

Journal of Food: Microbiology, Safety

Research Article

Safety Evaluation of Honey from *Jatropha Curcas* Nectar and its Implication for Honey Production in Ghana

Michael Kodwo Adjaloo^{1*}, George Asumeng Koffuor², Emmanuel Akomanin Asiamah³, Richard Annan-Dadzie⁴ and Benedicta Osei-Donkor⁵

¹Technology Consultancy Centre, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

²Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

³Department of Medical Laboratory Sciences, University of Allied Health Sciences, Ho, Ghana

⁴Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

⁵Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

*Corresponding author: Dr. Michael K. Adjaloo, Technology Consultancy Centre, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Tel: +233 243136701; E-mail: mkadjaloo@gmail.com

Received date: June 18, 2017; Accepted date: July 20, 2017; Published date: August 07, 2017

Copyright: ©2017 Adjaloo MK, et al. This is an open-access article distributed under the terms of the creative commons attribution license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Jatropha curcas L. (Euphorbiaceae) is a plant documented to have an interesting toxicity profile however; bees produce honey from the nectar of its flowers in a Jatropha curcas plantation in the Yeji municipality of the Brong-Ahafo Region of Ghana. This study therefore is aimed at ascertaining the safety for consumption of honey produced from the *J. curcas* plant. Grouped Sprague-Dawley rats administered orally with single doses of this honey (300-1500 mg/kg) were observed critically for 24 h in an acute toxicity study. Cage-side observation, hematological profile, liver and kidney function tests, and body and organ weight monitoring were also carried out on grouped rats given 300-800 mg/kg of honey daily for 30 days in a sub-chronic toxicity test. Results indicated no physical, clinical signs and symptoms of toxicity, morbidity, and mortality after acute and prolonged administration of the honey. Sub-chronic toxicity studies revealed no significant changes (p>0.05) in body weight and organ weight (stomach, heart, and kidney), hematological parameters, liver and kidney function. There was however a dose-dependent increase ($p \le 0.05-0.01$) in aspartate transaminase, and significant increments in liver weight at all treatment doses. Histopathological studies of stomach, heart, kidney and liver showed normal architecture with no pathologies. Honey produced from Jatropha curcas flower nectar would be deemed safe for consumption as it did not show significant toxicity symptoms in Sprague-Dawley rats.

Keywords: Aspartate transaminase; Alanine transaminase; Hematological profile; Liver and kidney function tests; Melliferous plant; Sub-chronic toxicity test

Introduction

Jatropha curcas L. (Euphorbiaceae), commonly known as Barbados nut, Purging nut, or Physic nut, is a multipurpose tropical large shrub with many attributes and considerable potential. It is native to Mexico and Central America, but is widely distributed in Latin America, India, South-East Asia and Africa [1]. In West Africa it is reported to be cultivated in Mali, Nigeria and Ghana [1,2]. As a drought resistant, perennial plant *J. curcas* grows even in the marginal or poor soil and can be used to reclaim land, as a hedge as well as a commercial crop [3,4]. Hence, it could provide employment, improve the environment and enhance the quality of rural life. It has been used widely in traditional medicine in the treatment of malaria, jaundice; dermatitis, rheumatism, and snake bite [5]. *Jatropha curcas* has of late received much attention as a major source of eco-friendly, biodegradable and renewable biofuel fuel [6,7].

Recent studies, however, have reported *J. curcas* to be toxic in mice, rats and rabbits as the seeds contain compounds such as protein (curcin) and phorbol-esters (diterpenoids) [8]. Rats fed with diet containing defatted whole seed of Jatropha meal caused severe pathological symptoms and death [9]. Topical application of a

petroleum ether extract of *J. curcas* on a shaved dorsal skin of rabbit showed erythema and oedema. The same extract in mice upon topical application exhibited swelling of the face, haemorrhagic eyes and skin erythema before death [10]. Acute toxicity and histopathological studies conducted on the crude aqueous extract of *J. curcas* leaves revealed a high mortality rate in mice [11], causing diarrhea and inability to keep normal posture, depression and lateral recumbence. The most marked pathological changes were catarrhal enteritis, erosions of the intestinal mucosa, congestion and haemorrhages in small intestines, heart and lungs and fatty changes in the liver and kidneys [11]. A case of *J. curcas* seed toxicity of a family of three showed that within ten to fifteen minutes, all of them had abdominal pain which was colicky in nature and diffuse, and vomiting [12]. The toxicity profile of *J. curcas* is thus very interesting.

As the jatropha plant is monoecious it depends on an array of flower visitors for pollination and fruit set [13,14]. In fact, the dependence of *J. curcas* on pollinators ranges from almost zero to high dependence [15]. However, honeybees appear to be the main pollinators of jatropha flowers [15,16], has proposed that to maintain the reproductive success of large acreage of *J. curcas* honeybees should be used as the prime pollinators, regardless of the pollination services provided by the local insect fauna. Since honeybees also use its nectar for the production of honey, *J. curcas* could be classified as a melliferous plant [17,18].

