Nephro-protective potency of natural honey: Report of preliminary study in white Wistar albino rats


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ABSTRACT

Over the last 20 years, man has been exposed to an array of chemicals in form of medicines, industrial waste and a variety of naturally occurring substances which adversely affect the kidney. Food additives, majorly flavorings and sweeteners, had been indicted as some of these natural/artificial occurring substances which causes nephro-toxicity but their adjunct role in food cannot be undermine; as such, there is need for a possible food flavourant/sweetener which will have close to zero nephrotoxic effect on the kidney and if possibly serve a nephro-protective role, thus helping the kidney to mop up the array of chemicals that it is daily expose to from other sources, with possibility of reducing acute injury and renal changes that might lead to end-stage renal failure and renal malignancies when toxicity goes unchecked.

Thirty-six weaned rats were used in this research work. They were grouped into 3 groups (12 rats each) randomly distributed without any prejudice. Group A (control) was fed with normal diet and water was supplied ad libitum. Group B (short term) was fed with normal diet and water was supplied ad libitum too, in addition, an oral administration of honey at a daily dosage of 250 mg/kg of rats was done for 21 days (3 weeks). Group C (long term) was also fed with normal diet and water was equally supplied ad libitum, in addition, an oral administration of honey at a daily dosage of 250mg/kg of rats was done for 12 weeks. The rats were sacrificed at the end of the 12th week, the various kidney toxicity markers were assayed for on the serum while the residual organ (kidney) was done on their respective kidney homogenates. A high and significantly different activities (P < 0.05) was observed on the serum fraction of the group A (control); however, an interestingly low and significant value was observed in both the plasma and kidney homogenates of group C (long term honey administration) rats with a close to zero toxicity value observed in its residual toxicity profiling, thus suggesting that oral honey consumption/administration in Wistar rats is greatly beneficial as an alternate food sweetener with a potentiality of exerting a nephro-protective effect.

Keywords: Honey, kidney, nephro-protective, functional food.

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INTRODUCTION

For the past two decades, it has become increasingly obvious that the kidney is adversely affected by an array of chemicals through exposure to medicines, industrial waste and a variety of naturally occurring substance. The level of exposure varies from minute quantities to very high doses. However, the full extent of the economic
impact of chemically induced or associated nephropathy is difficult to define because the diagnosis of early injury and documentation of the cascade of secondary degenerative changes have not been adequately identified (Ziemer and Gibson, 1998). Instead, most chemically associated with renal disease are only identified as an acute renal failure or as chronic renal failure at a very late stage, when therapeutic intervention is near impossible (Eknoyan et al., 1997). More important at this stage, the etiology may be observed by lack of reliable information on the likely causative agents, the levels and duration of exposure and other possible contribution and exacerbating factors. At present, epidemiological evidence indicates that nephro-toxicity leading to acute renal failure or chronic renal failure represents a substantial financial burden to society. Indeed, there are some indications that chemical exposure could play a much greater influence in the very high incidence of end stage renal disease encountered in nephrology and dialysis clinic than is currently considered to be the case (Bard et al., 2003).

Owing to its diverse function and small mass in relation to its resting cardiac output that it handles, the kidney is a target both for chemicals that are pharmacological active and for toxic material. The nephron and its related cells perform a diversity of physiological functions. It is the major organ of excretion and homeostasis for water-soluble molecules because it is a metabolic active organ. It can concentrate certain substances actively. In addition, its cells have the potential to bio-convert chemicals and metabolically activate a variety of compounds (Eknoyan et al., 1997). The cell responds to injury by repair, and the kidney responds to chemical lesion by renal and extra-renal adaptation to compensate for loss of that cell function. Although, there is a substantial capacity within the kidney for repair, there are also several circumstances where damage may be irreversible. In general, the proximal and distal tubules and urothelia can be repaired but the glomeruli and medulla may have a significantly lower repair facility. It is therefore possible to initiate a series of degenerative changes as of interfering with one or more of the physiological processes (Bard et al., 2003).

