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Research Article

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Effect of Glucose and Alcohol on the Growth and Development of 4 Hydroxyaminoquinoline N-oxide (4HAQO) Induced Pancreatic Adenocarcinoma in Rats

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ABSTRACT

The research work aimed at evaluating the effects of glucose and alcohol on 4 Hydroxyaminoquinoline 1 oxide (4HAQO) induced pancreatic carcinogenesis in rat models. Twenty Wistar albino rats were divided into four groups of five rats each. Three groups of rats were injected with 15 mg of 4HAQO through the tail vein weekly, for three weeks. One week after 4HAQO administration, rats were administered with either glucose or alcohol daily until experiment termination. Sixteen weeks after the first administration of 4HAQO all surviving rats were sacrificed; blood samples were assayed for glucose and pancreatic enzyme (lipase and amylase) levels. Excised pancreases were fixed in formalin, stained with hematotoxylin-eosin. Administration of 4 Hydroxyaminoquinoline to rats resulted in increased pancreas weights and pancreatic lesions as observed from histological studies. A significant decrease in blood glucose level was found in rats that received a glucose solution as compare to other 4HAQO injected rats. Significant increases in pancreatic serum lipase and amylase activity were found in groups that received glucose and alcohol. Pancreatic lesions were verified in all 4HAQO injected rats. Histological sections of pancreatic tissues showed the presence of lesions ranging from dilation of ducts, pleomorphic large cells, atypical acinar cells, reactive hyperplasia, necrosis, and dysplasia. Adenocarcinoma was found in one rat from the glucose group. Moderate to severe dilation of ducts and hyperplasia was seen in the 4HAQO control group, glucose, and alcohol groups. The association between glucose and pancreatic cancer was observed, also alcohol demonstrated carcinogenesis promoting effect.

Keywords: *4HAQO, Glucose, Alcohol, Pancreatic carcinogenesis.*

Abbreviations: *SEM: Standard Error of Mean; SPSS: Software Package of Social Science; 4HAQO: 4 Hydroxyaminoquinoline 1 Oxides; PCW: Percentage Change in Weight*

INTRODUCTION

Pancreatic cancer; is a group of cancers that start in cells of the pancreas. It is a devastatingly fatal form of cancer and is typically regarded as the most deadly and universally rapid-killing form of cancer. Most pancreatic cancers begin in cells that make the digestive fluids (acinar cells), and the most common of these cancers are called adenocarcinomas. Cancers that arise in the pancreatic cells that help control blood sugar levels (islets cells) are called pancreatic neuroendocrine tumors [1]. Pancreatic cancer recently moved from the fourth to the third leading cause of cancer-related death in the United States and is anticipated to become the second around 2020. It accounts for about 3% of all cancers in the US and about 7% of cancer deaths [2]. According to the American Cancer Society, an estimated 53,670 people were diagnosed with pancreatic cancer in 2017, of which 43,090 people died [3]. Because of the silent features, the patients with pancreatic cancer have no symptoms until progression to an advanced stage and therefore, the mortality rate is high. The overall 5-year survival rate is only 6% after diagnosis [4]. At the time of diagnosis, 10%-20% of the patients are eligible for potentially curative surgery.

The rat model shares several similar biological characteristics with humans including a ductal phenotype, the potential for local invasion and peritoneal metastasis with pancreatic cancer, genetic similarities include frequent and early k-ras mutations [5]. Chemical agent 4-Hydroxyaminoquinoline N-oxide, known as a potent pancreatic carcinogen is used. It has been well-documented that a single intravenous administration of 4HAQO preferentially induces pancreatic cells tumors in rats [6], where covalent binding of 4HAQO to DNA to form quinoline adducts has been indicated to be essentially significant for the initiation of rat pancreatic cells carcinogenesis by this agent and produces a phenotype of ductal adenocarcinoma with mutated K-ras [7].

Few studies have examined the intakes of added sugar, simple sugars, and refined sugars in relation to the risk of pancreatic cancer [8]. Moreover, studies that examine the relationship between pancreatic cancer and simple sugars and refined sugars or the risk of pancreatic cancer and sugar intake are case researches. However, it has not been firmly established whether or not alcohol intake is causally related to pancreatic cancer. A number of studies have tried to address the relation between alcohol intake and risk of pancreatic cancer, and most showed a negative result [9].

