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EFFECTS OF HONEY AND COCONUT OIL ON 4-HYDROXYAMINOQUINOLINE N-OXIDE (4HAQO) INDUCED PANCREATIC CARCINOGENESIS IN RATS

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ABSTRACT
Recently, there are many studies focusing on the use of natural products for cancer prevention and treatment. This research work evaluated the effects of honey and coconut oil on 4-Hydroxyaminoquinoline N-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 15mg of 4HAQO through the tail vein weekly, for three weeks. One week after 4HAQO administration, groups III and IV rats were administered with either honey or coconut oil daily for 12 weeks. 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 (0.34±0.01 to 0.53±0.11), significant increases in the activities of pancreatic serum lipase (33.33±1.76 to 57.33±.76) and amylase (80.33±3.17 to 120.60±2.40) with pancreatic lesions as observed from histological studies. Administration of honey and coconut oil for 12 weeks resulted in the reduction of pancreatic serum activities of lipase (36.00±2.30 for honey, 35.33±1.76 for coconut oil) and amylase (91.33±2.40 for honey, 88.67±4.67 for coconut oil). 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, and necrosis. Moderate to severe dilation of ducts and hyperplasia were seen in 4HAQO control group. There were mild necrosis and degeneration of certain cells from rats that were administered honey and coconut oil. Coconut oil and honey show suppression of 4HAQO –induced pancreatic carcinogenesis in rats, suggesting potential curative and/or protective effects.
Key words: 4HAQO, Coconut oil, Honey, Pancreatic carcinogenesis.

INTRODUCTION

Pancreatic cancer recently moved from the fourth to the third leading cause of cancer-related deaths in the United States and is anticipated to become the second around 2020 (American Cancer Society, 2017). It is one of the most lethal malignancies worldwide. The incidence of pancreatic cancer is gradually increasing, while the 5-year survival rate has remained stable at 7-8% (Siegel *et al.,* 2016), with an estimated deaths of 43,090 in the United Stated in 2017.

The etiology of pancreatic cancer is still poorly understood, however some studies have indicated some environmental factors in the occurrence of this disease (Li *et al*., 2004). The environmental and diet related factors include cigarette smoking, alcohol consumption, high consumption of processed foods, sugar and sugar-sweetened foods, high meat intake and occupational exposure to carcinogens like DDT and other organochlorine pesticides, while some of the factors are hereditary and genetic syndromes (WHO, 2014). Of concern is the fact that the devastating toll of pancreatic cancer is predicted to increase significantly unless more effective strategies for its prevention, early detection, and treatment are developed (National Cancer Institute, 2016). Few improvements have been made in the diagnosis and treatment of pancreatic cancer despite extensive efforts over the past few decades. Owing to the lack of clinically validated early screening methods, over 80% of patients are diagnosed at an advanced stage, at which time the cancer is generally considered unretractable (Du*et al.,* 2017). Recently, there are many studies focusing on the use of natural products for cancer prevention and treatment.

Natural products display special attributes (no/ almost no side effects, easily metabolized, very high LD_{50} etc.)in the treatment and prevention of a variety of human disorders including cancer. Coconut oil is a traditional product that has a long history of ethno pharmacological use. Pure coconut oil is extracted from fresh coconut flesh at low temperature and without the use of chemicals. Studies have shown that coconut oil inhibits the induction of carcinogenic agents in the colon and in mammary tumors in test animals (Law *et al.,* 2014). In chemically induced cancers of the colon and breast, coconut oil was shown to be more protective than unsaturated oil.This natural product is rich in antioxidants; with high polyphenol content, including high levels of lauric acid, ferulic acid, and p-coumaric acid (Kamalaldin *et al.,* 2015). It also exhibits anti-bacteria, anti-viral, anti-inflammatory, and anti-diabetes properties, and it has been used extensively as a topical application to treat skin disorders (Nevin and Rajamohan, 2010).

Honey is a natural product produced by bees from the nectar and other sugary substances derived from many plants. It has been traditionally used for centuries to promote health and fight disease, but the associated biochemical mechanisms for its possible protective and therapeutic effects are not yet clarified and remain an important challenge in research (Attalla*et al.,* 2012). Healing properties of honey are related to the antioxidant, anti-inflammatory, antimicrobial, and anticancer activities of flavonoids (Attalla *et al.,* 2012). Although honey is principally a concentrated aqueous solution of inverted sugars (glucose and fructose), it also contains other saccharides, amino acids, organic acids, vitamins, minerals, antioxidants, flavonoids, phenolic acids and carotenoids (Aljadi and Kamaruddin, 2004) that can also contribute to the healing power of honey and its useful inclusion in the diet to complement other polyphenols (Blasa *et al*., 2006).