The task of cleaning, or filtering, the blood is performed by millions of nephrons, remarkable structures that extend between the cortex and the medulla. In addition to cleaning the blood, the kidneys perform several other essential functions. One of such activity is regulation of the amount of water contained in the blood (Bard et al., 2003). The kidney also adjusts the body's acid-base balance to prevent such blood disorders as acidosis and alkalosis, both of which impair the functioning of the central nervous system. If the blood is too acidic, meaning that there is an excess of hydrogen ions, the kidney moves these ions to the urine through the process of tubular secretion. An additional function of the kidney is the processing of vitamin D; the kidney converts this vitamin to an active form that stimulates bone development. Several hormones are produced in the kidney. One of these, erythropoietin, influences the production of red blood cells in the bone marrow. When the kidney detects that the number of red blood cells in the body is declining, it secretes erythropoietin. This hormone travels in the bloodstream to the bone marrow, stimulating the production and release of more red cells (Boron and Boulpaep, 2003).

Foods are no longer appreciated by consumers only in terms of its taste and immediate nutritional needs, but also in terms of its ability to provide specific health benefits (White, 1983). Functional foods (which honey belongs) became an important food sector promoting health benefits via functional ingredients in these products. Honey is often produced by the honey bees in larger quantity than bee wax, pollen, royal jelly and bee venom which are other beehive products (White, 1979). Honey has three major uses (Cooper et al., 2002): as food, as medicine, as raw materials. As food, honey is a near perfect food (Ladas and Raptis, 1999). As medicine, its anti-bacterial ability and supersaturated sugar solution with high osmotic pressure builds up the immunity level of individual consumers (Dixon, 2003). The curative power of honey can only be ascertained therapeutically as it has positive effects on the diseases. As raw material, honey is hygroscopic and the confectioneries made with honey remain moist most of the time (Delaplaine, 2006). Thus, the aim of the research work is to investigate the functional potency of honey as a nephro-protective food supplement.

MATERIALS AND METHOD

Experimental subject

Thirty-six weaned white albino rats weighing between 45 and 60 g were obtained from the Animal House, Olabisi Onabanjo University, Remo Campus, Ikenne, Ogun State. The rats were randomly grouped into 3 groups labelled A, B and C, placed on the same diet having been acclimatised for 1 week. Baseline physical and biochemical analysis was then carried out on two randomly selected group representative rats. The honey used for this research works is the ILORAA varieties (a local crude and dark honey commonly consumed in Remo land of Ogun State, Nigeria). This honey variety is gotten from Apis mellifera scutellata species of honey bees (Morse and Hooper, 1985).

Feeding

All the rats were fed on normal commercial rats chop. They were all maintained on this diet for 12 weeks, water was provided ad libitum.

Group A

Control (CTL) animals and were fed for 12 weeks with rat chow and then sacrificed.
Table 1. Percentage relative organ to body weight ratio.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
<th>Heart</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.34 ± 0.16a</td>
<td>3.26 ± 0.09a</td>
<td>2.48 ± 0.12a</td>
<td>2.08 ± 0.08a</td>
<td>1.84 ± 0.08a</td>
</tr>
<tr>
<td>B</td>
<td>6.74 ± 0.22a</td>
<td>3.20 ± 0.08a</td>
<td>2.44 ± 0.11a</td>
<td>2.12 ± 0.07a</td>
<td>1.97 ± 0.08a</td>
</tr>
<tr>
<td>C</td>
<td>8.44 ± 0.18b</td>
<td>3.75 ± 0.08b</td>
<td>2.39 ± 0.12a</td>
<td>2.14 ± 0.08a</td>
<td>2.17 ± 0.11b</td>
</tr>
</tbody>
</table>

**Group B**
Short term honey (STH) animals, in addition to feeding, 250 mg/kg honey was daily administered orally for 3 weeks (week 9 to week 12), at completion.

**Group C**
Long term honey (LTH) animals, in addition to diet feeding, oral 250 mg/kg honey was daily administered orally for 12 weeks of the study.

At the end of the research, they were all fasted overnight and sacrificed by keeping in an anaesthesing bottle containing diethyl ether. The brain was then removed by cervical encapulation and the kidney homogenate was then prepared according to Olowookere (2007).

