Protective Effects Of Curcumin Against Benzopyrene Induced Liver Toxicity In Albino Rats.

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ABSTRACT

Benzo[a]pyrene, a member of the polycyclic aromatic hydrocarbon group which is usually generated during the partial burning of organic matter, has been shown to inflict damage on the liver. The study investigated the protective effect of curcumin, on liver toxicity induced by benzo[a]pyrene in rats. Thirty albino rats were randomly divided into five treatment groups with six animals each, designated as control, benzo[a]pyrene(2mg/kg), benzo[a]pyrene(2mg/kg) + curcumin(100mg/kg), curcumin(100mg/kg) + benzo[a]pyrene (2mg/kg), curcumin (100mg/kg). Rats were administered their respective doses orally, (benzo[a]pyrene, daily and curcumin, every other day) for 6weeks. Benzo[a]pyrene administration elicited significant increases (p<0.05) in activities of liver enzymes (ALT, ALP and AST) and significant reduction (p<0.05) in concentrations of total protein and albumin respectively when compared to control. Administration of curcumin brought about significant reversal of benzo[a]pyrene altered biochemical indices. Activities of liver enzymes (ALT, ALP and AST) and concentrations of serum total protein and albumin, of curcumin treated rats, were found to be comparable to that of the control after curcumin administration. Treatment of benzo[a]pyrene induced toxic effects with curcumin helped to restore the normal histological architecture of the liver tissues. These results portray curcumin to be a potent protective agent against benzo[a]pyrene induced alterations in livers of albino rats.

Keywords: Curcumin, benzo[a]pyrene, oxidative damage, liver function

1. INTRODUCTION

Fish, a dietary protein source, is now becoming a vital part of the average Nigerian diet, based on the fact that more people are becoming aware of the health risks involved in the frequent consumption of beef in adults(Olatunde,1998; Omojowo et al.,2009). Fish can be prepared for consumption using various cooking methods such as cooking in hot oil(frying), cooking in water(boiling), grilling, baking, smoking and even broiling. Increased dynamic knowledge in the field of science, has revealed that preparing foods with diverse cooking methods at increased temperatures elicits the formation of certain chemical compounds which are capable of altering the genetic material(DNA) such as polycyclic aromatic hydrocarbons( PAHs) and heterocyclic aromatic amines(Nwaogu and Onyeze,2015). A major focus has been drawn to the generation of
The properties of curcumin as an antioxidant have been documented. It is a strong and effective scavenger of reactive oxygen species including superoxide anion radicals and hydroxyl radicals. Scientific evidence has shown it to prevent erythrocyte lipid peroxidation (Borra et al., 2013). The use of Curcumin have been shown to diminish arsenic, gentamicin, and acetaminophen-induced oxidative stress.
oxidative stress in rats (El-Demerdash et al., 2009; Cekmen et al., 2009). It also debarred the generation of free radical due to myocardial ischemia and lung injury as a result of paraquat in rats (Manikandan et al., 2004). Asides that, curcumin protected against toxicity brought about by diazinon in blood, liver, and erythrocyte of male Wistar rats (Messarah et al., 2013). Therefore this study sought to find out the effects of exposure of benzo[a]pyrene on liver functional parameters of male rats and to determine if these negative effects could be improved upon by side treatment with curcumin. To succeed in fulfilling this objective, rats were treated benzo[a]pyrene and curcumin orally for 6 weeks, after which the activities of selected enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) were examined with the determination of the concentrations of total protein and albumin. Histological examination of tissues of the liver was also carried out.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Laboratory animals

The laboratory animals employed for this study were 30 Wistar albino rats. The rats were aged between 6 and 8 weeks with weights that ranged within 130g and 200g. They were procured from the animal house of the department of Biochemistry, University Of Ilorin, Ilorin, Kwara State. They were allowed to get used to conditions of the laboratory, patterns of feeding and procedures of handling for one week, i.e. 7 days, before the startup of the experiment.

2.1.2 Reagents

Benzo[a]pyrene (B[a]P) with purity ≥ 96% high-performance liquid chromatography), CAS Number 50-32-8, B-1760 and Curcumin with purity ≥ 65%. high-performance liquid chromatography, CAS Number 458-37-7, both procured from Sigma-Aldrich Chemie GmbH, were used for the study. Every other reagent used during the study was of analytical grade.

2.2 Design of the experiment

Thirty albino rats (Rattus norvegicus) with an average weight of 130-200g were segregated into five treatment groups with 6 rats each as follows.

Group 1 (Control): Received rat feed and portable water only.

Group II (Benzo[a]pyrene group): This group received 2mg/kg of B[a]P dissolved in olive oil daily (orally) from week 1 to week 3.

