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Assessment of Organ Damage Markers in Acetaminophen-induced Hepatotoxicity in Rats Pre-treated with Quail Eggs

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Authors' contributions

This work was carried out in collaboration between all authors. Author PEA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author CEN managed the literature searches and analyses of the study. Author ECN managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the response of organ damage markers to quail egg pre-treatment on acetaminophen–induced hepatotoxicity in rats. Thirty (30) albino *wistar* rats assigned to 5 groups of 6 rats each were used for this study. Groups 2-4 rats were orally pre-treated with 30, 15 and 7.5 mg/ml of quail egg solutions respectively for 7 days. On day 7 post treatment, 2000 mg/kg of acetaminophen (Paracetamol®) was orally administered to rats in groups 2-5. Groups 1 and 5 rats were pre-treated with distilled water (10 ml/kg) to serve as normal and negative controls respectively. Forty eight hours (48 h) post acetaminophen administration, blood samples were collected for biochemical [(total protein (TP), albumin (ALB), blood urea nitrogen (BUN), conjugated bilirubin (CB) and unconjugaed bilirubin (UB)] analyses. Results indicated significant ($P < 0.05$) decrease in BUN values of groups 2-4 rats (Quail egg-pretreated groups) when compared to that of

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the rats in group 5 (negative control). The total bilirubin values of rats in group 5 (induced untreated group) were significantly ($p < 0.05$) lower than those of groups 1-3 rats. The conjugated bilirubin levels of rats in group 2 were significantly ($P < 0.05$) higher than that of the negative control rats while no significant changes ($P > 0.05$) were observed in the TP and ALB values across all the groups. It was concluded that pre-treating acetaminophen-induced hepatotoxic rats with quail egg, ameliorated organ damage injuries with highest ameliorative effect at 30 mg/ml.

Keywords: Quail eggs; markers; acetaminophen; rats; hepatotoxicity.

1. INTRODUCTION

Quail eggs have been known for its several landmark health benefits like elimination and removal of stones from kidneys and gall bladder, remedy against stomach and duodenal ulcers, curative effects on tuberculosis, bronchial asthma and diabetes. Others include anti-cancer effects and stimulation of sexual libido [1].

It has been reported that quail eggs have strong therapeutic effect on various liver impairments/diseases [2]. Beneficial effects of quail egg have been attributed to some of its vital contents such as proteins, vitamins (A, B complex, E), calcium zinc and sulphur and antioxidants [1,3].

Acetaminophen (paracetamol®) has been shown to be safe when taken in normal therapeutic doses [4]. In the case of overdose, the sulfate and glucuronide pathways become saturated and some paracetamol are shunted to the cytochrome P450 system to produce N-acetyl-p-benzoquinoneimine (NAPQI). This compound, NAPQI deletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure [5]. Overdose of acetaminophen is the most common cause of acute liver failure in the United States [6]. Acetaminophen is over the counter analgesic drug that is often abused especially in the developing country [7].

Organ damage markers are used to assess/diagnose whether organ (s) is/ are injured or not. In the case of liver injury, assessing organ damage markers like alanine aminotransferase (ALT), aspartate amino transferase (AST), Alkaline phosphatase (ALP), bilirubin, total protein and many others can be used in determining the overall liver function [8].

In view of the adverse effects associated with synthetic drugs, natural products are considered to be safer, cheaper and more effective [2]. To this end, this study was therefore designed to evaluate possible efficacy of quail egg in mitigation of liver and kidney injuries induced by acetaminophen.

2. MATERIALS AND METHODS

Thirty (30) adult rats of mixed sexes and aged 10 to 16 weeks used in this study were obtained from animal house laboratory unit of Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were acclimatized for the duration of 7 days under standard environmental conditions with 12 hour light/dark cycle maintained on a regular grower feed (vital feed) and water *ad libitum.*

Freshly laid quail eggs of between 10 to 15 g used in this study were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka Farm.

All the instrument, reagents and chemicals used in this study were obtained from reputable company (Sigma Aldrich, England) through reliable sources.

2.1 Experimental Design

Thirty adult (30) rats of mixed sexes were assigned to 5 groups of 6 rats per group. The quail egg yolk and albumen were extracted and solubilized in distilled water to make a desired concentration of egg solution. Thereafter, the solution was passed through serial dilutions to obtain different concentrations (30 mg/ml, 15 mg/ml and 7.5 mg/ml) for the individual groups of the rats.