Honey is a sweet food made by bees using nectar from flowers. It is a complex mixture of carbohydrates, proteins, and lipids. It also

contains vitamins (e.g. ascorbic acid, niacin, pyridoxine), enzymes (e.g. invertase, glucose oxidase, catalase, and phosphatases), as well as amino and organic acids (e.g. gluconic acid, acetic acid). Volatile chemicals, phenolic acids, flavonoids, carotenoid-like substances and minerals which may function as antioxidants are also present in honey [19]. The chemical composition of honey depends on the plant species visited by the honeybees [19]. If bees get their nectar from plant containing toxic substances, the resulting honey produced could be toxic honey. For example, honey produced from the nectar of Rhododendron ponticum contains alkaloids that can be poisonous to humans, while honey collected from Andromeda flowers contains grayanotoxins, which can cause paralysis of limbs in humans and eventually leads to death. In addition, Melicope ternata and Coriaria arborea from New Zealand produce toxic honey that can be fatal [20,21] have observed that phytochemicals are present in fruits, vegetables and many other plants.

Jatropha curcas is found in almost every community in Northern Ghana as a border plant, or as a live fence of gardens and other portions of the house or farms. It attained the status as a crop in Ghana after having been popularized through the usage of oil from its seeds as fuel for diesel engines and lamps in the rural areas where there is no electricity [6]. About 900 ha plantation of jatropha has been cultivated for biodiesel production by a biofuel company in the Yeji municipality, of the Pru District in the north east of Brong-Ahafo Region of Ghana, A 20-hive apiary of the West African honey bee, Apis mellifera adansonii, has been established at a distance of 3 m from the plantation to enhance pollination and hence reproductive success. Substantial honey is produced annually in the apiary which could be attributed to the flowers of the nearby plantation. Jatropha flowers are known to offer both nectar and pollen as rewards for flower visitors [16,22]. This together with its clustered floral arrangement could make the jatropha plant much preferred among flora in the vicinity [18,23]. Moreover, pollinators generally visit the flowers of nearby trees first, before moving to others [24].

As several toxicities are reported to be associated with the study plant [9-12], it is worth ascertaining the toxicity profile of this honey produced from the plantation to establish its safety before consumption. It may be that the toxic substances in plants which are lethal to humans have no effect at all on bees [25]. This is possible because the metabolism of bees and humans is sufficiently different that bees can safely collect nectars from plants that contain compounds toxic to humans. Many humans have eaten toxic honey and become seriously ill as a result [26]. Study has shown that the source of nectar for honey production ultimately affects the composition of the honey [27].

The study was therefore carried out to evaluate the safety of honey produced for consumption by the honeybees from the nectar of *Jatropha curcas* L. flowers in the plantation.

Materials and Methods

Study area

Yeji Municipality, is located between latitude 7° 27' 38" S and 8° 22' 55" N, and Longitude 1° 24' 13" W and 0° 34' 15" E, and is adjacent to Lake Volta. It has a tropical climate, with high temperatures averaging 23.9°C and a double maxima rainfall pattern of an average of 1000 mm. It is clothed with the guinea savannah woodland. Yeji is a town with a population of about 35,000 inhabitants. The strategic location of

Page 2 of 7

the town has turned it into an important market centre and a major transport hub, which serves as a transit point between the north and south of Ghana for goods and people.

Honey collection

Honey combs were harvested from the beehives in the *Jatropha curcas* plantation in the Yeji municipality, Brong-Ahafo Region, Ghana, at 8 am on 11th February, 2016. The honey was then extracted from the combs using honey press and filtered to remove any particulate matter. The sample was stored in clean air-tight glass containers for the study.

Experimental animals and husbandry

Sprague-Dawley rats (180-2220 g) of either sex obtained and kept in the Animal house of the Department of Pharmacology, KNUST, Kumasi, Ghana, were used. The animals were housed in groups of five in stainless steel cages ($34 \times 47 \times 18 \text{ cm}^3$) with soft wood shavings as bedding at a room temperature of $25 \pm 2^{\circ}$ C, with relative humidity of 50-70%, and lighting of 150–200 Lx (sequence being 12 h dark and light cycle). The animals were fed with normal commercial pelleted rat chow (Agricare Limited, Tanoso, Kumasi), and given water *ad libitum*.

Dosing of honey

The dosing of the honey was based on the observation that an individual could consume about 20-40 ml (equivalent to 50-100 g of honey) at a time. Doses administered to experimental animals were calculated based on the most recent body weight.

Acute and delayed toxicity assessment

Sprague-Dawley rats were randomly divided into five groups, A-E (n=5) and kept in the experimental environment for an acclimation period of 1 week. The animals were starved overnight, but were allowed access to water *ad libitum*. Group A, the control, received normal feed and water without honey. Groups B, C, D and E were treated orally with a single dose of 300, 500, 800, or 1500 mg/kg honey respectively. Cage-side observation was made at 15, 30, 60, 120 and 180 min, and 24 h. The animals were also observed daily for 14 days for delayed toxicity symptoms.