Group III (Curative group): Rats in this group received 2mg/kg of B[a]P (dissolved in olive oil) daily from week 1 to week 3 (orally), and later treated with 100 mg/kg of Curcumin (dissolved in DMSO) at alternate days (orally) from week 4 to week 6.
Group IV (Prophylactic group): The rats in this group received 100 mg/kg Curcumin (dissolved in DMSO) on alternate days from week 1 to week 3 after which they received 2mg/kg B[a]P (dissolved in olive oil) daily from week 4 to week 6.

Group V (Curcumin only): The animals in this group were administered (orally) 100 mg/kg of Curcumin (dissolved in DMSO) on alternate days from week 1 to week 6. Animals in all the groups were allowed rat feed and water ad libitum.

2.3 Methods

2.3.1 Collection of blood sample and extraction of the organs from rats.

2.3.1.1 Processing of tissues

Twenty-four hours, (day 43) after the experiment was completed, animals were placed in a diethyl ether chamber where they were anesthetized, after which they were sacrificed. Samples of blood was taken by cutting the jugular vein with a sharp sterile blade while serum was gotten by centrifugation of blood samples at 1500 x g for 10 minutes. The rats were then opened up and the livers were extracted and placed into a beaker containing ice-cold 0.25M sucrose solution. Each liver was weighed respectively, after which known weights was cut, chopped into small bits and later homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The homogenized tissues were diluted with 0.25M sucrose solution respectively to obtain a 1 in 5 dilution and kept in a deep freezer at -4°C until required for use.

The activity of alanine aminotransferase (ALT) was evaluated using the method described by Reitman and Frankel, 1957. The activity of aspartate aminotransferase (AST) was determined in line with the method of Reitman and Frankel, 1957. The activity of alkaline phosphatase (ALP) was determined using the method of Wright et al., 1972. Serum total protein concentration was determined based on the procedure described by Gornall et al., 1949. The serum albumin concentration was determined according to the method described by Grant and Kacchman, 1987.

2.3.2 Histopathological examination of liver tissues.

Biopsies of the livers were fixed in 10% neutral-buffered formalin and prepared for histology in line with standardized procedure (Bancroft and Gamble, 2008). The fixed tissues were quickly drained of water using increasing concentrations of alcohol, cleared by xylene and dipped in paraffin wax. The tissues were finally cut into 4–5 mm sections by a microtome, fixed on the slides and stained with hematoxylin and eosin. The slides were looked upon under a light microscope and photomicrographs were taken by pathologists who were blinded to control and treatment groups.

2.4 Statistical analysis.

The results were expressed as mean ± standard deviation, and the test of statistical significance was carried out using analysis of variance (ANOVA) at 95% confidence interval (P ≤ 0.05).
hoc testing was performed for intergroup comparisons using Turkey’s test. All statistical calculations were performed with SPSS 17.0 for Windows (Ozadamar, 1991).

3.0. RESULTS

Figure 1 shows the effect of B[a]P and curcumin treatments on mean serum alkaline phosphatase activity in rats. The result shows that control rats and B[a]P treated rats had the lowest and highest mean activity of serum alkaline phosphatase respectively. Rats treated with B[a]P had significantly (p<0.05) higher mean serum alkaline phosphatase activity when compared with control and other groups. There was no significant (p<0.05) difference in the mean activity of serum alkaline phosphatase of the other experimental groups.

Figure 2 depicts the effect of B[a]P and curcumin treatments on mean serum alanine aminotransferase activity in rats. Control rats and B[a]P treated rats had the lowest and highest mean activity of serum alanine aminotransferase respectively. Rats treated with B[a]P had significantly (p<0.05) higher mean serum alanine aminotransferase activity when compared with control and other groups. The mean activity of serum alanine aminotransferase of the curative group and the prophylactic group did not differ significantly (p<0.05) from each other.

Figure 3 shows the effect of B[a]P and curcumin treatments on mean activity of aspartate aminotransferase of rats. Control rats and B[a]P treated rats had the lowest and highest mean activity of serum aspartate aminotransferase respectively. Rats treated with B[a]P only had significant (p<0.05) higher mean serum aspartate aminotransferase activity when compared with other groups.

Figure 4 shows the effect of B[a]P and curcumin treatments on the mean protein concentration of rats. Control rats and B[a]P treated rats had the highest and lowest mean protein concentration respectively. Rats treated with B[a]P only had significantly (p<0.05) lower mean protein concentration when compared with control and other groups. The curative group and the prophylactic group did not differ significantly in their mean protein concentrations.

Figure 5 shows the effect of B[a]P and curcumin treatments on the mean albumin concentration in B[a]P treated rats. Control rats and B[a]P treated rats had the highest and lowest mean concentration of albumin respectively. The mean concentration of B[a]P treated rats was significantly (p<0.05) lower than all other groups.
Figure 1: Effect of Benzo[a]pyrene and Curcumin treatment (curative, prophylactic and curcumin only) on mean serum alkaline phosphatase activity in albino rats.