The rats were pretreated with aqueous solution of quail eggs of varying concentrations for 7 days using stomach tube. On the day 7, 2000 mg/kg of acetaminophen (Paracetamol®) was administered orally to all the rats in groups 2-5. Acetaminophen was not administered to rats in group 1 (Positive control). Forty eight hours (48 h) post acetaminophen administration, blood samples for biochemical determinations were collected from the media canthus of the eyes of all the rats through the retrobulbar plexus as described elsewhere [9].

2.2 Blood Sample Collection

Blood samples were collected from the retrobulbar plexus of the median canthus of the eye of the rats. A microcapillary tube was carefully inserted into the medial canthus of the eye to puncture the retrobulbar plexus and thus enable outflow of about 2 ml of blood into a clean glass tube. The blood was kept at room temperature for 30 minutes to clot. Afterwards, the test tubes containing the clotted blood samples were centrifuged at 3000 revolution per minute using a clinical table centrifuge. The clear serum supernatant was then carefully aspirated with syringe and needle and stored at 4℃ in a clean sample bottle for the serum biochemistry assays.

The experimental protocol used in this study was approved by the ethics committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Enugu state, Nigeria. ECUN: 2010/173451.

The Table 1 summarizes the experimental design.

Table1. Administration of varying doses of quail egg solution and intoxication with paracetamol®

Group	Treatment
1	Pretreated with distilled water + NO Paracetamol® intoxication (Positive control)
2	Pretreated with 30 mg/ml quail egg solution + 2000 mg/kg Paracetamol®
3	Pretreated with 15 mg/ml quail egg solution + 2000 mg/kg Paracetamol®
4	Pretreated with 7.5 mg/ml quail egg solution + 2000 mg/kg Paracetamol®
5	Pretreated with distilled water + 2000 mg/kg Paracetamol® (Negative control)

2.3 Serum Biochemistry Tests

Total proteins were determined by the direct Biuret method [10]. The serum albumin was determined by the bromocresol green method as described elswhere [11]. Blood urea nitrogen was determined by the modified Berthelot-Searcy method [12]. Total serum bilirubin was determined following the Jendrassik-Grof method [13] for the *in-vitro* determination of direct and total bilirubin in serum. All determinations were done using test kits (Randox, UK).

2.4 Statistical Analysis

The data generated were analyzed with the One-way Analysis of Variance (ANOVA) and the variant means separated with Duncan's Multiple Range post hoc test. $P < 0.05$ was considered significant. The results were presented as Mean (± standard error of the mean) in Figures.

3. RESULTS

The results showed that the serum total protein levels of the rats in all the groups were statistical comparable ($p > 0.05$) (Fig. 1). There was no significant ($p > 0.05$) difference between the albumin levels of the quail egg-pretreated rats and the normal and the negative controls (Fig. 2). The results showed that the blood urea nitrogen of group 5 rats (induced untreated) were significantly ($p < 0.05$) higher than that of the other rats in groups 1-4. The blood urea nitrogen of the rats in groups 1-4 were comparable ($p > 0.05$) (Fig. 3). The results showed that the total bilirubin values of rats in group 5 (induced untreated group) were significantly ($p < 0.05$) lower than those of groups 1-3 rats. The total bilirubin values of rats in group 4 were statistically comparable ($p > 0.05$) with that of the groups 3 and 5 (Fig. 4). The conjugated bilirubin levels of rats in groups 4 and 5 were comparable ($p > 0.05$) to each other but significantly ($p < 0.05$) lower than those of groups 1-3. The conjugated bilirubin levels of rats in groups 1 and 2 compared very well ($p > 0.05$) but were significantly ($p < 0.05$) higher than that of the groups 3-5 rats. The rats in group 3 had significantly ($p < 0.05$) higher levels of conjugated bilirubin when compared with those of the groups 4 and 5 rats but significantly ($p < 0.05$) lower when compared with those of groups 1 and 2 (Fig. 5).

4. DISCUSSION

The non significant ($P > 0.05$) decrease in the serum total protein and albumin values in group 5 rats (negative control) compared to those of the rats in groups 1-4 indicated that the damage done to the hepatocytes by the acetaminophen was not chronic. Researchers have reported that the synthetic abilities of the liver such as protein synthesis are impaired in chronic liver diseases [7]. Hepatocytes are saddled with the responsibility of synthesizing liver proteins [14,15]. Any damage to the liver will cause changes in the dynamics of these products (serum proteins).