Sub-chronic toxicity assessment

Sprague-Dawley rats were randomly grouped into four. Group A, the control, received normal feed and water without honey. Groups B, C, and D were treated orally with 300, 500 and 800 mg/kg honey daily, for 30 consecutive days. Cage-side observations were made daily. Weekly recording of body weight of the rats was taken before honey administration during the study period, and at the end of the study period. Change in body weight was calculated for each group and compared to the control. On day 31, blood was drawn from the jugular vein for hematological assessment and serum biochemical analysis, after which the animals were humanely sacrificed by cervical dislocation. The heart, liver, kidney and stomach were harvested after dissection, freed of fat and connective tissue, blotted with clean tissue paper and weighed. The organ-to-body weight index (OBI) was calculated.

 $OBI = \frac{Absolute organ weight (g)}{Rat body weight on day of sacrifice (g)} \times 100$

Hematological assessment

About 1.5 ml of blood collected from each rat in the various treatment groups were put into ethylene diamine tetra acetic acid (EDTA) vacuum blood collecting tubes (EDTA K3, Anhui Medipharm Co., Ltd, China (Mainland)). The tubes were rolled gently from side to side to mix the blood with the EDTA. They were then sent to the Clinical Analysis Laboratory (CAn LAB), Department of Biochemistry and Biotechnology, KNUST for hematological analysis using the Sysmex XP 300 fully automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

Liver and kidney function assessment

About 2.5 ml of the blood from each rat were also collected into serum separator tubes (Anhui Medipharm Co., Ltd, China (Mainland)) and centrifuged at 4000 rpm at 25°C for 10 min to obtain serum, which was collected assayed for biochemical indicators for liver and kidney function at the CAN lab KNUST, using Kenza BioChemisTry, a semi-automated biochemistry analyzer (Biolabo diagnostics, France).

Histopathological assessment

The harvested liver, kidney, heart and stomach of rats from the various treatment groups were fixed in 10% phosphate buffered formalin (pH 7.2) for histopathological assessment at the Department of Pathology, Komfo Anokye Teaching Hospital, Kumasi, Ghana. Sections of these organs, after routine processing (dehydrated through a series of ethanol solutions, embedded in paraffin, sectioning, and staining with haematoxylin-eosin) by a Laboratory technologist, were made into slides and examined microscopically, using the Leica DM 750 microscope (Leica Microsystems CM5 GmbH, Wetzlar, Germany), by a Pathologist and photographs taken.

Ethical Considerations

Laboratory study was carried out in a level 2 biosafety laboratory. Protocols for the study were approved by the Committee on Animal Research, Publication and Ethics (CARPE); Reference number FPPS/ PCOL/011/2016. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). All the technical team observed all institutional biosafety guidelines for protection of personnel and laboratory.

Data analysis

Graph-Pad Prism Version 6.0 was used for all statistical analyses. Data were presented as mean \pm SEM and analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison test (post hoc test). P \leq 0.05 was considered statistically significant.

Results and Discussion

Cage-side observation in an acute and delayed toxicity assessment revealed no treatment-related physical, behavioral, and clinical signs of toxicity after a single dose treatment with 300-1500 mg/kg honey. No deaths were recorded. Body weights between honey-treated rats and the control were not significantly different (p>0.05) (Figure 1). Subsequent observation for up to 14 days did not reveal any delayed toxicity symptoms.

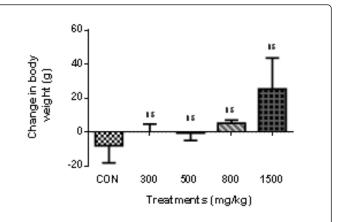


Figure 1: Effect of a single administration of 300, 500, 800, and 1500 mg/kg of honey on body weight to SD rats in an acute and delayed toxicity test. There were no significant changes (ns p>0.05) between honey-treatment and control. One-way ANOVA followed by Dunnett's *post hoc* test.

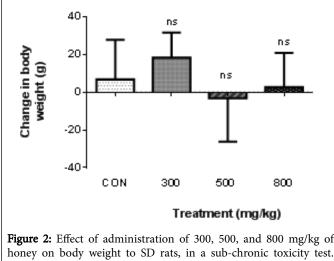
This suggests that the honey, within limits, has no lethal effect and the lethal dose (LD_{50}) , if any, is proposed to be beyond 1500 mg/kg. According to Obici (2008) [28] substances with an LD_{50} value of more than 1000 mg/kg given by oral route are generally considered to be safe for consumption.

