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Role of Oxidative Stress in Hepatic and Renal Damage in Wistar rats treated with Fake Paracetamol Syrup.

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ABSTRACT

Hepatic and renal tissues are vital to the survival of a mammalian species. Being organs responsible for the metabolic processing and excretion of xenobiotics, they are mostly affected by the devastating effects of a foreign agent. The aim of this study is to investigate the possible harmful effects of bonadabe paracetamol syrup on the hepatic and renal functions of female Wistar rats. Rats (200 g) used for the study were divided into 3 groups of 7 rats each. Groups 1, 2, 3 were administered with fake bonadabe paracetamol syrup, genuine drug and distilled water respectively. Serum activities of ALT, AST, γ-GT, ALP and levels of total protein, albumin, bilirubin, globulin, urea, creatinine and uric acid; markers of hepatic and renal functions were determined in serum samples. In addition, activities/levels of markers of oxidative stress were determined; namely glutathione-S-transferase, glutathione reductase, superoxide dismutase, glutathione peroxidase, catalase, and MDA, as well as reduced and oxidized glutathione. Histologic examinations of hepatic and renal tissues were carried out using hematoxylin-eosin staining technique. Results of the study showed gross hepato-renal damage; biochemistry results were significantly different at $p \leq 0.05$, when data were subjected to analysis of variance. Moreover, markers of oxidative stress were significantly different. Histology results also confirmed tissue damage. The concurrent increase in markers of hepatic and renal damage and decrease in the levels of antioxidant suggest that hepato-renal damage featured by the fake drug administered rats may be oxidative-stress mediated.

Keywords: fake paracetamol syrup; kidney; liver, antioxidant status.

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INTRODUCTION

A drug has been defined as a substance used as medication or in the preparation of medication. In most cases when the therapeutic dose is exceeded drugs are toxic to the system, according to Paracelsus drugs are poisons in small aliquot[1]. The fact that most of the therapeutic agents are capable of yielding reactive species and therefore induce oxidative stress is one of the characteristics for which drugs are well known. Paracetamol, a mild analgesic and anti-pyretic agent, is metabolized through two major pathways that yield non-toxic end-products, the third pathway though yield the highly reactive intermediate metabolite N-acetyl-P-benzoquinoneimine (NAPQI), which is very toxic mostly to the hepatic and renal cells[2, 3]. Oxidative stress which usually manifests as lipid peroxidation sets in when imbalance between the levels of oxidants and antioxidants occurs[4-6].

Some of these oxidants that have been implicated in the process of hepato-renal damage are superoxide ions, hydroxyl radical, hydrogen peroxide e.t.c; whereas endogenous antioxidants include small molecular weight compounds as well as antioxidant enzymes[7, 8]. Antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase, catalase) especially have been recognized to be the first-line cellular defense against oxidative stress, this is because they decompose oxygen radical and hydrogen peroxide before they interact to form the more reactive hydroxyl radical. A relationship usually exists between tissues like the liver or kidney and endogenously derived-oxidants in xenobiotic-exposed states; usually these tissues generate high level of oxidants compared with many other tissues, making them susceptible tissues i.e. suitable targets for the assessment of toxic effects of an agent. Both the hepatic and renal cells are known to be rich in the cytochrome P450s that are necessary to yield many of the highly dangerous reactive species responsible for oxidative stress. This study is embarked on, to identify if fake paracetamol syrup is capable of causing damaging-effect on the hepato-renal system even when administered within tolerable level of 90 mg/kg. In addition, by estimating the activities of antioxidant enzymes and levels of products of oxidation this study will help to explore if such harmful effects are oxidative stress-induced.

MATERIALS AND METHODS

Chemicals/Drugs

All chemicals used for the study were of the highest purity commercially available. The fake bonadabe paracetamol used for the study was obtained from National Agency for Food and Drug Administration and Control (NAFDAC), Western region office, Ibadan but the genuine drug was

purchased from a reputable Pharmacy in Osogbo. The fake drug was so identified based on the World Health Organization (WHO) definition of counterfeit medications as being a drug that is 'deliberately and fraudulently mislabelled with respect to identity and/or source [9].