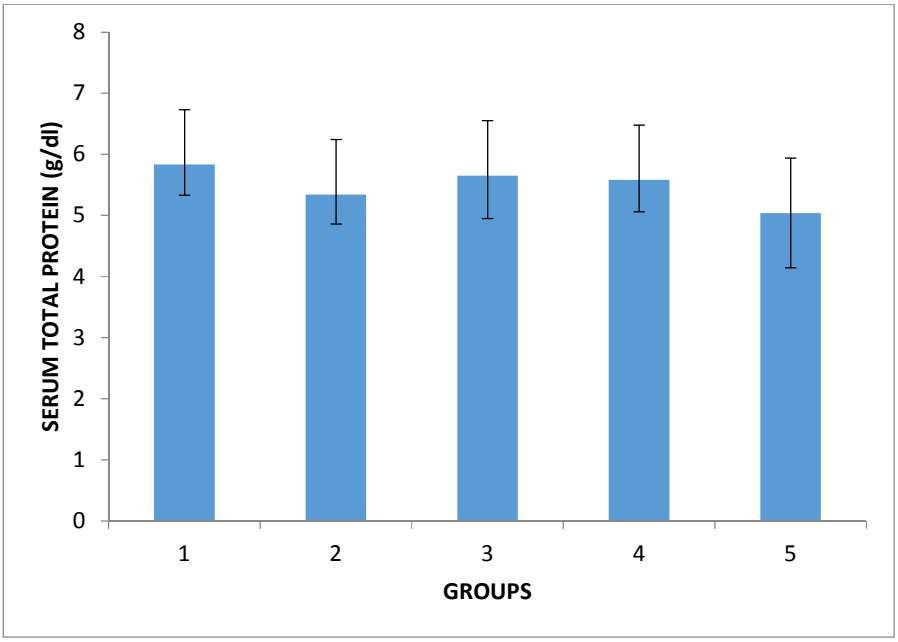

Fig. 1. The effects of Quail egg pretreatment on the total protein of acetaminophen-induced hepatotoxic rats

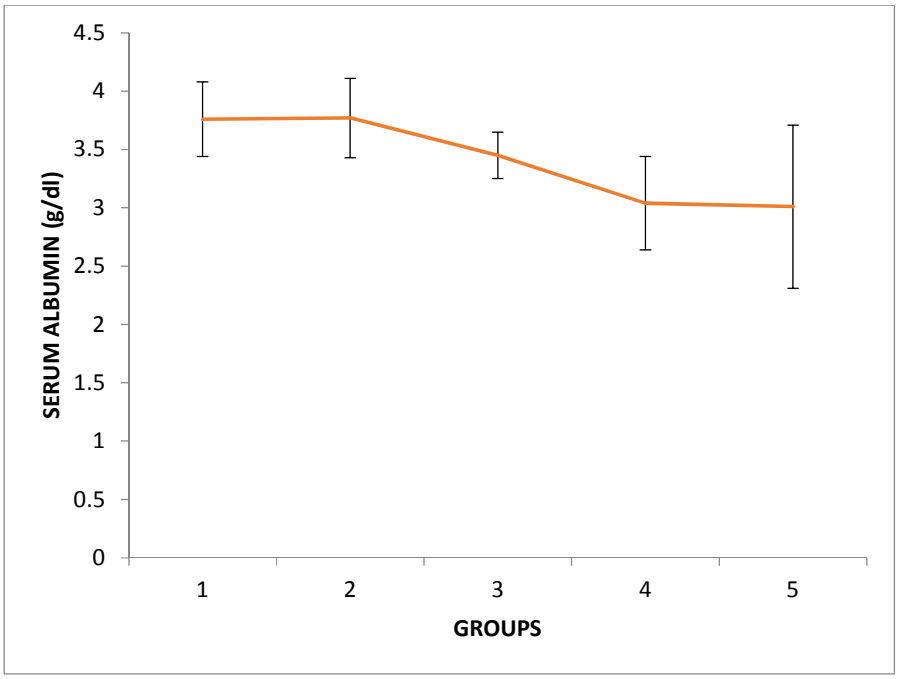

Fig. 2. The effects of quail egg pretreatment on the serum albumin levels of acetaminophen-induced hepatotoxic rats