Chemical agent used in this study is 4-Hydroxyaminoquinoline N-oxide, known as a potent pancreatic carcinogen. It has been well-documented that a single intravenous administration of 4HAQO preferentially induces pancreatic cell tumor in rats (Takayoshi *et al*., 2001), where covalent binding of 4HAQO to DNA to form quinoline adducts has been indicated to be essentially significant for the initiation of rat pancreatic cell carcinogenesis. This agent produces a phenotype of ductal adenocarcinoma with mutated *K-ras* (Qin *et al*., 1990).

This research work aims at evaluating the effects of honey and coconut oil on 4-Hydroxyaminoquinoline N-oxide (4HAQO) – induced pancreatic carcinogenesis in rat models.

MATERIALS AND METHODS

Twenty (20) Wistar Albino rats (weighing between 160 and 170g) were purchased from the Animal House of the Biological Sciences Department, Bayero University, Kano. The rats were maintained under standard laboratory conditions and fed with commercial grower feeds (vital feed finisher) and given water *ad lib*. The standard assay kits for the measurements of serum lipase and amylase were sourced from Spectrum Diagnostics, Cairo Egypt. A glucometer (Accu-Chek)was sourced from Roche Diagnostic Corporation, USA. 4Hydroxyaminoquinoline N-oxide (4HAQO) was obtained fromSigma -Aldrich (St. Louis, MO USA) sourced through Bristol Scientifics Plc.

Pure Honey (Hon=bee, Kano)-100%, tested by putting drops in water and watching how the drops quickly settled at the bottom without melting, indicating 100% purity. It was purchased from Islamic shop, Kabuga Kano.

Extra virgin Coconut Oil (EL-Hawag oils, Cairo). Cold pressed and 100%pure, purchased from Islamic shop, Kabuga Kano.

Experimental Design

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 (normal control). Group II served as the carcinogenesis control group while groups III and IV were the treatment groups. The protocol was carried out using the method of (Attalla *et al.,* 2012; Sophia *et al*., 2014).

Group I: Rats were not administered with 4HAQO, or the solutions (normal control)

Group II: Rats were injected with 15mg/kg body weight of 4HAQO weekly for three weeks(carcinogenesis or 4HAQO control)

Group III: Rats were injected 15mg/kg body weight of 4HAQO weekly, for three weeks, after one week, they were given honey (2g/kg body weight) daily, orally for 12 weeks

Group IV: Rats were injected 15mg/kg body weight of 4HAQO weekly, for three weeks, after one week, they were given coconut oil (1ml/kg) daily, orally for 12 weeks.

Dosage and Administration

According to the OECD (Organization of Economic Corporation and Development) guidelines, dosage of drug (mg) should be constituted in an appropriate volume not usually exceeding 10 ml/kg (1 ml/100g) body weight of experimental animals (mice and rats) for non-

aqueous solvents in oral route of administration. However in the case of aqueous solvents, 20 ml/kg (2 ml/100g) body weight can be considered (Erhirhie *et al.,* 2014).

The rats were weighed before the start of the experiment, and weekly during the course of the experiment. The blood glucose levels of all the rats in all groups were determined before 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 honey and coconut oil to groups III and IV began a week after the weekly administration of 4HAQO, and continued as such for 12 weeks. Sixteen weeks after the first administration of 4HAQO, all surviving animals were sacrificed; blood samples were obtained to determine levels of fasting blood glucose, serum lipase and amylase, and the pancreatic tissues were collected for histological examinations.

The change in body weight of the rats was calculated as follows (Eleazu and Eleazu, 2013):

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

The relative pancreatic weight of the animals was calculated as:

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

Induction of carcinogenesis in rats

4-Hydroxaminoquinoline N-oxide (4HAQO) was administered intravenously to the rats according to a previously established protocol (Takayoshi *et al*., 2001). In brief, 15 rats weighing between 160 and 170g were given 4HAQO dissolved in 0.005M Hydrochloric acid into the tail vein at a dose of 15mg/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 to 4 *u*m, and routinely stained with hematoxylin and eosin (H&E). Serial sections were prepared and examined (Auwioro, 2010).

Biochemical analysis

The blood samples were collected, and sera were 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-Chek® Roche Diagnostic Corporation, USA).

Statistical Analysis

The experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using one way ANOVA using standard statistical software package of social science (SPSS 16.0) with significant difference measured at P< 0.05.