Figure 2: Effect of Benzo[a]pyrene and Curcumin treatment (curative, prophylactic and curcumin only) on the mean alanine aminotransferase activity in albino rats.
Figure 3: Effect of Benzo[a]pyrene and Curumin treatment (curative, prophylactic and curcumin only) on mean aspartate aminotransferase activity in albino rats.

Figure 4: Effect of Benzo[a]pyrene and Curumin treatment (curative, prophylactic and curcumin only) on mean protein concentration in albino rats.
3.1. Histopathological findings.

Figure 6 showed normal histology features of hepatic cells of control rats, which exhibited the well-organized lobular architecture with apparently healthy liver parenchyma. Figure 7 depicted gross breakage in the hepatic cells which was milder in rats pre-exposed to B[a]P(Curative) and rats post exposed to B[a]P(Prophylactic)(Figures 8 and 9 respectively). The curcumin treated rats (Figure 10) showed normal hepatocytes with few inflammatory cells.
Figure 8: Photomicrograph of Liver section of curative rats hematoxylin and eosine (H&E) Staining. (Magnification 600x)

Figure 9: Photomicrograph of Liver section of prophylactic rats hematoxylin and eosine (H&E) Staining. (Magnification 600x)

Figure 10: Photomicrograph of Liver section of Curcumin only treated rats hematoxylin and eosine (H&E) Staining. (Magnification 600x)
4.0. DISCUSSION

The present study examined the effect of Benzo[a]pyrene and Curcumin (curative, prophylactic and curcumin only) administration on liver function indicators such as activities of liver enzymes [alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST)], albumin and total protein concentration alongside with histological examination of tissues of the liver. Curcumin, an antioxidant and cancer-preventing substance, has been documented to possess a safe-guarding benefits against liver damage and peroxidation of microsomal membrane lipids caused by ferric nitrite-triacetate (Iqbal et al., 2003); protective action against detrimental effects of cisplatin (Antunes et al., 2000). Curcumin could also exercise anti-oxidative benefits either directly as a chemical antioxidant based on its capacity to clear up reactive oxygen and nitrogen free radicals or by regulating cellular defenses which bring about antioxidant benefits (Priyadarsini et al., 2003; Awasthi et al., 2000).

Based on its role in the conversion of environmental xenobiotic, the liver is highly susceptible to injury. When liver cell plasma membrane is harmed, diverse enzymes normally located in the cytosol, are discharged into the bloodstream thus accelerating the levels of such enzymes in the blood. The determination of the activities of serum (plasma) marker enzymes like AST, ALT, and ALP has been found to be a potent tool for the determining the efficacy of the liver (Ulican et al., 2003; Porchezhan and Ansari, 2005). Such determination can also be used as an indicator of tissue cellular damage brought about by a chemical compound long before it manifests by histological techniques (Akanji, 1986; Olatunji et al., 2014).

The significant increases in the mean plasma activities of ALP, ALT, AST respectively (Figures 1, 2 & 3) manifested in the benzo[a]pyrene administered rats, shows B[a]P-induced damage and eventual escape of such enzymes from the liver, through the disrupted plasma membrane of the hepatic cells, into the general blood circulation. This could also be as a result to increased synthesis of such enzymes due to organ impairment (Ploa and Hewitt, 1989; Vasanth et al., 2012). It could also be due to necrotic damages in the hepatocytes of the rats (Ujowundu et al., 2011). Therefore, the increased mean enzymes activities could be considered to be a sign of oxidative stress (Vasanth et al., 2012), as a result of generation of free radicals which manifests during the biotransformation of benzo[a]pyrene. Mariyamma et al., 2009 reported elevated levels of plasma transaminases brought about by oxidative stress, which brings about alterations in the membrane integrity, thereby changing the membrane permeability leading to gradual loss of intracellular enzymes.

This loss in enzyme activity in the liver of the B[a]P treated rats, may negatively influence the movement of metabolites or required ions across the cell membrane, thus leading to inadequate supply ions and metabolites to these organs (Akanji et al., 1993; Yakubu, 2006). It could also adversely affect other metabolic processes in which such enzymes are involved in specified processes such as the formation of nuclear proteins, nucleic acids and phospholipids as well as in the breakage of phosphate esters (Shi et al., 2006). The significant decrease in the mean activities of serum liver enzymes of curcumin treated group, post-exposed group to benzo[a]pyrene (curative) and pre-exposed group to B[a]P (prophylactic) may be traced to the antioxidant activity of curcumin (Keith et al., 1999). Curcumin may also have improved the
molecular mechanism of the action of such enzymes. This is an indication that curcumin is helpful for liver regeneration to reverse damage to the liver.

