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Effect of controlled-diet in the treatment of carbon tetrachloride-induced hepatotoxic rats

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ABSTRACT

The aim of this study was to investigate the effect of controlled-diet in the treatment of rats with hepatotoxicity caused by CCl₄. The serum level of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, direct bilirubin, and total bilirubin was measured using standard methods and compared among group I (Normal control), group II (CCl₄ control; hepatotoxic rats without treatment), group III (Hepatotoxic rats treated with 5.2 mg/kg of standard drug only), and group IV (Hepatotoxic rats treated with 5.2 mg/kg of standard drug + controlled diet). The result showed that the level of the biochemical parameters was significantly lower ($p < 0.05$) in group III compared with group II. However, the result showed no significant difference ($p > 0.05$) in the level of measured biochemical parameters between groups III and IV. These results reaffirm the hepatocurative effect of the standard drug (Livolin). It also indicates that the controlled diet used in this research has no positive (curative) effect in the treatment of liver disease.

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1. Introduction

The liver plays a fundamental role in intermediary metabolism (Chantal et al., 2010). It supports almost every organ in the body and is therefore necessary for survival (Cotran et al., 2005). Considering the numerous functions it performs and its strategic location, the liver is prone to so many diseases which could be caused by chemicals, microorganisms, toxins, food, alcohol, genetic abnormalities, and drugs (Kerry and Janice, 2013; Kumar et al., 2004). The initial damage usually results in leakage of certain enzymes from the liver cells into the bloodstream. The elevation of these liver enzymes on a blood test indicates that a patient may have liver disease. Measuring of substance(s)

made or processed by the liver may also give insight into the severity of the disease (Nelson and Cox, 2005).

Liver diseases are a major problem of worldwide proportion (Kerry and Janice, 2013). The concern now is how to achieve effective management and treatment of liver disease. Many synthetic drugs are produced to that effect. Some of these synthetic drugs stand the test of time. Among these drugs is Livolin (Olukiran et al., 2014).

Despite the continues and successful efforts in producing hepatocurative drugs, it was realized that sometimes and at some stages, drugs alone cannot be sufficient; dietary therapy may prove beneficial, most especially in preventing further damage and in ameliorating the consequences of liver failure

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(British Liver Trust, 2011; Delany and Andrea, 2005). This is more apparent because some patients with liver failure lose the ability to process certain metabolites or remove the normal byproducts from digested and absorbed foods from the circulation. Therefore, these byproducts remain in circulation and lead to metabolic and clinical abnormalities. In such cases, drugs alone cannot be sufficient, and foods that result in the accumulation of such byproducts need to be restricted. Therefore, this research was aimed at studying the effect of controlled-diet in the treatment of liver disease.

2. Materials and methods

2.1. Experimental animals

Male and female albino rats weighing between 200 and 250 g were purchased from Biological Science Department, Bayero University Kano. The rats were housed in a well-ventilated animal house in the Biological Science Department, Bayero University Kano, and were allowed to acclimatize for 1 week prior to the experiment and maintained on food and clean water *ad libitum*.

2.2. Preparation of controlled diet

Dried maize grain (3,000 g) was bought from Kabuga gate market, Kano. It was grounded into powder with a grinding machine. Spinach was also bought from Kabuga gate market and was dried under shade at room temperature. The dried spinach was grounded to a powdered form. The controlled diet was prepared by mixing the processed maize powder above (50 g) with the powdered spinach (5 g) and a small quantity of water was added to make a bolus.

2.3. Animal groupings and treatment

A total number of 20 rats were used, which were divided into four groups of five rats each.

Group I: Normal control (Received only food and water).

Group II: CCl_4 control (100 mg/kg CCl_4 once) administered subcutaneously as reported by Alhassan et al. (2009).

Group III: 100 mg/kg of CCl_4 once, administered subcutaneously as reported by Alhassan et al. (2009) + 5.2 mg/kg body weight of Livolin for 30 days.