Animals and Animal Care

Twenty-one female Wistar rats of average weight of 200 g were purchased from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan. These were divided into 3 groups, with each group consisting of 7 rats; rats were kept in cages and fed with standard rat pellets and water *ad libitum.*

Experimental Design

Group 1 rats were administered with fake drug while group 2 rats were given genuine bonadabe® paracetamol syrup (produced in Nigeria) at a dose level of 90 mg/kg[10]. Seven other rats served as the control and were administered with distilled water. Drug administration to each of the rats took place between 10.00 h and 12.00 h each day and the route of administration was by gastric gavage. This dose was administered daily for a period of 21 days. Whole blood drawn from each rat through retro-orbital bleeding was allowed to clot at room temperature and centrifuged at 3000 g for 10 minutes to obtain serum. Serum was sub-aliquoted and kept at -20ºC. This experiment was performed in accordance with guidelines established in the NIH Guide for the Care and Use of Laboratory Animals.

Clinical Chemistry and Histopathology

Activities of liver enzymes (alanine aminotransferase- ALT; aspartate aminotransferase- AST; alkaline phosphatase- ALP; gamma glutamyl transferase- γ-GT) were estimated to investigate the degree of liver injury at the end daily exposure. Aside the hepatic enzymes, indices like bilirubin, total protein, albumin and globulin were also determined. While level of activity of serum alkaline phosphatase (ALP) was assessed by Mc Comb and Bowers[11] method, serum activities of AST & ALT were determined using Bergmeyer et al[12] method, but those of bilirubin and albumin were carried out by employing modified Jendrassik-Groff[13] & standard bromocresol methods respectively. Respectively levels of total proteins, creatinine, and urea were measured using Biuret's method[14], Jaffé reaction and diacetyl monoxime oxidase method. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

The activity of glutathione S transferase was estimated using the method of Habig et al.[15], but that of glutathione reductase activity was by method of Zhou & Freed [16]. Serum activities of superoxide dismutase, glutathione peroxidase, catalase and MDA were quantified by the methods of Misra and Fridovich[17]; Rotruck et al.[18]; Sinha[19]; and Ohkawa et al.[20] respectively.

Moreover, reduced and oxidized glutathione were determined using the methods of Prins and Loos [21] and Owen Joshua and Butterfield [22] respectively.

Hepatic and renal tissue sections collected were fixed in 10% neutral buffered formalin. These were embedded in paraffin and stained with hematoxylin-eosin staining technique. The slides were viewed under the microscope at ×400.

Statistical analysis

Data obtained are expressed as mean ± SD (standard deviation). Level of significant difference among the three groups was determined using analysis of variance (ANOVA). SPSS package version 15 was used for this purpose. $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

When data obtained were subjected to analysis of variance, treatment of male Wistar rats with fake or genuine paracetamol syrup did not cause significant changes only in the concentrations of globulin, bilirubin and uric acid. All other hepato-renal markers such as total protein, alkaline phosphatase, γ-glutamyl transferase, alanine aminotransferase and aspartate aminotransferase as well as albumin, creatinine, and urea were significantly changed as shown in Table 1. In Table 2 serum concentrations of malondialdehyde, reduced glutathione, oxidized glutathione and reduced/oxidized glutathione ratio were also significantly different. Moreover, the activities of the antioxidant enzymes: glutathione peroxidase, superoxide dismutase, glutathione reductase, glutathione S transferase and catalase were significantly different as presented in Table 3.

Table 1: Serum concentrations of markers of hepatic and renal damage in rats treated with fake and original paracetamol syrup.

	Control	Fake drug	Genuine	P-value	F-value
AST (IU\L)	32.12±5.20	58.53±22.91	35.16±3.11	5.764	0.012*
ALT (IU\L)	31.33±4.47	66.63±13.89	32.20±6.31	14.163	0.004*
ALP (IU\L)	52.37±5.21	67.21±14.42	56.00±3.44	5.086	0.018*
γ-GT (IU\L)	22.64±3.42	55.58±15.34	24.93±2.62	12.601	0.006*
Total protein (g\L)	69.21±2.62	62.27±4.86	70.45±2.70	12.167	0.001*
Albumin (g\L)	40.29±2.06	33.28±2.97	41.56±4.09	14.014	0.000*
Globulin (g\L)	29.48±2.68	28.99±4.90	30.39±4.46	0.228	0.798
Bilirubin (µmol\L)	99.63±3.35	12.58±6.31	10.62±3.79	0.728	0.497
Urea (mg/dL)	22.62±3.42	40.74±11.52	25.66±3.49	9.229	0.002*
Creatinine (µmol\L)	28.59±5.83	39.06±6.46	30.24±4.39	1.350	0.047*
Uric acid (mmol\L)	153.90±14.12	172.36±28.10	148.98±11.29	2.857	0.084

Results are expressed as mean ± standard deviation. *p <0.05 is considered significant. Abbreviations: ALT- alanine aminotransferase; AST- aspartate aminotransferase; ALP- alkaline phosphatase; γ-GT- γ- glutamyl transferase. N = 7.

