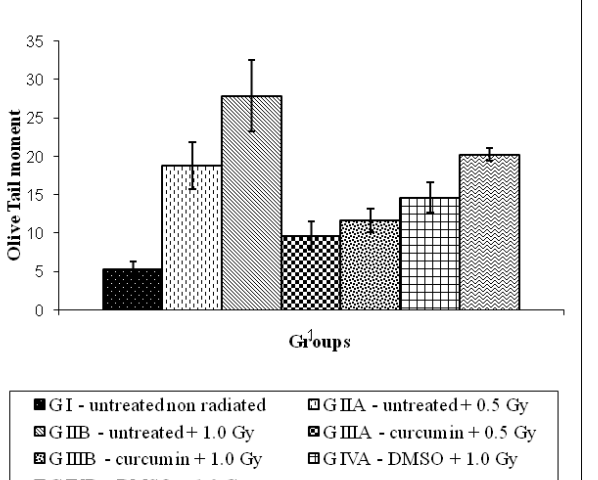


Fig. 4. Gamma radiation induced changes in Olive Tail moment of duck blood cells



exposed samples (GIVB).The corresponding values obtained in GIVB birds were 28.8±2.9 as % DNA in tail, 84.0±10 µm as tail DNA length, 18.78±2.9 as tail DNA moment and 20.3±0.8 as olive tail DNA moment.

DISCUSSION

Ionizing radiations like X-rays and Gamma rays, beta particles, alpha particles and neutrons are known to induce oxidative stress due to Reactive Oxygen Species (ROS) production within cells, resulting in imbalance of the pro-oxidant and antioxidants in the cells which ultimately leads to cell death. Major damages due to ionizing radiation are single strand breaks, double strand breaks, DNA-DNA and DNA-protein cross links and damages to nucleotide bases. Overproduction of ROS, thus leads to mutation and chromosomal aberrations. Radiation induced loss of viability of cells has been attributed to unrepaired lesions in DNA. Thiol compounds such as amifostine, phosphonol, *N*-acetyl-L-cysteine, captopril and mesna have been shown to exhibit antioxidant properties and reduce radiation damage in DNA (Kataoka *et al.*, 2007).

In the present study the damage inflicted on blood cells by Gamma radiation yielded significantly higher levels of DNA damage, in a dose dependent manner. Results also revealed that the exposure of blood cells collected from DMSO alone treated group as well as from curcumin treated group to ionizing radiation did not exhibit a wild variation from non-radiated group. However, the dose selected for curcumin would have been insufficient to quench all free radicals generated due to ionizing radiations. From the results obtained it was observed that pretreatment with curcumin protects the cell from Gamma radiation to a significantly greater extent. The inherent free radical scavenging property of DMSO (**Carolina** *et al.,* 2011) also supplemented the effects of curcumin as a radioprotective agent in a synergistic manner.

Turmeric has been used to treat various ailments in the Ayurvedic system of medicine in India. In the present work, pretreatment of ducks with curcumin at the dose rate of 40mg/kg body wt., i.v., resulted in decreased damage of cellular DNA significantly ($P< 0.05$), as induced by Gamma radiation and evidenced by all the comet assay parameters. This could be due to antioxidant sparing action of Curcumin. Curcumin being lipid soluble reacts with lipid peroxyl radicals and acts as a chain terminating antioxidant (Srinivasan*et al.,* 2006). Curcumin being hydrophobic not only get localized in the lipid bilayer membrane, but also easily get into the cytoplasm. The presence of curcumin in the cytosol directly scavenges the free radicals like superoxide anion, hydroxyl radical and lipid peroxyl radicals and the formation of phenoxyl radicals. The phenoxyl radicals of curcumin thus produced are stabilized over the extended conjugation (Khopde *et al.*, 2000). Curcumin stimulates gamma glutamyl cysteinyl synthase, the rate limiting step in the glutathione synthesis, thereby yielding protection to DNA against oxidative damage. Studies have shown that curcumin significantly enhance the synthesis of antioxidant enzymes such as super oxide peroxidase, catalase and glutathione peroxidase in rat liver (Reddy and Lokesh, 1994). These results are particularly interesting since turmeric is consumed in many parts of India. This may offer protection to individuals staying in areas where background radiation from natural radioactivity is higher. Supplementation of minimum dose of curcumin of turmeric in the diet of semi-intensively reared water fowl like geese and ducks would ensure protection against low levels of ionizing radiations.

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