RESULTS

The Body weight (in grams) changes of control rats, and 4HAQO -injected rats administered with honey and coconut oil is presented in Table 1. The normal control group, 4HAQO control group, and groups III and IV which were administered honey and coconut oil had insignificantly 31.64%, 27.50%, 25.00% and 28.03% increases in percentage body weight changes respectively.

Table 2 presents the pancreas and relative pancreas weights. The pancreas weights of group II rats were significantly different (P<0.05) from those of group I. Likewise the pancreas weights of treated groups were significantly different (P<0.05) with those of group II. However, the pancreas weights of treated groups were not significantly different from those of group I.

The blood glucose levels of all groups from the beginning to the end of the research period were not significantly different at p<0.05 (Table 3).

Table 4 presents the levels of serum lipase and P-α-amylase of control and treated groups. 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). Groups III and IV (treated groups) showed a significant decrease (P<0.05) in the activities of lipase and amylase when compared with group II rats.

Table 1:Body weight (g) changes of control rats, and 4HAQO -injected rats administered with honey and coconut oil

Groups	Weights			PCW (%)
	Week 0	Weeks 1-10	Weeks 10-16	
I: Normal control	163.33±2.58[a]	195.00±5.00[a]	215.00±5.00[a]	31.64
II: 4HAQO control	163.33± 5.16[a]	193.33±5.77[a]	208.30±5.77[a]	27.53
III: 4HAQO + honey (2g/kg)	166.67±2.25[a]	193.33±2.88[a]	208.33±2.88[a]	25.00
IV: 4HAQO +coconut oil (1ml/kg)	165.33± 2.22[a]	195.00±5.00[a]	211.67±2.88[a]	28.03

Values are expressed as mean ± STD (n= 3), PCW = percentage change in weight. Means with the same superscripts within rows are significantly different at $P < 0.05$

Table 2:Pancreas and relative pancreas weights of control and treated groups

Groups	Pancreas weight(g)	Relative pancreas weight (g/100g)
I: Normal control	0.34± 0.01[a]	0.16
II: 4HAQO control	0.53± 0.11[abc]	0.25
III: 4HAQO + honey (2g/kg)	0.35± 0.00[b]	0.17
IV: 4HAQO + coconut oil (1 ml/kg)	0.36± 0.14[c]	0.17

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 row

Table 3: Blood glucose concentrations (mg/dl) of control and treated groups

Group	Glucose concentrations (mg/dl)			
	Week 0	week 4	week10	week 16
I: Normal control	92.33±5.04	90.00± 4.61	94.00± 4.16	95.33± 4.37
II: 4HAQO control	92.00±6.43	92.00 ± 6.11	89.00± 3.78	94.66± 4.37
III: 4HAQO + honey (2g/kg)	98.67±1.76	90.66 ±9.82	91.33 ± 5.20	89.33± 0.74
IV: 4HAQO + cnut oil (1ml/kg)	90.00±2.31	95.33± 5.02	93.66 ± 2.96	92.00 ± 5.03

Values are expressed as mean ± SEM (n= 3)
Means with the same superscripts within Rows are significantly different at $P < 0.05$

Table 4: Serum lipase and p-α-amylase levels of control and treated groups

Groups	Lipase(U/L)	P-α-amylase(U/L)
I: Normal control	33.33 ± 1.76[a]	80.33 ±3.17[ab]
II: 4HAQO control	57.33 ± 1.76[abc]	120.60± 2.40[acd]
III: 4HAQO + honey (2g/kg)	36.00 ± 2.30[b]	91.33 ± 2.40[bc]
IV: 4HAQO + coconut oil (1 ml/kg)	35.33 ± 1.76[c]	88.67 ± 4.67[d]

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, c and d show statistical differences along the column

Histopathological 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 cells, necrosis, pleomorphic large cells, dilated ducts and necrosis.