Table 2: Serum concentrations of malondialdehyde, reduced glutathione, oxidized glutathione and reduced/oxidized glutathione ratio rats treated with fake and original paracetamol syrup.

	GSH (mol/ml)	GSSG (mol/ml)	GSH/GSSG Ratio	MDA (nmol/ml)
Control	1.60±0.21	0.17±0.03	20.34±3.98	20.32±1.61
Fake drug	1.23±0.18	0.10±0.01	12.08±1.83	47.92±14.38
Original drug	1.31±0.22	0.09±0.01	13.43±2.65	23.15±2.60
F-value	6.373	2.626	15.708	22.394
P-value	0.008*	0.027*	0.002*	0.016*

Results are expressed as mean ± standard deviation. *p <0.05 is considered significant. Abbreviations: GSH-reduced glutathione; GSSG-oxidized glutathione; GSH/GSSG-reduced/oxidized glutathione ratio; MDA- malondialdehyde. N = 7.

Table 3: Serum activities of antioxidant enzymes of rats treated with fake and original paracetamol syrup.

	SOD (U/mg protein)	CAT (µmol H_2O_2 consumed/(min·mg protein))	GPx (µmol GSH consumed/(min·mg protein)	GR (U/mg protein)	GST (U/mg protein)
Control	12.38±1.58	4.17±0.34	15.82±2.40	58.86±2.29	0.71±0.06
Fake drug	9.55±1.39	3.29±0.65	12.49±2.19	50.75±2.94	0.60±0.09
Original drug	10.98±1.17	4.21±0.61	14.11±2.20	57.02±3.67	0.69±0.06
F-value	3.395	6.319	3.723	4.645	4.673
P-value	0.025*	0.008*	0.032*	0.048*	0.023*

Results are expressed as mean ± standard deviation. *p <0.05 is considered significant. Abbreviations: SOD-superoxide dismutase; CAT- catalase; GPx- glutathione peroxidase; GR-glutathione reductase; GST- glutathione S transferase. N =7.

The histology results of Figures 1 and 2 showed that there were histologic changes in both the liver and kidney of rats treated with fake paracetamol syrup as shown in the photomicrographs. These specific histologic manifestations or changes included moderate diffuse vacuolar degeneration of hepatocytes (liver); the renal tubules are severely degenerated. There is severe, diffuse necrosis of the renal tubular epithelium (kidney). The photomicrograph results in both Figures 1 and 2 showed no visible lesion for not only the rats in the control group but also for genuine drug administered rats.

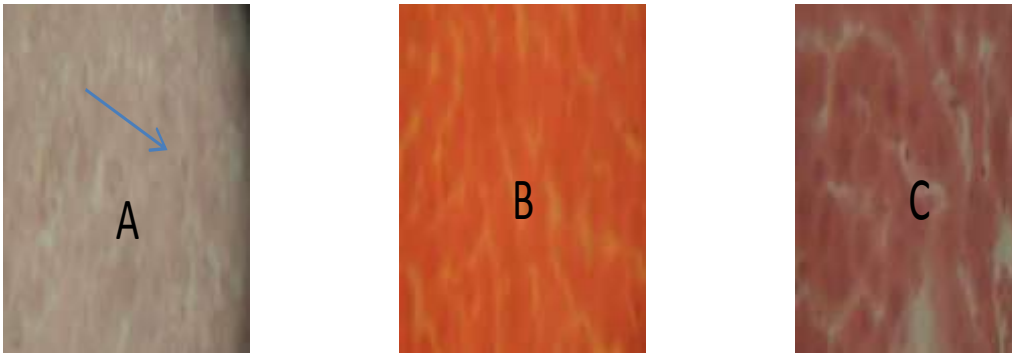

Figure 1: Photomicrographs of liver of rats dosed with fake paracetamol syrup(A), genuine paracetamol syrup(B) and control(C) showing moderate diffuse vacuolar degeneration of hepatocytes, no visible lesion and no visible lesion respectively