MATERIALS AND METHODS

Experimental animals

Twenty (20) Wistar albino rats weighing between 160 and 170 g were used in the study. They were purchased from The Nigeria Institute for Trypanosomiasis Research (NITR), Kaduna. The rats were kept in Animal House of Biological Science Department, Bayero University Kano, Nigeria, fed with commercial grower feeds (vital feed finisher) and given water ad libtum. The rats were maintained under standard laboratory conditions.

Solutions

- **Alcohol:** Dry Gin alcohol 43% ABV, was diluted in water at room temperature to a concentration of 15% ABV
- **Glucose:** Glucose D powder was dissolved in distilled water, at a concentration of 800 g/1000 ml

4Hydroxyaminoquinoline N-oxide (4HAQO) for pancreatic carcinogenesis was obtained from Sigma (USA). Glucose (Evans Medical PLC, Agbara, Ogun State) was purchased from ASUU pharmaceutical shop, Bayero University, Kano. Alcohol (Vargas Dry Gin, Onitsha) was purchased from Sabon Gari Area Kano.

Experiment protocol

Twenty (20) Wistar albino rats were randomly divided into four groups of five rats each. Three groups were administered with 4-Hydroxyaminoquinoline N-oxide (4HAQO) and designated as groups II, III, and IV while group I was not administered with 4HAQO. The protocol was carried out using the method of Attalla and Sophia [10,11].

- **Group I:** Rats were not administered with 4HAQO or the solutions
- **Group II:** Rats were injected with 15 mg/kg body weight of 4HAQO weekly for three weeks
- **Group III:** Rats were injected with 15 mg/kg body weight of 4HAQO weekly for three weeks. After one week, were given glucose (0.8 g/kg) daily, orally till the time of sacrifice
- **Group IV:** Rats were injected with 15 mg/kg body weight of 4HAQO weekly for three weeks. After one week, were given alcohol (15% ABV/kg) daily, orally till the time of sacrifice

The rats were weighed before the start of the experiment, and weekly during the curse of the experiment. The blood glucose levels of all the rats in all groups were determined before the commencement of the injection of 4HAQO by use of a glucometer. Prior to the administration of respective groups with the test solutions, the fasting blood glucose levels were determined, and also monitored closely till experiment termination. The administration of solutions (glucose and alcohol) to respective groups began a week after the repeated three weeks administration of 4HAQO and continued as such for 12 weeks. Sixteen weeks after the administration of 4HAQO, all surviving animals were sacrificed; Blood samples were obtained to determine fasting blood glucose, serum lipase, and amylase levels, and the pancreatic tissues were collected for histological examinations.

The change in body weight of the rats was calculated as follows [12].

$$Percentage\,change\,in\,weight = \frac{Initial\,weight - Final\,weight}{Initial\,weight} \times 100$$

The relative pancreatic weight of the animals was calculated as:

$$Relative\,pancreatic\,weight\left(g/100g\right) = \frac{Weight\,of\ \ pancreas}{Final\,body\,weight} \times 100$$

Induction of carcinogenesis in rats

4-Hydroxaminoquinoline N-oxide (4HAQO) was administered intravenously to the rats according to a previously established protocol [6]. In brief, 15 rats weighing between 160 g and 170 g, were given 4HAQO, dissolved in 0.005 M Hydrochloric acid into the tail vein at a dose of 15 mg/kg body weight, weekly for 3 weeks. Five (5) control rats were not administered with 4HAQO.

Histology

The surviving rats were sacrificed; their pancreas was immediately collected weighed and fixed in 10% phosphate-buffered formaldehyde

solution, embedded in paraffin, sectioned at 3 µm to 4 µm, and routinely stained with hematoxylin and eosin (H and E). Serial sections were prepared and examined [13].

Biochemical analysis

The blood samples were collected, and serum was separated. Serum lipase and P-α-amylase were estimated using standard assay kits (Spectrum Diagnostics, Cairo Egypt). Blood glucose levels were measured using glucometer (an Accu-Check® Roche Diagnostic Corporation, USA).

Statistical analysis

The experiments data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using one way ANOVA as in standard statistical software package of social science (SPSS) with significant difference measured at (P<0.05).

RESULTS

Body weight changes in different experimental animal groups, between week one and week ten, post administration of 4HAQO, all the rats of all groups show the same pattern of growth, with no significant difference in their body weights when compared to the normal control rats in group I. In the subsequent weeks, the weights differed significantly between the groups. The final body weights of rats from group IV, which were administered with alcohol, had a significant decrease in the percentage body weight change (15.37%) when compared to other groups. The normal control group, 4HAQO control group and group III which were administered with glucose had 31.64%, 27.50%, and 24.50% increase in body weight change respectively (Table 1).