In a sub-chronic toxicity assessment, cage side observation, again, did not show any observable treatment-related toxicity. There were no physical, behavioral, or clinical signs and symptoms of toxicity. The animals remained alert with no motor or neurological changes, and no adverse gastrointestinal tract disorders. Cage side observation in subchronic toxicity assessments are made as an initial step in the detection of physical, behavioral, and clinically signs and symptoms of toxicity, including mortality [29]. Physical signs of toxicity include unkemptness, skin erythema, loss of hair, swollen paws and limbs and other inflammatory skin conditions [30-32]. Behavioral changes affect centering, rearing, and grooming, as well as sniffing and mounting in males [33]. Behavioral changes could be neurological i.e. autonomic or CNS effects (depression or excitation) and this could have resultant effects such as: inability to keep posture, bizarre walking, lateral recumbence, tremors, salivation, diarrhea, anorexia, tearing, rhinorrhea, decreased locomotory activity, sedation, and hyperactivity [33]. Allergic reactions such as itchiness and body irritations, conjunctivitis, and other inflammatory dermatological conditions could also be noticed by cage-side observation as licking, scratching, and biting of the affected area make the animals unkempt and smelly [29,31]. Cage-side observation during a daily administration of the honey for 30 days also did not reveal any toxic signs and symptom, and no death was recorded which also gives an indication that the honey is safe for consumption. Conclusions however, cannot be drawn as other parameters such as changes in body and some vital organ weights, hematological profile, liver and kidney function assessments, and histopathological assessments of some vital organs have to be conducted to confirm deductions from cage-side observations.

There were no significant honey-treatment associated changes (p>0.05) in body weight (Figure 2) compared to the control. Honey treatment also did not cause any significant changes to the weights of the stomach, heart, and kidney as indicated by the non-significant

Page 4 of 7

differences in calculated OBI between the honey-treated animals and the control. Liver weights were however significantly elevated (p \leq 0.05-0.001) (Figure 3).



honey on body weight to SD rats, in a sub-chronic toxicity test. There were no significant changes (ns p>0.05) between honeytreatment and control (One-way ANOVA followed by Dunnett's *post hoc* test).

In toxicological studies, a decrease in body weight associated with treatment is an indication of toxicity in the animal [28,35,36]. Changes in organ weight are also indices of toxicity. The OBI of the liver was elevated in rats at all doses of the honey treatment. It is worth noting that hepatocellular hypertrophy and increased liver weight, common findings in toxicity studies, are generally not adverse findings but rather evidence of adaptation in a healthy liver due to increased

endoplasmic reticulum in response to a xenobiotic [37]. Enlargement of the liver without pathological alteration (as seen in the histopathological study) could be due to variety of substances (food, drugs, and some chemicals) which causes an increase in the metabolizing capacity of the enzyme systems which are associated with microsomal fraction derived from the endoplasmic reticulum of the liver parenchymal cells thus an increase in liver size [37]. Chronic administration of the honey could have caused the increase in liver weight which does not indicate hepatocellular damage but an adaptation of the liver.

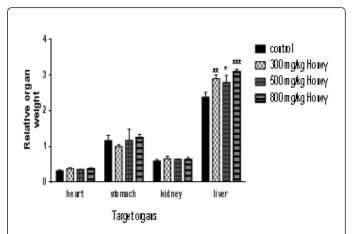


Figure 3: Relative organ weight of the heart, stomach, kidney and liver of SD rats after 30 days of honey treatment in a sub-chronic toxicity study. Values plotted are mean \pm SEM (n=5). * implies p \leq 0.05, ** p \leq 0.01, *** implies p \leq 0.001; compared to the control (One-way ANOVA followed by Dunnett's post hoc test.

| Parameter | Control | 300 mg/kg | 500 mg/kg | 800 mg/kg |
|---|---------------|---------------|--------------|--------------|
| WBC (x10 ³ /uL) | 8.9 ± 2.08 | 9.43 ± 2.08 | 8.47 ± 1.00 | 9.3 ± 0.61 |
| RBC (x10 ⁶ /uL) | 7.36 ± 0.37 | 6.74 ± 0.28 | 8.74 ± 0.72 | 7.67 ± 0.05 |
| HGB (g/dL) | 13.43 ± 0.52 | 12.87 ± 0.35 | 15.57 ± 1.19 | 14.33 ± 0.26 |
| HCT (%) | 41.37 ± 1.67 | 39.3 ± 1.31 | 50.37 ± 4.25 | 46.5 ± 1.50 |
| MCV (fL) | 56.27 ± 0.58 | 58.37 ± 1.42 | 57.63 ± 0.19 | 60.7 ± 2.401 |
| MCH (pg) | 18.27 ± 0.29 | 19.13 ± 0.49 | 17.83 ± 0.27 | 18.67 ± 0.34 |
| MCHC (g/dL) | 32.47 ± 0.48 | 32.77 ± 0.20 | 30.97 ± 0.43 | 30.9 ± 1.16 |
| PLT (x10 ³ /uL) | 748.3 ± 102.3 | 700.7 ± 55.38 | 630.3 ± 168 | 505 ± 102.8 |
| LYM [#] (x10 ³ /uL) | 4.833 ± 2.77 | 7.533 ± 2.09 | 6.567 ± 0.71 | 4.333 ± 2.19 |
| NEUT# (x10 ³ /uL) | 4.067 ± 1.82 | 1.9 ± 0.0 | 1.9 ± 0.35 | 4.967 ± 2.38 |
| RDW-SD (fL) | 29.27 ± 0.27 | 30.67 ± 0.20 | 30.27 ± 0.12 | 31.13 ± 1.84 |

