A Comparative Study Of The Protective Effect Of Jubi Formula And Melatonin On Acute Acetaminophen Induced Liver Damage In Sprague-Dawley Rats

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Abstract

Acetaminophen is widely used as analgesics in human and can be abused. Incidences of accidental poisoning are also reported, and at high doses, it induced hepatotoxicity. Jubi formula and melatonin are said to have protective activities against the oxidative damage on the liver. The experiment was designed to compare the efficacy of melatonin and Jubi formula in protecting the liver against acetaminophen-induced liver damage. Twenty Sprague-Dawley rats with average weight of 180gm were randomly assigned to groups of I, II, III, and IV with each groups having five (5) rats each. Group I serve as the Sham Control. The Group II rats were given 2 mls of 0.9 % saline daily for five days and on the sixth day were given a single dose of 400mg/kgwt of acetaminophen and were sacrificed 2 hours thereafter. Group III and IV rats were given 1mg/kgwt of Jubi formula and melatonin daily for five days respectively and on the sixth day they were given 400mg/kgwt of acetaminophen and sacrificed 2 hours after. All the substances were given orally using gastric tube. Blood sample was collected by cardiac puncture for measurement of liver function test (LFT). The mean total bilirubin concentration for the control was 0.66±0.02 and for group II was 0.63±0.02. There was statistical significant difference between them. Serum glutamate oxaloacetic transaminase (SGOT) level was elevated in groups II and III (18.2±2.0 and 16.8±2.0 respectively) but in group in IV, it was 11.1±1.0. There was no statistical significance difference between the group and the control group. Serum glutamate pyruvic transaminase (SGPT) was not elevated in all the groups and similar observations were found in Alkaline Phosphatase. The results indicate that exogenous administrated melatonin have a potent hepatoprotective activity against acetaminophen-induced hepatocellular injury than Jubi formula.

Keywords: Acetaminophen, Melatonin, Jubi formula, Hepatotoxicity, Sprague-Dawley Rats, Liver function test

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Introduction

Hepatoxin are agents that cause liver injury in both man and animals in a predictable manner[1]. The liver performs many diverse functions essential for life and many of these specific functions are assessed by laboratory procedures[2,3]. Acetaminophen is widely used as analgesics in human and can be abused. Incidences of accidental poisoning are also reported, and at high doses, it induced hepatotoxicity. It is detoxified in the liver through sulfation and glucoronidation, while a small amount are converted by cytochrome P450 catalyzed oxidation to an electrophylic, highly toxic metabolite[4].

Melatonin isolated from bovine pineal tissue[5] and its levels in all species of animals especially when measured in darkness is associated with large rises in pineal melatonin, independent of whether the animals are nocturnally or dicturnally active[6,7,8]. Melatonin scavenges and neutralizes the most damaging free radicals, the hydroxyl radicals five times better than glutathione and twice more effective in deactivating the peroxyl radicals than vitamin E[9, 10]. It is highly effective in human to reduce free radicals (Oxidative) damage[11,12] couple with it virtual absence of toxicity[13,14,15]. Furthermore, pro-inflammatory mediators (TNF-α, IL-1, NO) in serum and liver homogenates were significantly reduced by melatonin[16].

Jubi formula is a Nigerian herbal mixture discovered by Okunbena which contains about five (5) different herbal ingredients namely: Perguenna nigresces (Ogbo), Shorgumbi color (Oka-baba), Herugara madegosicanriencesis (Adidun), annacardium ocudentale (Fetiga) and Wathens indica (Ewe-epo). The mechanisms of its actions were not known but it was thought to

stimulate elimination of toxic waste in the system through the kidneys and the bile ducts[17].

The efficacy of melatonin and Jubi-formula in the protection of hepatic injury following toxic dose of acetaminophen in Sprague Dawley rats was the crux of this experiment.

Materials and Methods

The procedure was carried out according to the University of Lagos guidelines of the Medical Ethics Committee for the use of animals in experiment. Twenty Sprague-Dawley rats with average weight of 180gm obtained from the animal unit of the College of Medicine, University of Lagos were allowed to acclimatize in the animal room of Department of Anatomy for a week. Rodent chow and water were given ad libitum.

The animals were randomly assigned to groups of I, II, III, and IV with each groups having five (5) rats each. Group I served as the Sham Controls. The Group II rats are given 2 mls of 0.9 % saline daily for five days and the sixth day they were given single dose of 400mg/kgwt of acetaminophen and were sacrificed 2 hrs thereafter. Group III rats were given 1mg/kgwt of Jubi formula daily for five days and on the sixth day they were given 400mg/kgwt of acetaminophen and sacrificed 2 hrs thereafter. Group IV rats were administered 1mg/kgwt of melatonin daily for five days, and on the sixth day, the were given 400mg/kgwt of acetaminophen and sacrificed 2 hrs later. All the substances were given orally using gastric tube.