Table 1: Body weight (g) changes of control rats, and 4HAQO injected rats, administered with glucose solution and alcohol.

Weights				
Groups	**Week 0**	**Weeks 1-10**	**Weeks 10-16**	**PCW (%)**
Normal control	163.33 ± 2.58^{a}	195.00 ± 5.00^{a}	215.00 ± 5.00^{a}	31.64
4HAQO control	163.33 ± 5.16^{a}	193.33 ± 5.77^{a}	208.30 ± 5.77^{a}	27.53
4HAQO+glucose (0.8 g/kg)	166.00 ± 3.22^{a}	191.67 ± 2.88^{a}	206.67 ± 2.88^{a}	24.5
4HAQO+alcohol (15%ABV/kg)	167.67 ± 2.25^{abc}	188.33 ± 2.88 ^{b}	193.33 ± 2.86 ^{c}	15.37
Values are expressed as mean ± STD (n=3) Means with the same superscripts within rows are significantly different at P<0.05 The superscripts a, b, and c show statistical differences along the row				

Table 2: Blood glucose concentrations (mg/dl) of control rats and 4HAQO injected rats administered with glucose solution and alcohol.

Glucose concentrations (mg/dl)				
Group	**Week 0**	**Week 4**	**Week10**	**Week 16**
Normal control	92.33 ± 5.04	90.00 ± 4.61	94.00 ± 4.16	95.33 ± 4.37
4HAQO control	92.00 ± 6.43	92.00 ± 6.11	89.00 ± 3.78	94.66 ± 4.37
4HAQO+glucose (0.8 g/kg)	95.33 ± 8.66a	96.60 ± 2.40^{b}	83.33 ± 3.52^{c}	62.00 ± 5.77^{abc}
4HAQO+alcohol (15% ABV/kg)	93.66 ± 4.33	92.66 ± 10.34	98.00 ± 1.15	109.33 ± 4.80
Values are expressed as mean ± SEM (n=3) Means with the same superscripts within Rows are significantly different at P<0.05 The superscripts a, b, and c show statistical differences along the row				

Blood glucose concentrations (mg/dl) of control rats and 4HAQO injected rats administered with glucose solution and alcohol is presented in Table 2. One week post-4HAQO administration had no significant difference in the blood glucose levels compared to control rats. However, between weeks 11th and 16th the blood glucose levels of group III rats, which were administered glucose solution post-4HAQO administration, had a significantly lowered blood glucose levels compared to both control groups.

Table 3: Serum lipase and p-α-amylase activity of control rats, and 4HAQO injected rats administered with glucose, alcohol, honey and coconut oil.

Groups	**Lipase(U/L)**	**P-α-amylase(U/L)**
Normal control	33.33 ± 1.76^{abc}	80.33 ± 3.17^{abcd}
4HAQO control	57.33 ± 1.76^{ad}	120.60 ± 2.40^{a}
4HAQO+glucose (0.8 g/kg)	66.33 ± 0.88^{b}	111.33 ± 4.80^{bd}
4HAQO+alcohol (15%ABV/kg)	69.33 ± 1.76^{cd}	125.00 ± 2.64^{cd}
Values are expressed as mean ± SEM (n=3) Means with the same superscripts within columns are significantly different at P<0.05 The superscripts a, b, and c show statistical differences along the column		

Table 3 presents the effects of alcohol and glucose solutions on the activities of serum lipase and amylase of 4HAQO induced carcinogenesis in experimental rats. Pancreatic enzymes (amylase and lipase) were found to be significantly increased (P<0.05) in group II (4HAQO control rats) when compared with the group I (Normal control rats). It indicates that the 4HAQO has created an effect on

the pancreatic cell, and it leads to increasing amylase and lipase levels. Administration of glucose and alcohol to rats in groups III and IV showed a significant increase ($P<0.05$) in lipase and amylase levels with respect to group II.