Decline in hepatic function tends to influence levels of albumin and plasma proteins, bringing about distortions in their plasma concentration (Oluwatosin et al., 2007; Nwaogu and Onyeze, 2010). Thus there were pronounced decreases in the mean concentration of albumin and total protein (Figures 4 & 5) in the rats treated with benzo[a]pyrene when compared to the control. The significant decrease, supports the trend displayed in liver diseases and damage relating to dysfunctional liver capacity (Oluwatosin et al., 2007; Nwaogu and Onyeze, 2010). The reduced mean concentrations of albumin and total protein in the study is supported by the work of Ujowudu et al., 2012; Aycicek et al., 2005.

Albumin which is majorly formed by the liver, is a major antioxidant component of the plasma, and could be carrying out a major role in the total antioxidant capacity of plasma (Aycicek et al., 2005; Ekam et al., 2012). Thus decreased mean concentration of total protein and albumin levels respectively in B[a]P treated rats, could emerge due to dysfunctional producing ability of the liver brought about by oxidative stress (Loekle et al., 1983).

Another factor that may have led to decline in albumin levels, is the function of albumin as a binder of numerous endogenous and exogenous substances (xenobiotic) while acting as an antioxidant. This essential molecule might have been utilized to clear off the resulting free radicals. The levels of toxic reactive oxidative species (ROS), might have increased greatly beyond the ability of the animals’ antioxidant systems, leading to the mop up of albumin, resulting to its decrease. In spite of that, increased concentrations of these proteins were noticed in curcumin-treated groups (curative, prophylactic and protective), indicating a safe-guarding action of the liver by curcumin.

A close examination of the histological sections showed gross structural disintegration in the liver cells of rats treated with B[a]P only as evidenced by presence of large open spaces, when compared with the control rats. Sunmonu and Oloyede (2007); Poli and Parola (1997) explained these open spaces as areas of tissue disintegration. This observation indicates that tissue fragmentation increases with greater intensity of B[a]P metabolites during its conversion into other forms in the rats treated with benzo[a]pyrene.

Reports show that reactive oxygen specie (ROS) are a crucial causative factor required for changes in the histology of the liver (Mohammed, 2013; Halliwell, 2001). Membranes are majorly susceptible to effects of reactive oxidative species (ROS), peroxidation of unsaturated fatty acids in biological membranes which results in diminished fluidity and distortions of membrane configuration and capacity, which is implicated in serious pathologies (Suzuki et al., 1998; El-Agamy, 2010) including lesions in liver. Due to direct damage of ROS on plasma membranes, the permeability of hepatic cells increases, leading to gradual escape of enzymes into blood circulation.

In the current study, greater activities of enzymes associated with functions of the liver, were observed in rats exposed to B[a]P (Figures 1, 2, 3), which points to damage to hepatocytes and subsequent permeability. It can be inferred that the observed activities of these enzymes were
due to damage brought about by B[a]P to membranes of hepatocytes. This conclusion is supported by histological findings (Figure 7) indicating degenerative changes in livers of rats exposed to B[a]P. These results are consistent with those of other studies, in which B[a]P treatment resulted in significantly greater activities of ALP and transaminases in blood of rats.

Curcumin helped to successfully bring to normal the activities of these enzymes, which are used as markers for damage to membranes in liver. Hepato-protective and therapeutic properties of curcumin against B[a]P-mediated toxicity has been credited to its ability to clear free radicals, in which it controlled the gradual transition of the oxidative stress procedure obtaining a healthy antioxidant status and chelate metal ions, thus hindering the occurrence of the Fenton reaction (Weifeng et al., 2006; Garcia-Nino and Pedraza-Chaverrí, 2014).

The findings of the study are in line with previous reports on the hepato-protective effects of curcumin against heavy metals and diethyl nitrosamine-induced toxicity (Kadasa et al., 2015; Harvey, 1985). Curcumin has been found to manifest its protective benefits against severe oxidative damage via its powerful antioxidant property, whereby it clears off oxygen free radicals. The occurrence of milder capsular spaces in rats pre-exposed to B[a]P rats (Curative) and rats post exposed to B[a]P (Prophylactic) (Figures 8 and 9 respectively) may be ascribed to the safe-guarding action of curcumin. The curcumin treated rats (Figure 10) showed normal hepatocytes with few inflammatory cells.

5. CONCLUSION

In conclusion, the data obtained from this study revealed that Curcumin treatment, (curative, prophylactic and Curcumin alone) protected against benzo[a]pyrene-induced hepatic injury. This can be attributed to the antioxidant and free-radical scavenging properties of curcumin. Thus, dietary inclusion of Curcumin could exert protective effects against hepatic toxic effects resulting from B[a]P exposure. In addition, we recommend the use of curcumin as active ingredient in the pharmaceutical industries for the production of new drugs used as therapeutics for cancer treatment.

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