Table 1: The effects of 300, 500 and 800 mg/kg of honey treatment on the hematological profile of Sprague-Dawley rats in a sub-chronic toxicity test. Values are mean \pm SEM (n=5). There were no significant changes in hematological parameters between the honey-treated rats compared to the control (One-way ANOVA followed by Dunnett's *post hoc* test.

Hematological studies revealed no significant differences (p>0.05) between the control and honey-treated groups for all measured (Table

1). A hematological study is very necessary in safety assessment as it has a higher predictive value (91%) for toxicity in humans [38] as most

substances find their way into the blood irrespective of the route of administration. Damage to and destruction of the blood cells results in a variety of consequences such as a reduction in the oxygen-carrying capacity of the blood, reduction in immune system function, and impairment of hemostatic function.

Values obtained in a liver function test showed a significant elevation ($p \le 0.05$ -0.01) of Aspartate transaminase (AST) for the 500 and 800 mg/kg honey-treated groups relative to the control. All other parameters measured were not significantly different from the control (Table 2). The liver is the major site for the metabolism of most chemicals. It is prone to toxicity because metabolism of drugs does not always lead to detoxification. Results from the liver function test indicated a honey-treatment elevation in serum AST with all other parameters being non-significantly different from the control. AST is not a specific indicator of hepatocyte damage (i.e. non-specific), as is also present in other tissues such as the heart, skeletal muscle, kidney, brain and red blood cells [39]. This finding may therefore not be indicative of hepatocellular damage; for hepatocellular damage, there must be an increase in serum levels of AST and Alanine transaminase (ALT) [40]. ALT is localized in the cytosol of hepatocytes making it a more sensitive marker of hepatocellular damage as compared to AST [41].

| Honey treatment | | | | | | | |
|-----------------|---------------|--------------|---------------|------------|--|--|--|
| Parameter | Control | 300 mg/kg | 500 mg/kg | 800 mg/kg | | | |
| ALB (g/l) | 27.33 ± 2.19 | 29 ± 1.53 | 28.33 ± 0.67 | 31 ± 1.16 | | | |
| GLOB (g/l) | 46 ± 1.73 | 43.33 ± 5.33 | 40.33 ± 2.40 | 42.3 ± 1.5 | | | |
| TP (g/L) | 73.33 ± 2.60 | 72.33 ± 4.49 | 68.67 ± 1.76 | 73.3 ± 0.3 | | | |
| ALT (u/L) | 11.67 ± 1.66 | 10 ± 5.0 | 6.667 ± 1.667 | 8.3 ± 1.67 | | | |
| AST (u/L) | 90 ± 5.0 | 100 ± 0.0 | 118.3 ± 1.67* | 108 ± 4** | | | |
| ALP (u/L) | 131.7 ± 17.64 | 125 ± 30.55 | 111.7 ± 13.33 | 110 ± 25.7 | | | |
| GGT (umol/L) | 5 ± 0.0 | 5 ± 0.0 | 5 ± 0.0 | 5 ± 0.0 | | | |

Table 2: The effect of honey treatment on liver function test performedon Sprague-Dawley rats in sub-chronic toxicity test. Values are mean \pm SEM (n=5). *implies $p \leq 0.05$, ** $p \leq 0.01$, compared to the control(One-way ANOVA followed by Dunnett's post hoc test.ALB=Albumin, GLOB=Globulin, TP=Total Protein, ALT=AlanineTransaminase, AST=Aspartate Transaminase, Alkaline Phosphatase,GGT=Gamma-Glutamyl Transferase.

| Honey treatment | | | | | | | |
|-----------------|--------------|--------------|---------------|-------------|--|--|--|
| Parameter | Control | 300 mg/kg | 500 mg/kg | 800 mg/kg | | | |
| Urea | 10.57 ± 1.21 | 9.23 ± 1.16 | 10.03 ± 3.14 | 10.83 ± 5.0 | | | |
| Creatinine | 52.2 ± 2.186 | 46.9 ± 2.566 | 50.43 ± 3.139 | 38.23 ± 5.0 | | | |

Table 3: The effect of honey treatment on kidney function test performed on Sprague-Dawley rats in sub-chronic toxicity test. Values are expressed as mean \pm SEM (n=5) compared to the control by the One-way ANOVA.