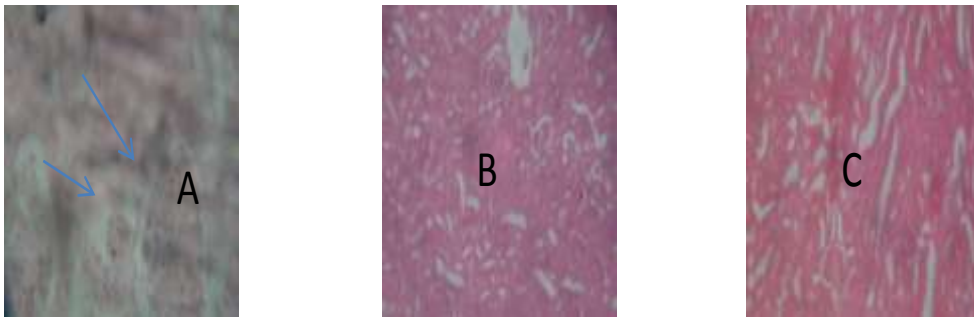

Figure 2: Photomicrographs of kidney of rat with fake paracetamol syrup(A), genuine paracetamol syrup(B) and control(C) showing severe, diffuse necrosis of the renal tubular epithelium, no visible lesion and no visible lesion respectively

Liver disease can be a serious medical problem and data obtained from a number of studies have indicated that liver injuries can occur as a result of the use or abuse of drugs. Even conventional and/or synthetic drugs such as steroids, vaccines, antivirals, and other medications cause serious side effects, many times manifesting as liver disorders [23]. The probability of liver diseases occurring as a result of fake drug consumption then becomes much higher. The fact that liver disease is one of the major health problems globally may not be unconnected with the wide range of functions (e.g. biotransformation and detoxification of endogenous and exogenous harmful substances, plasma protein synthesis, and glycogen storage) this vital organ carries out in the body [24.] Hepatic injury is associated with distortion of various metabolic functions. In addition, it is well known that reactive oxygen and nitrogen species play a crucial role in initiation and progression of many liver diseases especially chemical-induced ones [25-27].

Acetaminophen, a widely used analgesic and antipyretic drug, is primarily metabolized by the liver and excreted by the kidneys. Its safety at lower dose level is well established, but excessive

exposure to acetaminophen can lead to liver damage. Acetaminophen toxicity is linked to one of its intermediate metabolites, N-acetyl-p-benzoquinone imine (NAPQI) which is conjugated by hepatic glutathione to yield a non-toxic product called mercapturic acid. At overdose level, the capacity of both processes of glucuronidation and sulfation is exceeded leading to the formation of excess NAPQI. Therefore, liver and kidney damage in the case of acetaminophen toxicity can be inferred to be linked with depletion of glutathione, which leads to a situation in which excessive NAPQI binds with hepatic and renal cell proteins and manifests as hepato-renal injury [28-30].

Whereas past studies have linked hepatotoxicity and abnormal renal presentations with toxic doses of paracetamol, rats administered with tolerable levels of fake paracetamol also featured abnormal hepato-renal presentations, with histology results showing necrosis of the renal tubular epithelium as well as diffuse vacuolar degeneration of hepatocytes. This is in agreement with results of blood biochemistry; hepatic enzymes such as ALT, AST, ALP, and γGT were significantly different when the three groups of rats were compared using ANOVA. Other markers of hepatic and renal damage (albumin, total protein) were also significantly different, a further indication of toxic effect of exposure to fake drug. These results raise the need for a more effective measure to be embarked upon to prevent human exposure to fake drugs, since neither the histology nor blood biochemistry of rats treated with genuine drug showed any form of abnormality.

While 0% mortality was recorded among the rats used for the study but a much worse occurrence has been associated with fake paracetamol in Nigeria. In 1990 deaths of over 100 paediatric patients administered with fake paracetamol were reported. Rats administered with fake drug also presented with abnormal antioxidant system as evident by decreases in the activities of SOD, GST, GPx, GR and catalase as well as significant decrease in the levels of reduced glutathione and significant increases in the levels of oxidized glutathione and MDA. This suggests that hepato-nephrotoxicity exhibited by the rats in the fake drug group could have been oxidative stress-induced. Oxidative stress-induced renal and hepatic damage has been reported to occur from the metabolism of some other agents [3]. Renal indices like urea and creatinine were also significantly different.

Since metabolism of chemicals takes place largely in the liver, this accounts for the organ's susceptibility to metabolism-dependent drug-induced injury. Drug-induced liver injuries are very common and constitute approximately one-half of the cases of acute liver failure and mimic all forms of acute and chronic liver diseases [31]. Most of the therapeutic agents or their metabolites

that have been linked with hepatic injury induce their hepatotoxicity by interfering with the cell antioxidant systems causing free radical formation and oxidative stress initiation. While the major component of paracetamol toxicity is CYP2E1-mediated metabolism of paracetamol to NAPQI [32, 33], the quantity of paracetamol administered to these rats was at therapeutic level. This means that the hepatotoxic effects could only have been due to the other components of fake drugs.