**Physico-analysis**

**Weight gain determination of the rats**
The 12 control rats were weighed weekly with the Digital Mettler S20 weighing balance for the period of 12 weeks and likewise the 12 rats each of both short and long term administered were also weighed for the period of twelve weeks. After sacrificing the rats, the weight of the various organ was taken and the percentage relative organ to body weight ratio determined mathematically as shown below (Olowookere, 2007):

\[
\text{Weight of organ} \quad \times 100 \\
\text{Final body weight of rat}
\]

**Plasma preparation**
The blood in the EDTA bottle was centrifuged at 4000 rpm for 15 min at room temperature (using a table centrifuge) and the plasma was separated from the blood using decantation method, labeled and store in the refrigerator under suitable condition.

**Organs homogenization**
Homogenization of the organ was done with aid of Sorvail Homogenizer (Olowookere, 2007). The Kidneys were macerated, that is, cut into smaller pieces. After the organs had been macerated, the homogenization mixture which was made up of 0.02 M phosphate was added to various organs in gradual process in a dilution factor of 1:4 w/v organ-working solution. Each mixture was collected into separate test tubes and placed in a centrifuge. The samples were then spun for 5 min at a speed of 4000 rpm so that the homogenate settles from the supernatant. The supernatant were then separated from the homogenate residue.

**Biochemical analysis**
Plasma urea nitrogen assay (PUN) was carried out by the method of Burtis and Edward (1999), creatinine was determined by the method of Jaffe (1986) in A.O.A.C (2000) and total urinary protein was estimated by the method of Kruger (1994). The urine sample of the various member of each group was collected daily as described by Henry (2001).

**Statistics analysis**
Values are expressed as Mean ± S.E.M (standard error of mean). Mean values are compared using one way ANOVA. Levels of significance were evaluated using Duncan’s Multiple Range Test (DMRT) at P < 0.05.

**RESULTS**
Table 1 showed a significant increase in the organ to body weight ratio of liver, kidney and spleen in animal fed with natural honey for long term.

**Body weight gain**
As shown in Figure 1, there was a progressive increase in body weight in animal fed with honey for short and long term. The increment in weight gain between Groups B and C pronounced at week 9 when honey administration commenced for short term group rat.

As shown in Figure 2, there was a significant reduction in the total plasma creatinine level which was progressive as the duration of honey administration increases.

As shown in Figure 3, there was a significant reduction in the total plasma urea nitrogen level which was progressive as the duration of honey administration increases.

As shown in Figure 4, there was a significant reduction in the total urinary protein level which was progressive as the duration of honey administration increases.

**Residual kidney toxicity assay**
The various kidney toxicity markers such as protein,
creatinine and urea nitrogen in its homogenate were significantly changed as the duration of honey administration increases (Table 2).

**DISCUSSION**

The kidney is unusually susceptible because of its role in filtering harmful substances from the blood. Exposure to chemical substances can cause adverse effects on the kidney, urethra, or bladder (kidney toxicity). Toxic injury to the kidney is known to occur as a result of exposures to halogenated hydrocarbons (Britigan et al., 1992), such as carbon tetrachloride and trichloroethylene, and the heavy metals cadmium and some known food contaminants entering the body through the oral route. In addition, the deleterious role of ageing in causing neurotoxicity had earlier been established (Oloowokere, 2007; Tonks et al., 2003). Thus, ageing was used as a promoter of kidney toxicity in this research work. Figure 1 shows the weekly body weight of the experimental animals. A steady, consistent, and progressive increase in body weight was observed in all the three groups; however, these significantly increase in body weight was more pronounced in both short term honey and long term honey animals when compared with control group of rats.

The result of the effect of honey administration on the various blood samples of the various grouped animal using the various integrity markers, vis-a-viz; plasma urea nitrogen (PUN), creatinine and total urinary protein activities of the rats, is shown in Figures 2, 3 and 4. The plasma/blood urine nitrogen reflects the amount of nitrogen that is present in the body in the form of a waste product called urea. BUN is used to determine if there are extra nitrogenous wastes in the blood stream, which should have been filtered out of the kidneys which is
Figure 2. Total plasma creatinine level.