Generally, there were no significant change in kidney function between control and honey-treated groups as indicated by plasma urea and creatinine measured (Table 3). Creatinine and urea levels in blood are used as a measure of kidney function as these substances are excreted by the kidney (creatinine is a more specific marker) [33]. Elevated levels are an indication of kidney malfunction or damage. The honey therefore did not have any detrimental effect on the kidney.

Histopathological assessment showed no observable treatmentrelated changes in the architecture of the heart, stomach, kidney and liver of honey-treated animals compared to the control (Figures 4-7).

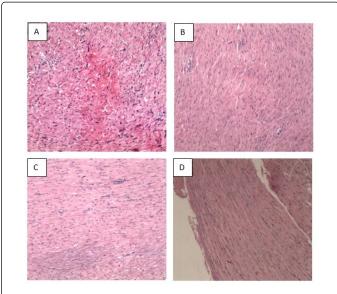


Figure 4: Photomicrographs of the heart tissue of SD rats showing normal histological architecture after treatment with 300 mg/kg (B), 500 mg/kg (C), 800 mg/kg (D) of honey daily for 30 days in a sub-chronic toxicity test. (A) is the control which had no treatment.

Histopathological assessment of some selected organs including the liver was conducted. The criteria for assessing histopathological changes include necrosis, cloudy swelling, fatty infiltration of cells and inflammatory infiltration among other parameters [42]. There was no inflammatory cellular infiltration of the liver when histopathologic examination of the liver was done. Comparing the morphological structure of the liver in the honey-treated rats to the control, there were no abnormalities; the capsule and hepatic lobules were normal with no necrosis or cellular degeneration. There was no hyperplasia in connective tissues and no fatty liver was observed. This could confirm that the increase in AST was not an indication of liver damage. The morphology of the heart, stomach and kidneys of the honey-treated rats were also not significantly different from that of the control, suggesting "no toxicity" in these organs.

The findings of the study on the whole suggest that though *J. curcas* may contain some toxic active substances in the seeds [8,9] and leaves [11,12], the nectar of *J. curcas* may not have such active principles. These findings are consistent with other studies on chemopreventive properties and toxicity of Kelulut Honey in Sprague Dawley rats induced with Azoxymethane [43] a study on the single-dose oral toxicity of super key in Sprague-Dawley Rats [44] and Toxicological evaluation of honey as an ingredient added to cigarette tobacco [45]. The use of Sprague Dawley Rats in toxicity tests is consistent with universal standards because they have many similarities with humans in terms of metabolic pathways, and many anatomical and

physiological characteristics allowing for comparisons in absorption, excretion, and distribution. Its convenient size, relative docility, short life span and gestation period, makes it economic to maintain, and there is a large database of its characteristics, which is invaluable in the interpretation of the relevance of animal data for humans.

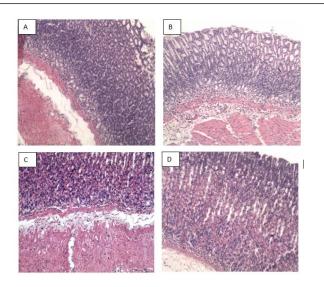


Figure 5: Photomicrographs of stomach tissue of SD rats showing normal histological architecture after treatment with 300 mg/kg (B), 500 mg/kg (C), 800 mg/kg (D) of honey daily for 30 days in a subchronic toxicity test. (A) is the control which had no treatment.

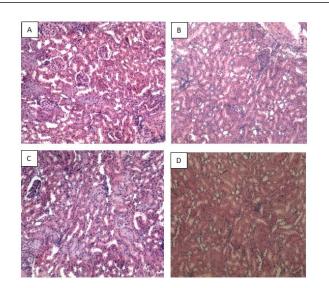


Figure 6: Photomicrographs of kidney tissue of SD rats showing normal histological architecture after treatment with 300 mg/kg (B), 500 mg/kg (C), 800 mg/kg (D) of honey daily for 30 days in a subchronic toxicity test. (A) is the control which had no treatment.

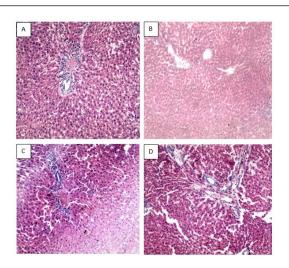


Figure 7: Photomicrographs of liver tissue of SD rats showing normal histological architecture after treatment with 300 mg/kg (B), 500 mg/kg (C), 800 mg/kg (D) of honey daily for 30 days in a subchronic toxicity test. (A) is the control which had no treatment.

Nectar production is believed to involve some intricate biological processes determined by plant characteristics in response to prevailing environmental conditions [46,47]. The absence of toxic active substances therefore has implications for the beekeeping industry as honey production could be enhanced. *Jatropha curcas* can therefore be employed in poverty reduction programmes and hence rural development because of its multiple uses.