Histological studies

Examination of histological sections obtained from the pancreatic tissues demonstrated the presence of pancreatic lesions with various morphological features ranging from normal pancreas acini, atypical acinar large cells to carcinoma.cells, hyperplasia, hemorrhage, dilation of ducts; pleomorphic (Ia) Control rats show a section of normal pancreas acini and normal pockets of islet cells. (IIa) 4HAQO-injected rats show atypical acini in increased numbers. The acini reveal enlargement of cells, nuclear enlargement exhibiting nuclear pleomorphism and hyperchromasia. Hemorrhage poorly formed islets with poorly cohesive formations supported by scanty fibrous stroma. Dilated pancreatic duct is seen (IIb) with acini hyperplasia. (IIIa) 4HAQO injected rats, which received (0.8 g/kg) glucose solution (group III) show atypical hyperplasia, dilation of pancreatic ducts. (IIIb) pointing out invasive pancreas adenocarcinoma, desmoplastic stroma with atypical glands. (IVa) 4HAQO injected rats, which received alcohol (15% ABV/kg), shows hemorrhage. (IVb) section show extreme dilation of duct marked nuclear pleomorphism and hemorrhage (Figure 1).

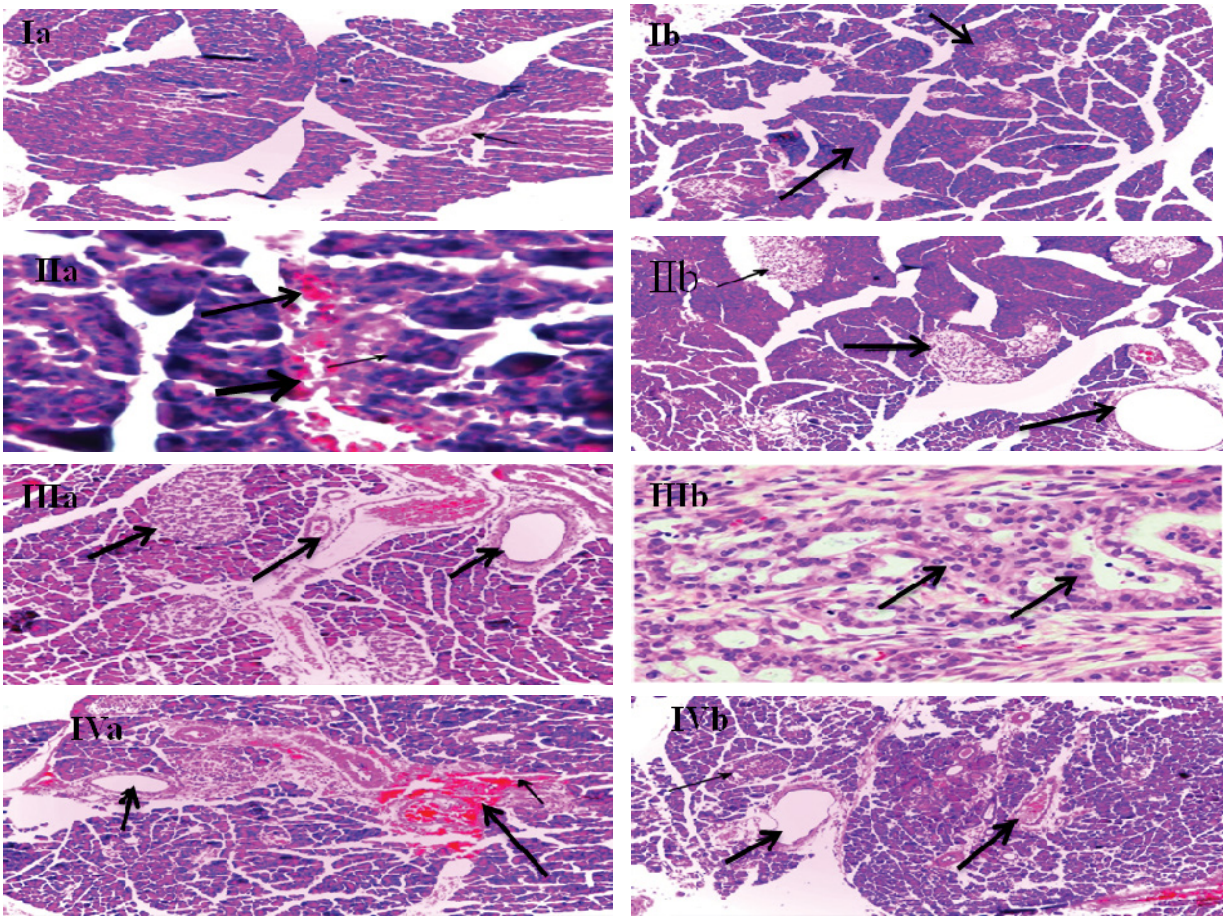

Figure 1: Histopathological features of pancreatic tissues of control rats, 4HAQO injected rats which were administered with glucose solution and alcohol (HE, original magnification x100).