Blood collection

A total of 5 mls of venous blood was obtained from each rat via cardiapuncture using lithium heparinised tubes. Blood collection for each rat was performed at approximately the same time of the day throughout the study period to reduce variability within each rat. Immediately after the collection, plasma and erythrocytes were separated by centrifugation. Centrifuged samples were stored at 4°C, until analysis was done. Liver function test (LFT) was analyzed using electro-photo spectrometer.

Data analysis

For statistical data comparisons, data were evaluated by one-way ANOVA, followed by least significant differences tests. All values are given as mean ± S.E.M with n values indicating the number of subjects analyzed. P values < 0.05 are considered significant.

Results

The mean total bilirubin concentration for the control was 0.66±0.02 and in group II was 0.63±0.02. There was statistical significant difference between them. The mean concentration of total bilirubin for group III was 0.65±0.02 and group IV was 0.64±0.01. There are statistical significance difference between group III, group IV and with the control group. The conjugated bilirubin concentration for the control was 0.22±0.01, group II was 0.22±0.01, while group III was 0.20±0.01, and those in group IV was 0.24±0.01. There was no statistical significance difference between all the groups. Serum oxaloacetic transaminase (SGOT) for the control was 9.4±2.0 and group II was 18.2±2.0. There was statistical significance difference between them. The SGOT for group III was 16±2.0 and there was statistical significance difference between group III and the control but no difference between group III and group II (p value < 0.05). SGOT value for group IV was 11.1±1.0 and there was no statistical significance difference between it and the control group, however there was significance difference between it and those in group II. Serum glutamate pyruvic transaminase (SGPT) for the control was 7.5±0.2, group II was 10.8±2.0, those for group III was 10.0±1.0, and the group IV was 9.7±2.0. There were no statistical significance differences between all the groups. Alkaline phosphatase level for the control was 77±1.0, and for group II was 80.0±2.0. There was no significance difference between them. The value for group III was 84.0±1.0 and group IV. There was statistical significance difference between them, with the control group and group II.

Discussion

Hepatic injury induced by various toxic agents, including acetaminophen, has been attributed in past to the production of pro-inflammatory cytokines and other mediators by resident kupffer cells within the liver. However, recent evident from laboratories has demonstrated that hepatoprotective factors, such as interleukin (IL)-10 and cyclo-oxygenase-derived mediators are up regulated in response to hepatic damage[18]. Elevated bilirubin level is an indication of biliary obstruction and haemolysis[19], and this was found to be elevated in all the experimental groups except the control group. This demonstrates that acetaminophen do actually induced hepatic damage in all experimental animals but for the control. The elevated level may be due to acute or severe hemolysis resulting from the destruction of

Table I: - Liver function test results of the Sprague-Dawley rats in their respective groupings

Contents	Control	Group II	Group III	Group IV	Units
Total Bilirubin	0.66±0.02[a]	0.63±0.02	0.65±0.02	0.64±0.01	gm/dl
Conjugated Bilirubin	0.22±0.01	0.22±0.01	0.20±0.01	0.24±0.01	mg/l
SGOT	9.4±2.0	18.2±2.0[b]	16.8±2.0[b]	11.1±1.0	µg/l
SGPT	7.5±0.2	10.8±2.0	10.0±1.0	9.7±2.0	µg/l
Alkaline Phosphatase	77.0±1.0	80.0±2.0	84.0±1.0	84.4±5.0	µg/l

a = Mean±SD b = $p < 0.05$

the red blood cells (RBC) within the hepatocytes. However, the value was slightly lower in the group with melatonin; this may be as a result of the protective effect of melatonin.

The conjugated bilirubin level were normal for all the experimental groups and the control, the elevation of the total serum bilirubin may then be due to the raised serum level of unconjugated bilirubin. The elevated level of Serum glutamic oxaloacetic transaminase (SGOT) in groups II and III is an indication of hepatocellular injury thus, demonstrating the non protective action of Jubi formula. However, the reduction in the concentration of SGOT levels in the group IV and the control group was an indication that melatonin has protective activity against acetaminophen-induced hepatic injury. The mechanism by which melatonin protect the hepatocytes could be due to its antioxidant activities, it ability to scavenges and neutralizes the free radicals thus protecting the hepatic cells[20]. Similar observations were found and reported in other studies[16, 21]. The elevation of serum glutamic pyruvic transaminase (SGPT) in all the experimental groups is an indication of hepatocellular injury and this is consistence with some studies that found elevations in the levels both SGOT and SGPT following acetaminophen hepatotoxicity[19,22].

The alkaline phosphatase level was within normal limit for all the experimental groups and the control. However, there was the need to assess the phosphate level as hypophosphates was a frequent feature of acetaminophen induced hepatotoxicity and may be involved in the pathogenesis of the hepatic failure that normally occurs as a complication.[23] These results demonstrated that exogenous administration of melatonin have potent hepatoprotective effect against acetaminophen induced liver injury by it increased activity of cytochrome P450 and it anti-inflammatory mediators effect, but same can not be said of Jubi formula.

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