Conclusion

This study has shown that honey derived from *Jatropha curcas* has no significant toxicity profile in Sprague-Dawley rats, and therefore suggests that it should be safe for human consumption. Also, recognizing *J. curcas* as a melliferous plant adds to the already known multiple uses, and hence could be a tool for the promotion of apiculture as a source of extra income to farmers. Ultimately, it could contribute to economic empowerment of rural communities in Ghana and Africa.

Acknowledgement

We are very grateful to Mr Frank Agyemang Bonsu and staff of Clinical Analysis Laboratory (CanLab), Department of Biochemistry and Biotechnology, KNUST, Ghana, as well as Mr. Gordon Daaku, and the staff of the Department of Pharmacology laboratory and Animal House, KNUST, Kumasi, Ghana.

References

- 1. Warra AA (2012) Cosmetic Potentials of Physic Nut (Jatropha curcas Linn.) Seed Oil: A Review. Am J Sci Ind Res 3: 358-366.
- Timko JA, Amsalu A, Acheampong E, Teferi MK (2014) Local Perceptions About the Effects of Jatropha (Jatropha curcas) and Castor (Ricinus communis) Plantations on Households in Ghana and Ethiopia. Sustainability 6: 7224-7241.
- Pratt JH, Henry EMT, Mbeza HF, Mlaka E, Satali LB (2002) Malawi agroforestry extension project marketing and enterprise program main report. Malawi Agroforestry 47: 44-46.

Page 6 of 7

Page 7 of 7

- 4. Achten WMJ, Nielsen LR, Aerts R, Lengkeek AG, Kjaer ED, et al. (2010) Towards Domestication of Jatropha curcas. L Biofuels 1: 91-107.
- 5. Thomas R, Sah NK, Sharma PB (2008) Therapeutic Biology of Jatropha curcas: A Mini Review. Curr Pharm Biotechnol 9: 315-324.
- 6. Openshaw K (2000) A Review of Jatropha curcas: An Oil Plant of Unfulfilled Promise. Biomass and Bioenergy 19: 1-15.
- Jongschaap REE, Corré WJ, Bindraban PS, Brandenburg WA (2007) Claims and Facts on Jatropha curcas L: Global Jatropha curcas Evaluation, Breeding and Propagation Programme. Plant Res Inter BV Wageningen.
- King AJ, He W, Cuevas JA, Freudenberger M, Ramiaramanana D, et al. (2009) Potential of Jatropha curcas as a Source of Renewable Oil and Animal Feed: Review Paper. J Exp Bot 60: 2897-2905.
- 9. Rakshit KD, Darukeshwara K, Rathina R, Narasimhamurthy K, Saibaba P, et al. (2008) Toxicity Studies of Detoxified Jatropha meal (Jatropha curcas) in rats. Food Chem Toxicol 46: 3621-3625.
- 10. Gandhi VM, Cherian KM, Mulky MJ (1995) Toxicological Studies on Ratanjyot Oil. Food Chem Toxicol 33: 39-42.
- 11. Azubike NC, Okwuosa CN, Achukwu PU, Maduka TC, Chike O (2015) Acute toxicity and histopathological effects of crude aqueous extract of Jatropha curcas leaves in mice. Res J of Med Plant 9: 340-346.
- 12. Shah V, Sanmukhani J (2010) Five Cases of Jatropha Curcas Poisoning. J Assoc Physicians Ind 58: 245-246.
- Bhattacharya A, Datta K, Datta SK (2005) Floral biology, floral resource constraints and pollination limitation in Jatropha curcas L. Pak J of Biol Sci 8: 456-460.
- Pranesh KJ, GururajaRao MR, Sowmya HC, Balakrishna G, Savithramma DL, et al. (2010) Studies on floral display and mode of reproduction in jatropha (Jatropha curcas L.). Elect J of Plant Breed 1: 832-838.
- Vaknin Y (2012) The Significance of Pollination Services for Biodiesel Feedstocks, with Special Reference to Jatropha curcas L.: A Review. Bio Ener Res 5: 32-40.
- Kumar V, Belavadi V, Ashok Kumar CT (2012) Honey Bees as Effective Pollinators of Jatropha. Entomol Acad Ind 19: 27-31.
- Ab van Peer (2010) Growing Jatropha Including propagation methods for Jatropha curcas. Technical Report supported by the Global Sustainable Biomass Fund of NL Agency.
- Adjaloo MK, Yeboah-Gyan K (2003) Foraging Strategies of the African Honeybee, Apis mellifera adansonii (L) in the Humid Semi-Deciduous Forest Environment of Ghana. Jof Sci Tech 23: 16-25.
- Eteraf-Oskouei T, Najafi M (2013) Traditional and Modern Uses of Natural Honey in Human Diseases: A Review. Iran J Basic Med Sci 16: 731-742.
- 20. Islama N, Khalil I, Islamb A, Gan SH (2013) Toxic compounds in honey. J of App Toxicol 34: 733-742.
- 21. Bode AM, Dong Z (2015) Toxic phytochemicals and their potential risks for human cancer. Cancer Prev Res (Phila) 8: 1-8.
- 22. Rincón-Rabanales M, Vargas-López LI, Adriano-Anaya L, Vázquez-Ovando A, Salvador-Figueroa M, et al. (2016) Reproductive biology of the biofuel plant Jatropha curcas in its center of origin. PeerJ 4: e1819.
- Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86: 361-377.
- 24. Young AM (1986) Cocoa pollination. Cocoa Growers' Bulletin 37: 5-23.
- 25. Grayanotoxins (2015) Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. US FDA.
- Kettlewellh BD (1945) A Story of Nature's Debauch. The Entomologist 88: 45-47.
- 27. Adgaba N, Al-Ghamdi A, Tadesse Y, Getachew A, Awad AM, et al. (2017) Nectar Secretion Dynamics and Honey Production Potentials of Some Major Honey Plants in Saudi Arabia. Saudi J of Biol Sci 24: 180-191.