Figure 3. Total plasma urea nitrogen level.
usually associated with kidney dysfunction. One of the symptoms of this kidney problem is the failure to filter as much urea as is necessary. An excess of nitrogen compounds in the blood may lead to uremia. The serum creatinine is present after the chemical creatine is broken down by the body in order to make energy for the muscles. The kidneys are normally able to filter out large amounts of creatinine on a daily basis. However, when kidney problems are present, the creatinine levels will increase, reflecting less creatinine being filtered out through the kidneys.

It was observed that the various toxicity marker results follow similar pattern: Administration of honey even on short term duration is seen here to have significantly effect on all the kidney integrity markers. While the various kidney toxicity markers such as creatinine, plasma urea nitrogen (PUN) were greatly reduced to close to zero level on administration of honey on long term (Table 2), their corresponding residual kidney protein was greatly increased to cause a significant morphological and metabolic change. This is in agreement with the reports of Tony et al. (2003); Harman et al. (2005) on honey as having the potential to stimulate inflammatory cytokine production from monocytes, thus increasing the tissue protection from various scavenging oxidants. This is also in agreement with the findings of Al-Mamarya et al. (2002) and Molan (1992) who reported an antioxidant property of honey while Gheldof et al. (2002) identifies the presence of antioxidants such as ascorbate, selenium and catalase in honey. However, it was Tonks

Table 2. Result of total kidney protein (mg/ml) of the 3 groups of experimental rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Short term honey</th>
<th></th>
<th>Long term honey</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (A)</td>
<td>Test (B)</td>
<td>Control (A)</td>
</tr>
<tr>
<td>Residual kidney protein</td>
<td>1.18 ± 0.30°</td>
<td>1.77 ± 0.25°</td>
<td>1.14 ± 0.27°</td>
<td>2.36 ± 0.32°</td>
</tr>
<tr>
<td>Residual kidney urea</td>
<td>1.45 ± 0.17°</td>
<td>0.88 ± 0.11°</td>
<td>1.38 ± 0.14°</td>
<td>0.39 ± 0.08°</td>
</tr>
<tr>
<td>Residual kidney creatinine</td>
<td>4.38 ± 0.06°</td>
<td>2.46 ± 0.06°</td>
<td>13.44 ± 0.06°</td>
<td>3.62 ± 0.58°</td>
</tr>
</tbody>
</table>
et al. (2003) who attributed the nephro-protective potency of honey to its ability to stimulate inflammatory cytokine production from monocytes of the kidney.

Protein in urine (proteinuria), especially at high levels, can indicate kidney disease or another serious condition. The kidneys filter many substances, including waste products, from the blood. These waste products are then excreted in urine. Normally, during this filtering process, the kidneys retain components, including proteins, that the body needs (Brenner and Floyd, 1999). But some diseases and conditions can allow proteins to slip through the filters of the kidneys, causing protein in urine. Low levels of protein in urine are normal, and are extremely desirable (< 65 mg/dl), but an unwanted situation like indiscriminate usage of birth control pills, drugs, ageing, diabetics, consumption of contaminated food (from natural and artificial food adjuncts like sweeteners, flavourants, etc) and indiscriminate usage of stimulants may lead to this unwanted state of kidney necrosis (Brittenger et al., 1992). However, the prolong administration/consumption of honey was found to help the kidney in its metabolic performance/organ integrity by the findings that honey reduced to the barest minimum the unwanted protein in the urine (proteinuria) which is an indices of kidney malfunction (Figure 4).

Conclusion
Kidney dysfunction is a condition that its causes are too diverse and inclusive, particularly with reference to ageing and exposure to food oxidants, and pollutants from day to day social activities; thus, a more economical approach towards its prevention cum remediation will be to be on a natural common products-functional food (e.g. honey) which might help to improve the kidney integrity/bio-regeneration. We therefore recommend that substituting sugars with honey at high concentration in day to day diet might help to achieve this desired kidney detoxification/remediation, bearing in mind the economic cost and risk that this kidney necrosis present to individual and the society at large.

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