Significant increase in the BUN values of rats in group 5 (untreated and acetaminophen-induced) when compared with the normal control rats that was observed is a pointer to possible kidney involvement in acetaminophen-induced toxicity. The effects usually reported following toxic injury to the kidney reflect decreased elimination of wastes such BUN levels [16]. The BUN is considered important biomarker of kidney dysfunction [17]. Acetaminophen-induced kidney

impairment had earlier been reported [18,19]. The common adverse effects of traditional non-steroidal anti-inflammatory drugs on renal function included reductions in renal blood flow, glomerular filtration rate and are mediated via inhibition of renal cyclooxygenase [18]. The amelioration of acetaminophen toxicity as evidenced in the quail egg pretreated group 2 may be attributed to the antioxidant contents of quail egg [1]. Antioxidants are known to mop up free radicals (which are incriminated in cell damage) [20].

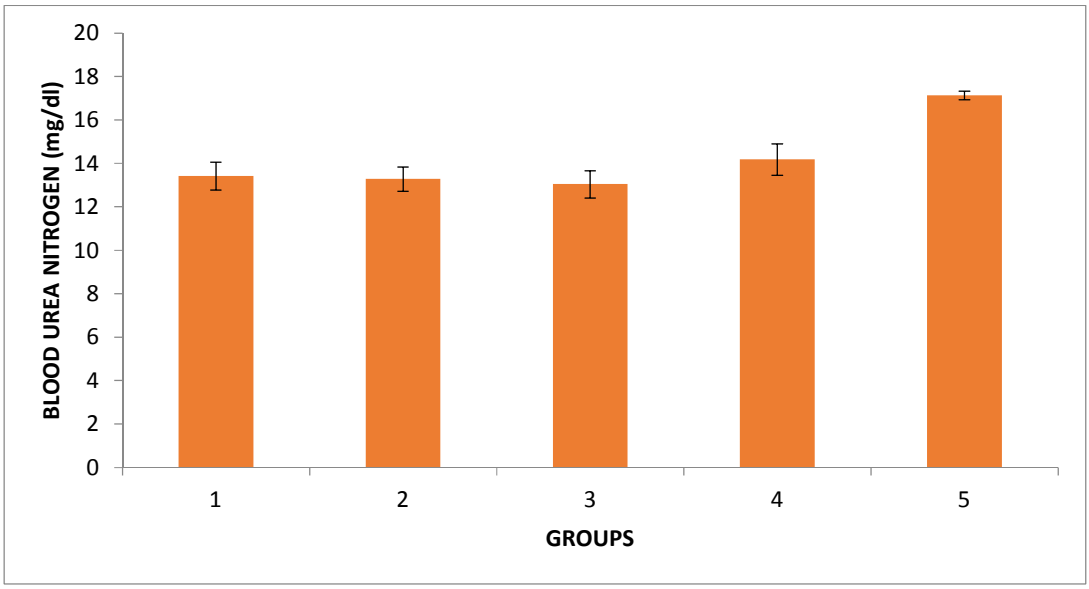

Fig. 3. The effects of Quail egg pretreatment on the blood urea nitrogen of acetaminophen-induced hepatotoxic rats