According to Dash et al.[34], in many cases of chemical-induced destructive process of liver injury, lipid peroxidation has been postulated to be a critical event. The elevation in MDA concentration in fake drug-exposed animals suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Moreover, the significant decrease in the activity of SOD may be due to inactivation of SOD [35] or the antagonistic effect of adulterant with copper and zinc, which are important metals for the activity of SOD molecule [36.] The decrease in the activity of catalase may be as a result of accumulation of superoxide radicals and hydrogen peroxide. The elevation in lipid peroxidation as shown by significant increase in MDA levels in rats treated with fake drug compared with control rats and rats treated with genuine product could be due to insufficient disposal of peroxides as significant decrease in the activity of GPx was also observed [37] On the other hand, the decrease in reduced glutathione in the same category of rats when compared with control rats as well as rats treated with genuine product could probably be due to increased utilization of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents. The significant decrease in GPx activity may also be an indication of enhanced utilization of GSH by GPx (38).

Increase free radical generation has usually been associated with oxidative stress-induced diseases but according to Devasagayam et al. [39], while ROS are well known for their role in many pathological states, they also play important roles in normal cell signaling and homeostasis. For instance, in the vasculature, it is known that they may act to limit the duration of the response to nitric oxide, an important mediator in vascular functions, including regulation of smooth muscle tone and blood pressure, platelet activation, and vascular cell signaling [40.] However, beyond normal physiological roles, when excessive production of ROS occurs as it can be deduced to have taken place in rats administered with fake paracetamol syrup as well as in many other cases of toxicant exposure, radiation damage, and disease it can consequently lead to local oxidative stress and adaptive responses.

One of the adaptive responses in this case is the modulation of the antioxidant levels. According to Deavall et al. [41] cells have different types of defense mechanisms that eliminate free radicals to either prevent or limit intracellular damage and ameliorate the dangerous effects of ROS, components of this defense mechanism include low molecular weight antioxidants e.g. vitamin E, ascorbic acid, and glutathione and antioxidant enzymes e.g. glutathione peroxidase, superoxide dismutase (SOD), and catalase. Many of the indices that were found to be significantly different in fake paracetamol exposed rats. Mitochondrial manganese superoxide dismutase (MnSOD), an example of such defense system, is an enzyme that converts superoxide radicals to hydrogen peroxide, which is further degraded into water by peroxidases [42]. As a result of the activities of these mechanisms physiological levels of ROS are low. However, with increased concentrations of ROS, defense systems can be overwhelmed leading to cellular damage.

Normally functioning cells can tolerate the amount of ROS derived physiologically, but if an imbalance occurs as it has been associated with fake drug treatment in these rats, then significant damage to the cell may take place, damage that may significantly modify intracellular targets such as DNA, proteins, and lipids as well as modulate survival signaling cascades in general. Deavall et al.[41] though observed that at the molecular level, the degree of damage that occurs will depend on many factors such as the site of ROS production, reactivity of the target, and the availability of metal ions.

While there are specific mechanisms for the removal of modified proteins and lipids through normal cellular turnover, DNA damage that can possibly occur as a result of oxidative stress requires specific repair mechanisms. For example when there is mitochondrial DNA oxidation, events such as mutations, rearrangements, and transcriptional errors that impair important mitochondrial components can occur which eventually can lead to more oxidative stress and eventual death of the cell. This therefore suggests that even though fake drug administration may not instantly lead to death it can be the commencement of the initiation of DNA-related disorders. Such possibility though was neither explored nor investigated by this study.

In instances when molecular modifications in surviving cells result in changes in gene expression, especially in cases of severe or extended exposure to oxidants, prosurvival or proapoptotic response pathways may be activated. The onset and development of many diseases such as cardiovascular disease, neurological degenerations (e.g. Alzheimer's disease, ischemic stroke), and cancer, as well as the ageing processes that been linked with oxidative stress-induced damage to DNA and macromolecules can then be predicted as a result of fake

paracetamol syrup exposure. While the role of oxidative stress in many pathological conditions is well documented, they also play beneficial physiologic roles. For example, generation of both ROS and RNS seems to be an important feature of some desirable immunological responses where, in response to activation by pathogens, phagocytes produce reactive species (including superoxide, nitric oxide, and peroxynitrite) that can cause extensive damage to infected cells.

CONCLUSION

Data obtained from the study seem to suggest that the fake bonadabe paracetamol syrup administered to the female rats is capable of damaging both the hepatic and renal cells, since both the synthesizing ability of the liver and the excretory roles of both the kidney and liver were deranged. It is therefore being advocated that a more serious and determined approach be embarked upon by all stakeholders to curb the menace of fake drugs.

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