- Obici S, Otobone FJ, da Silva Sela VR, Ishida K, da Silva JC, et al. (2008) Preliminary Toxicity Study of Dichloromethane Extract of Kielmeyera coriacea Stems in Mice and Rats. J Ethnopharmacol 115: 131-139.
- 29. Chan PK, Hayes AW (1989) Principles and Methods of Toxicology. 2nd edn, Raven Press, New York.
- 30. Richardson JD, Vasko MR (2002) Cellular Mechanisms of Neurogenic Inflammation. J Pharmacol Exp Ther 302: 839-845.
- Steinhoff M, Ständer S, Seeliger S, Ansel JC, Schmelz M, et al. (2003) Modern Aspects of Cutaneous Neurogenic Inflammation. Arch Dermatol 139: 1479-1488.
- Koffuor GA, Woode E, Mensah AY (2011) Neurobehavioral and Safety Evaluation of a Polyherbal Antihypertensive Mixture in Ghana. Euro J Exp Bio 1: 20-30.
- Koffuor GA, Woode E, Obirikorang C, Asiamah E (2011) Toxicity Evaluation of a Polyherbal Antihypertensive Mixture in Ghana. J Pharm Bioall Sci 1: 34-48.
- Pandey VC, Singh K, Singh JS, Kumar A, Singh B, et al. (2012) Jatropha curcas: A Potential Biofuel Plant for Sustainable Environmental Development. Renew Sustain Ener Rev 16: 2870-2883.
- 35. Raza M, Shabanah OA, El-Hadiyah TMH, Al-Majed A (2002) Effect of Prolonged Vigabatrin Treatment on Hematological and Biochemical Parameters in Plasma, Liver and Kidney of Swiss Albino Mice. Scientia Pharmaceutica 70: 135-145.
- 36. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, et al. (2002) A 90day oral gavage toxicity study of D-methylphenidate and D, Lmethylphenidate in Sprague-Dawley rats. Toxicology 179: 183-196.
- 37. Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, et al. (2012) Liver Hypertrophy: A Review of Adaptive (Adverse and Non-adverse) Changes - Conclusions from the 3rd international ESTP expert workshop. Toxicol Pathol 40: 971-994.
- Olson H, Betton G, Robinson D, Thomas K, Monro A, et al. (2000) Concordance of Toxicity of Pharmaceuticals in Humans and in Animals. Regul Toxicol Pharmacol 32: 56-67.
- 39. Crook MA (2012) Clinical Biochemistry and Metabolic Medicine. 8th edn, CRC press, Florida, USA.
- 40. Kumar P, Clark M (2005) Clinical Medicine 6th edn, Elsevier Saunders, USA.
- Giannini EG, Testa R, Savarino V (2005) Liver Enzyme Alteration: A Guide for Clinicians. CMAJ 172: 367-379.
- Greaves P (2007) Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation. 3rd edn. Academic Press, New York.
- 43. Saiful Yazan L, Muhamad Zali MF, Mohd Ali R, Zainal NA, Esa N, et al. (2016) Chemopreventive Properties and Toxicity of Kelulut Honey in Sprague Dawley Rats Induced with Azoxymethane. Biomed Res Int 2016: 4036926.
- 44. Kim J, Lee J, Kim S (2015) A study on the single-dose oral toxicity of super key in Sprague-Dawley Rats. J Pharmacopuncture 18: 63-67.
- 45. Stavanja MS, Ayres PH, Meckley DR, Bombick BR, Pence DH, et al. (2003) Toxicological evaluation of honey as an ingredient added to cigarette tobacco. J Toxicol Environ Health A 66: 1453-1473.
- Macukanovic-Jocic MP, Djurdjevic L (2005) Influence of Microclimatic Conditions on Nectar Exudation in Glechoma hirsuta W.K. Arch Biol Sci 57: 119-126.
- 47. Shuel RW (1992) The Production of Nectar and Pollen. In: Graham JM (Ed): The Hive and the Honeybee. Michigan Bookcrafters, Chelsea.