Group IV: 100 mg/kg of CCl_4 once, administered subcutaneously as reported by Alhassan et al. (2009) + 5.2 mg/kg body weight of Livolin for 30 days + controlled diet for 30 days.

2.4. Collection of blood samples

After 30 days, the rats were sacrificed by cervical decapitation. The blood of each rat was collected in a separate centrifuging tube. The blood samples were centrifuged and the sera were pipetted into separate well-labeled containers. These sera were used in the estimation of the various biochemical parameters.

2.5. Determination of biochemical parameters

All the biochemical parameters [aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB)] were determined using Randox test kits specific for each of the parameters. The procedures were carried out according to the manufacturer's protocol.

2.6. Statistical analysis

Statistical analysis was carried out using Minitab software and Means were compared using one-way analysis of variance. The values were expressed as the Mean \pm standard deviation (SD). p values < 0.05 were considered as statistically significant.

3. Results

Table 1 shows a comparison between the normal control group (group I) and the CCl_4 control group (group II). The result showed a significant increase ($p < 0.05$) in the level of serum AST, ALT, ALP, TB, and DB in group II compared with group I. This indicates that hepatotoxicity is induced in group II.

The results in Table 2 shows that the levels of AST, ALT, ALP, DB, and TB are significantly lower ($p < 0.05$) in group III compared with group II. This suggests that 5.2 mg/kg of the standard drug may have a hepatocurative effect. However, the comparison between groups III and I shows no significant difference ($p > 0.05$) except in the level of ALT where it was significantly higher ($p < 0.05$) in group III than group I.

The result also showed that the level of AST, ALT, ALP, DB, and TB was significantly lower ($p < 0.05$) in group IV compared with group II. This suggests that the 5.2 mg/kg of Livolin + controlled diet may have a hepatocurative effect. Also, the comparison between groups IV and I showed no significant difference ($p > 0.05$) except in the level of ALT where it was significantly higher ($p < 0.05$) in group IV than group I.

Furthermore, the comparison between groups III and IV showed no significant difference ($p > 0.05$) in the level of biochemical parameters. This may indicate that the addition of the controlled-diet to the treatment has no effect since there is no difference in the

Table 1. Comparison of biochemical parameters between the normal control group (group I) and CCl₄ control group (group II).

Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TB (mg/dl)	DB (mg/dl)
Group I	36.36 ± 1.17 ^a	44.75 ± 1.19 ^b	78.55 ± 1.20 ^c	0.95 ± 0.04 ^e	0.50 ± 0.07 ^f
Group II	105.2 ± 2.74 ^a	93.22 ± 1.31 ^b	114.53 ± 3.75 ^c	1.97 ± 0.05 ^e	1.26 ± 0.18 ^f

Values are expressed as mean ± SD.

Values in the same column bearing the same superscript are significantly different at $p < 0.05$.

$n = 5$.

Table 2. Comparison of biochemical parameters between normal control group (group I), CCl₄ control group (group II), group administered 5.2 mg/kg Livolin (group III), and group administered 5.2 mg/kg of Livolin + controlled diet (group IV).

Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TB (mg/dl)	DB (mg/dl)
Group I	36.36 ± 1.17 ^a	44.75 ± 1.19 ^{ac}	78.55 ± 1.20 ^a	0.95 ± 0.04 ^a	0.50 ± 0.07 ^a
Group II	105.23 ± 2.74 ^{abc}	93.22 ± 1.31 ^{ad}	114.53 ± 3.75 ^{abc}	1.97 ± 0.05 ^{abc}	1.26 ± 0.18 ^{abc}
Group III	39.31 ± 0.10 ^b	48.36 ± 1.50 ^a	79.06 ± 0.80 ^b	0.99 ± 0.18 ^b	0.50 ± 0.05 ^b
Group IV	41.15 ± 1.57 ^c	50.86 ± 2.52 ^{cd}	81.44 ± 1.58 ^c	0.97 ± 0.11 ^c	0.51 ± 0.12 ^c

Values are expressed as mean ± SD.