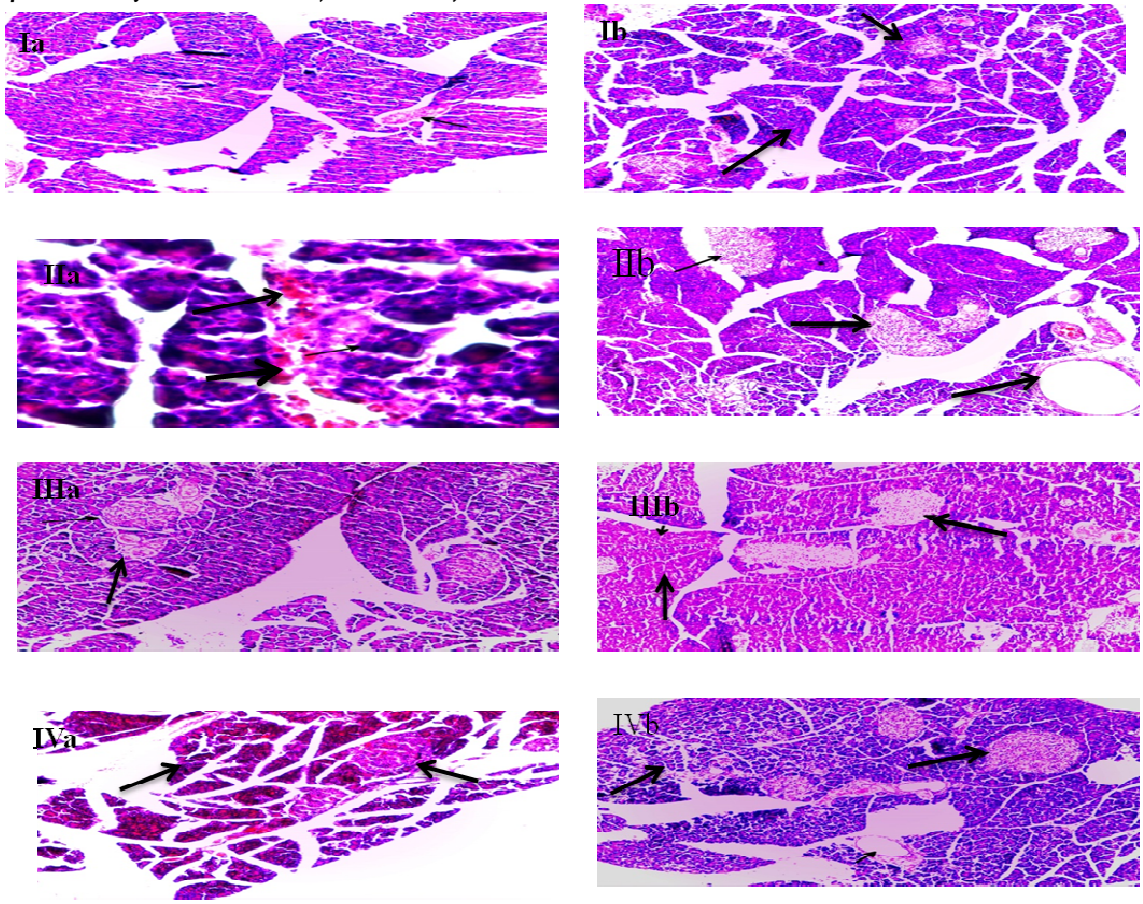

Figure1: **Histological features of pancreatic tissues of control rats, 4HAQO injected rats which were administered with honey and coconut oil(HE, original magnification ×100).** (Ia) Normal control rats (Group I) shows a section of normal pancreas acini and normal pockets of islet cells (Ib). (II) 4HAQO-control rats (Group II) shows atypical acini in increased numbers. The acini reveal enlargement of cells, nuclear enlargement exhibiting nuclear pleomorphism and hyperchromasia, haemorrhage, poorly formed islets with poorly cohesive formations supported by scanty fibrous stroma. Dilated pancreatic duct is seen (IIb) with acini hyperplasia. (III) 4HAQO – injected rats which were administered honey (2g/kg): (IIIa) shows mild necrosis of acinar cells and well-formed islet cells, (IIIb) shows mild to severe necrosis of acinar cells, well-formed islet cells. (IV) Coconut oil treated group (1ml/kg): (IVa) Shows remarkable pancreatic acinar cells, with normal islets, (IVb) Section shows periductal inflammation, hyperplasia and degenerated areas.

DISCUSSION

In this research, no marked loss in body weight was observed, although Pancreatic cancer is usually associated with weight loss, either from loss of appetite, or loss of exocrine function resulting in poor digestion (Bond-Smith *et al.*, 2012). This could be due to the relatively short duration of the research period (4 months), symptoms were yet to manifest in earnest. However a marked change in pancreatic weights was recorded, when normal control rats were compared to the 4HAQO induced carcinogenesis rats. A multitude of medical conditions are associated with change in size and weights of organs (Caglar *et al.,* 2014). Pancreatic atrophy and neoplasia are associated with changes in pancreas weights (Heuck, *et al.*, 2004). Also deviations from the normal ranges of pancreatic weights may indicate the presence of certain pathological changes (Caglar *et al.,* 2014); it indicates that the 4HAQO has created an effect on the pancreatic tissue, and it led to increased pancreas and relative pancreas weights.