DISCUSSION

Pancreatic cancer is usually associated with weight loss, either from loss of appetite, or loss of exocrine function resulting in poor digestion [14]. In this research, no marked loss in body weight was observed, although rats administered with alcohol showed a decrease in the percentage body weight change. Table 2 shows the initial blood glucose levels before the commencement of 4HAQO injection. Also one week after the completion of repeated 3 weeks injection of 4HAQO, the blood glucose levels were determined. Afterward, the blood glucose levels were closely monitored till the ends of the experiment, this was to check if the carcinogenesis could have diabetic effects. One week post-4HAQO administration had no significant difference in the blood glucose levels compared to control rats. However, between weeks 11th and 16th the blood glucose levels of group III rats, which were administered glucose solution post-4HAQO administration, had a significantly lowered blood glucose levels compared to both control groups. These decreases in blood glucose levels may be due to an increase in plasma insulin concentrations. Li D [15] Stated that hyperinsulinemia and insulin resistance could be a consequence of pancreatic cancer, that hyperinsulinemia and activation of the insulin-like growth factors pathway contributed to tumorigenesis and, subsequently, more rapid progression of cancer [16].

The pancreas is chiefly and intimately associated with the proper functioning of the digestive tract. Enzymes which are released from

the pancreas are amylase, lipase, and proteases [17]. An increase in the serum concentration of pancreatic enzymes (amylase and lipase) is commonly an expression of inflammatory or neoplastic pancreatic disease [18]. In this research, the induction of carcinogenesis was found to increase significantly the serum activity of lipase and p-amylase. This increase in the serum activity of lipase and amylase is comparable to the rise in activity found in other studies involving chemically induced carcinogenesis [19]. The high value of amylase and lipase enzyme is seen in pancreatitis, pancreatic cancer and pancreatic duct obstruction [20]. Consequently, the higher serum lipase and amylase activity was seen in rats that were given alcohol and glucose solutions may be due to hyperplasia and pro-inflammatory metabolic effects of glucose and alcohol. Oxidative and non-oxidative pancreatic damage due to the metabolism of alcohol can initiate inflammatory and fibrotic cascades that may result in subsequent carcinogenesis [21]. Few studies have examined the intakes of added sugar, simple sugars, and refined sugars in relation to the risk of pancreatic cancer [8]. Moreover, studies that examine the relationship between pancreatic cancer and simple sugars and refined sugars or the risk of pancreatic cancer and sugar intake are case researches. High consumption of high-sugar beverages and foods is associated with evidence of increased inflammation and oxidative stress [22]. Increased glucose flux and metabolism promotes excessive proliferation and inflammations of pancreatic tissue [23]. In this research, the increase in the activities of these enzymes may be due to inflammation caused by increased glucose and alcohol metabolism, through carcinogenesis in acinar cells. In this research an association between glucose and pancreatic cancer has been observed, as the adenocarcinoma recorded, was seen in the rats that were administered glucose solution. When the alcohol group was compared to the glucose group, the pancreatic lesions point out characteristics of malignancy, although no tumors were recorded from the rats that were administered with alcohol. However, pancreatic lesions of alcohol group score or grade was higher than that of the 4HAQO induced control group, which could demonstrate the possible promoting effect of alcohol in this experimental model. The association between the consumption of alcohol and pancreatic cancer has been widely researched. However, it has not been firmly established whether or not alcohol intake is causally related to pancreatic cancer. A number of studies have tried to address the relation between alcohol intake and risk of pancreatic cancer, and most showed a negative result [9]. Studies have examined the association between the consumption of alcohol and pancreatic cancer [9,24,25] although the vast majority of epidemiological studies do not confirm this association [25]. There is only indirect epidemiological evidence of this association, as the consumption of alcohol increases the risk of pancreatitis by initiating inflammatory responses that result in overt chronic pancreatitis, and pancreatitis increases the risk of pancreatic cancer [26-28]. In addition, oxidative and non-oxidative pancreatic damage due to the metabolism of alcohol can initiate inflammatory and fibrotic cascades that may result in subsequent carcinogenesis [21]. This association could be partly explained by the following reason: alcohol causes pancreatitis (tissue inflammation) and the concomitant presence of 4HAQO and an accelerated process of cell division because DNA repair mechanisms to be overloaded [7].

CONCLUSION

In this research, the association between glucose and pancreatic cancer was observed, also alcohol demonstrated carcinogenesis promoting effect.

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