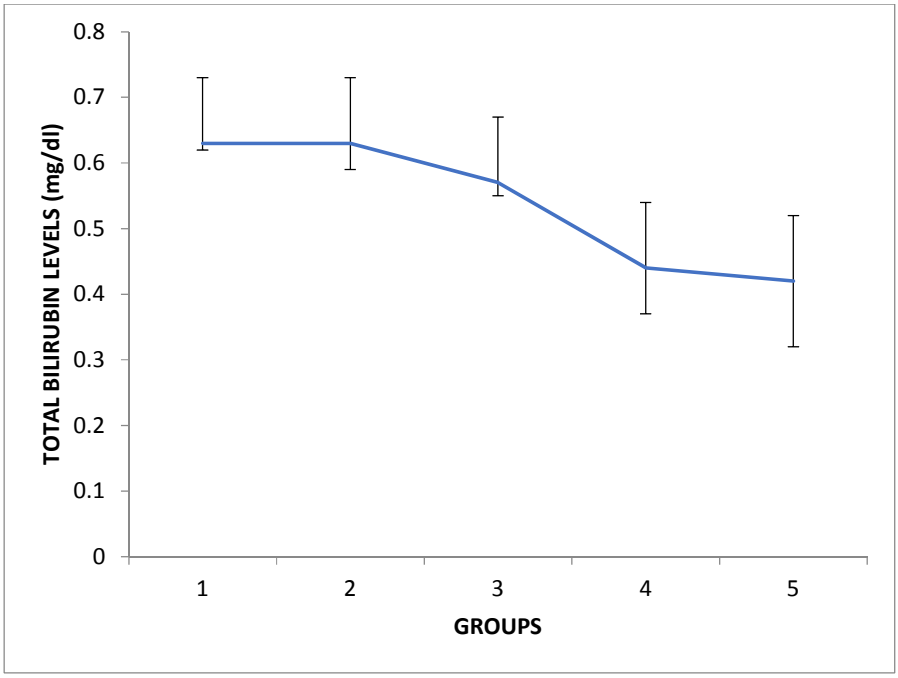

Fig. 4. The effects of Quail egg pretreatment on the total bilirubin levels of acetaminophen-induced hepatotoxic rats

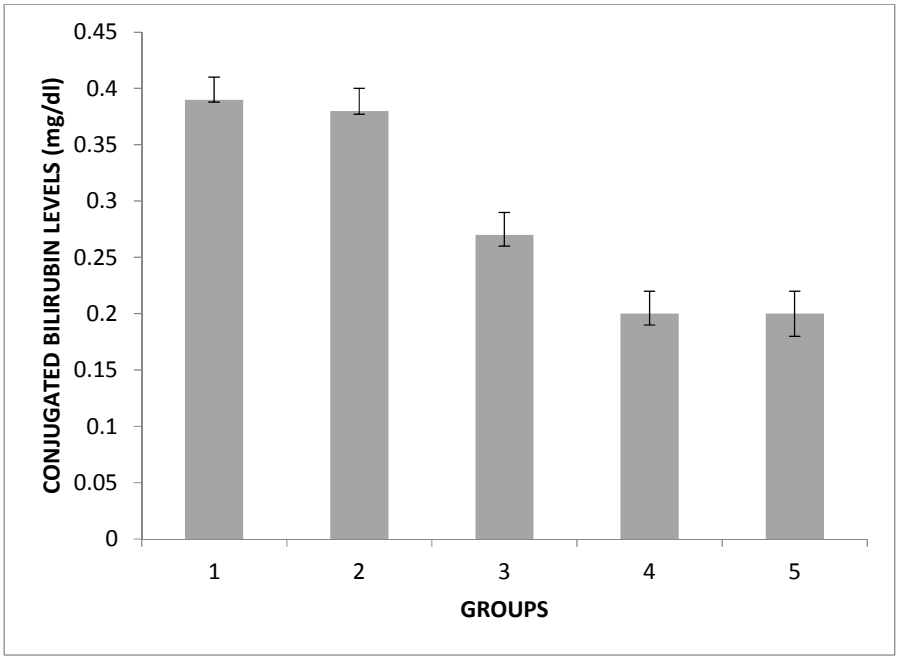

Fig. 5. The effects of quail egg pretreatment on the conjugated bilirubin levels of acetaminophen-induced hepatotoxic rats

The significant (P < 0.05) decrease in the conjugated bilirubin concentration in group 5 rats when compared to conjugated bilirubin concentration of group 1 rats may be due to the inability of the enzyme glucuronyl transferase to conjugate bilirubin with glucuronic acid in the liver to make it water soluble following hepatocyte dysfunction [21]. Conversely, the conjugated bilirubin levels of the pretreated groups (especially group 2 and 3 pretreated with 30 and 15 mg/ml of quail egg solution respectively) were significantly (P < 0.05) higher compared to those of the negative control rats. This indicates better hepatocyte function in the former (pretreated groups) than in the later (negative control). Quail egg solution contains vitamin E, iron, and zinc which restore hepatocyte functions, hence ensuring the ability of the liver to take up, process and secret bilirubin into bile [22].

Similarly, the total bilirubin values of the quail egg pretreated groups were significantly (P < 0.05) higher than that of the negative control group. The increase may probably be as a result of elevated conjugated bilirubin levels in these quail egg-pretreated groups as discussed earlier.

5. CONCLUSION

It was concluded that quail egg could ameliorate liver and kidney injuries occasioned by overdose of acetaminophen. In this study, the ameliorative potential of quail egg solution was profound at the concentration of 30 mg/ml.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experimemts have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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