Values in the same column bearing the same superscript are significantly different at $p < 0.05$.

$n = 5$.

level of biochemical parameters between the hepatotoxic rats treated with the drug only, and those treated with the drug + controlled diet.

4. Discussion

The result of this research (Table 1) showed that the administration of 100 mg/kg of CCl₄ subcutaneously once to normal (healthy) rats induced hepatotoxicity after 48 hours. This is evident because of the significant increase ($p < 0.05$) in the level of serum AST, ALT, ALP, TB, and DB in group II (CCl₄ control; hepatotoxic rats without treatment) compared with group I (normal control). This is in consistence with the report of Alhassan et al. (2009) who confirmed that high doses of CCl₄ (90–120 mg/kg) can induce massive liver damage and may persist for a long period. The hepatotoxic effects of CCl₄ occur when CCl₄ is metabolized by the cytochrome-P-450 systems in the liver into a highly reactive metabolite—trichloromethyl radical. CCl₃•, which binds covalently to macromolecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, thus initiating lipid peroxidation, thereby resulting in the damage of the liver cells (Muriel and Escobar, 2003). This damage leads to the leakage of liver enzymes such as ALT, AST, and ALP—which are nonplasma—from the injured cells into the bloodstream, thus raising their level in the serum (Keith and Robert, 2001; Nelson and Cox, 2005). The concurrent elevation of serum AST together with ALP

and ALT more likely indicates hepatotoxicity (Nduka, 1999). Also, the measurement of these enzymes may provide information about the severity of the damage (Nelson and Cox, 2005). Furthermore, the increase in total and DB level in the CCl₄ control group (group II) compared with the normal control group (group I) indicates the presence of hyperbilirubinemia. These observations are all indicators of hepatic cell damage and are in accordance with the report of Venukumar and Latha (2002).

The result of this research also showed that the administration of 5.2 mg/kg/day of standard drug (Livolin) to hepatotoxic rats results in a significant decrease ($p < 0.05$) in the level of AST, ALT, ALP, DB, and TB. This suggests that Livolin may have a hepatocurative effect. This is in accordance with the findings of Olukiran et al. (2014). Livolin is not antiviral but it contains an important phospholipid called phosphatidylcholine. This lipid is one of the most important substances for liver protection and health and is a primary constituent of cell membranes. It acts by several mechanisms, such as exerting potent antioxidant effects, inhibiting the tendency of stellate cells to progress to cirrhosis, decreasing apoptotic death of liver cells (thereby prolonging the life of liver cells), and stabilizing the cell membrane.

Liver plays a central role in metabolism (Chantal et al., 2010). Therefore, food substances—carbohydrates, fats, protein, vitamins, and inorganic salts—that must be taken by an individual for maintenance of good health are processed in part or fully by the liver.

However, disease state (liver disease inclusive) may prevent adequate utilization of materials even when they are adequately taken (Nduka, 1999), and in some cases, instead of them to improve health, they may further complicate and create a new disease condition. In such conditions, the intake of those diets (food substances) that are thought to complicate the disease need to be restricted. It has been documented that some patients with liver failure may lose the ability to properly carry out protein metabolism and other functions necessary for survival (Delany and Andrea, 2005). The controlled diet used in this research contains minimal proteins and lipids. However, the result showed that there is no significant difference ($p > 0.05$) in the level of biochemical parameters between hepatotoxic rats treated and fed with uncontrolled diet and those treated and fed with the controlled diet. This indicates that the controlled diet used in this research has no effect in treating liver disease.

5. Conclusion

The present research reveals that a controlled diet of maize and spinach may not help in the treatment of carbon tetrachloride-induced hepatotoxicity in rats.

6. Recommendation

1. It is recommended that different formulations of controlled-diet should be used in subsequent research.

Author's contribution

All the Authors have contributed tremendously during the research.

Conflict of Interest

There is no conflict of interest among the authors in whatever form.

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