Induction of carcinogenesis had no significant effect on the blood glucose levels, the blood glucose levels of all normal control and treated groups show no significant difference when compared with the carcinogenesis control group.4HAQO is indeed an inducer of pancreatic carcinogenesis in rats affecting only the ductal and acinar cellsas shown by the increased levels of exocrine enzyme levels (lipase and p-α-amylase). This finding of selective inducement of ductal and acinar cell carcinogenesis in the pancreas has previously been reported by Qin *et al.*, 1990.

Elevated serum levels of pancreatic enzymes are frequently used for the diagnosis of pancreatic diseases. An increase in the serum concentration of pancreatic enzymes (amylase and lipase) is commonly an expression of inflammatory or neoplastic pancreatic disease (Frulloni *et al.,* 2005). In this research, induction of carcinogenesis was found to increase significantly the serum levels of lipase and p-amylase. This increase in the serum levels of lipase and amylase is comparable to the rise in levels of same found in other studies involving chemically induced carcinogenesis (Nozawa *et al*., 2012; Sophia *et al*., 2014). High values of amylase and lipase enzyme are seen in pancreatitis, pancreatic cancer and pancreatic duct obstruction (Muniraj *et al.,* 2015). In this research, the increase in the levels of these enzymes may be due to inflammation, through carcinogenesis in acinar cells. Administration of honey and coconut oil to 4HAQO induced carcinogenesis of rats significantly reduced the serum levels of amylase and lipase. Increased serum levels of p-amylase and lipase are good indicators for acute and recurrent or chronic pancreatitis and are used to monitor the treatment of the disease (Gültepe *et al.,* 2016). This decrease may be due to the properties of coconut oil that may help modulate inflammation indirectly and modulate cancer risk. This natural product is rich in antioxidants; with high polyphenols content, including high levels of lauric acid, ferulic acid, and p-coumaric acid all of which possess reducing abilities (Zakaria *et al.,* 2011). Its exhibits of anti-bacterial, anti-viral, and anti-inflammatory have been reported (Intahpuak *et al*., 2010). In the same vein, the reduction in serum lipase and amylase levels in rats that were treated with honey could be due to the healing properties of honey, which are attributed to its various pharmacologically active constituents, especially flavonoids and phenolic constituents (Omotayo *et al*., 2014). These honey constituents have been shown to exert anti-inflammatory, antioxidant, antiproliferative, antitumor, antimetastatic and anticancer effects (Samarghandian *et al.,* 2011).

In the histopathological analysis (plates 1), the pancreatic lesions observed were of ductal and acinar cell origin, with severe dilation of ducts developed in rats of group II, the 4HAQO control group. Moderate dysplasia is seen, hemorrhage, pleomorphism was found in rats from group II. There were mild necrosis, pleomorphism and degeneration of certain cells from rats of groups III and IV treated with honey and coconut oil respectively. Their histological sections show mild necrosis of the acinar cells with well-formed islet cells. This necrosis or death of cells could be due to the protective/curative effects of honey and coconut oil, which indicates that coconut oil and honey are effective at suppressing cell growth and inducing cell death of pancreatic cells undergoing carcinogenesis. In agreeing with this research findings, El-kott *et al*. (2012) showed that DEN-induced severe liver injury and carcinogenesis in rat liver were prevented by bee honey, suggesting that honey is a protective antioxidant against liver toxicity and an anti-tumor agent. Antiproliferation effect of honey in colon cancer cells is found to vary depending on honey's botanical and geographical origin (Jaganathan and Mandal, 2009). Natural products such as honey and coconut oil have potential anticancer effects (Kamalaldin *et al*., 2015;Ahmed *et al*., 2013). Studies reporting anticancer effect of honey range from tissue cultures and animal models, to clinical trials (Ahmed *et al*., 2013). Honey has been shown to have anti-inflammatory, antimicrobial, antimutagenic, antioxidant, and antitumor effects (Hegazi *et al*., 2016). The polyphenols in honey are considered as one of the main factors responsible for the anticancer activity of honey (Jaganathan and Mandal, 2009).

CONCLUSION

In this research, coconut oil and honey showed suppression of 4HAQO –induced pancreatic carcinogenesis in rats, suggesting potential protective and/or curative effects. 4HAQO is indeed an inducer of pancreatic carcinogenesis in rats affecting only the ductal and acinar cells as shown by the increased levels of exocrine enzyme levels (lipase and p-α-amylase), while glucose levels in all the rats in all the groups remained within the normal range throughout the duration of the research period, suggesting non-interference with β cells of the islets of Langherans by 4HAQO.

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