Original Article

Histopathological effects of acetaminophen abuse in male Wistar rats, and prevalence in human subjects: An experimental and cross-sectional study

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Abstract

Aim: This study aimed to examine the histopathological effects of acetaminophen (ACMP) abuse in select organs of male Wistar rats. The second goal was aimed at determining the prevalence of ACMP abuse in human subjects. **Materials and Methods:** A cross-sectional design (structured questionnaire and oral interview) was used for data collection from 1911 male to 1009 female subjects, aged (15–72) years in Benin City, Nigeria, between June, 2014 and April, 2015. The animal study was done using 60 adult male Wistar rats with a mean weight of (228.34 g). ACMP was orally administered to 10 Groups of rat in the following order: Groups A_1 and A_2 (400 mg/kg), B_1 and B_2 (800 mg/kg), C_1 and C_2 (1200 mg/kg), and D_1 and D_2, (1600 mg/kg) body weight in rat. Water and feed were provided *ad libitum* for the duration of ACMP administration that lasted for 21 days (sub-acute exposure) in Group A_1, B_1, C_1, and D_1. The administration lasted for 42 days (sub-acute and acute exposures) in Groups A_2, B_2, C_2, and D_2 while Groups E_1 and E_2 served as the control. At termination, all rats were sacrificed by cervical dislocation, grossed, and processed histologically. **Results:** The prevalence of ACMP abuse within the study population (males and females, in Benin City, Nigeria) stood at 97.3% and was significantly affected by contributory factors like: Age-group, income, profession, etc. Grossly, renal and hepatic necrosis was observed in the high-dose/acutely exposed treated rats (C_2 and D_2). Histopathology findings revealed hepatocellular distortion at the central vein of the liver tissue and tubular expansion and increased glomerular space in the kidney. Decrease in body weights of the rats in Groups C_2 and D_2 were statistically significant ($P < 0.05$). **Conclusion:** There was a high incidence of ACMP abuse in the males and females population in Benin City, Nigeria. Prolonged oral consumption of ACMP in animals resulted in hepatocellular and renal deleterious effects and may be of a similar hazard in humans.

Key words: Acetaminophen, cross-sectional survey, gross examination, histochemical methods, histological techniques

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INTRODUCTION

Acetaminophen (ACMP) commonly referred to as paracetamol (N-acetyl-para-aminophenol) is a white crystalline solid or powder (Venkatesan and Deecaraman, 2014). It was first introduced as a prescription drug in the United States in 1955 and was approved by the Food and Drug Administration for sale as a nonprescription drug in 1960 (Venkatesan and Deecaraman, 2014). ACMP is available as oral, rectal, and injectable formulation (Payasi *et al*., 2010). The conventional oral dose for an adult is 325–1000 mg and 650 mg rectally (Insel, 1996). In children, the single dose is 40–480 mg, depending on age and weight. Infants under 3 months of age, a dose of 10 mg/kg body weight are recommended (Insel, 1996; Reynolds, 1996). It is a commonly used synthetic, nonopioid, centrally acting analgesic, and antipyretic agent (Reynolds, 1996). Other uses include the manufacture of azo dyes and photographic chemicals, as intermediate for pharmaceuticals, and as a stabilizer for hydrogen peroxide (National Toxicology Program, 1991). ACMP is widely used because many people mistakenly believe it to be entirely harmless. However, the use of ACMP is one of the most common causes of poisoning worldwide (Kett *et al*., 2011). It is estimated that ACMP poisoning results in about 56,000 injuries, 25,000 hospitalizations, and 450 deaths yearly in United States of America (Kett *et al*., 2011). ACMP poisoning can be due to ingestion of excessive repeated or too frequent doses (Kett *et al*., 2011).

It has been reported that repeated supratherapeutic intake of ACMP is a significant clinical problem (Heard, 2008; Olaleye and Rocha, 2008). Overdose or long-term users have well-known adverse effects which include: Hepatotoxicity, depletion of reproductive competence, and alteration of testicular structure (Heard, 2008). Ultrastructure and seminal quality impairment have also been reported (Olaleye and Rocha, 2008). Hepatocellular deleterious effects are the utmost notable feature of ACMP overdose (Anderson *et al*., 2005). Severe overdose can cause terminal liver damage, and in exceptional cases, a standard dose can act in like manner and the danger can be on the increase with alcohol intake (Venkatesan and Deecaraman, 2014). ACMP poisonousness is the leading cause of an acute liver damage (Anderson *et al*., 2005), while, renal effects as a result of ACMP overdose are less common than hepatic effects (Venkatesan and Deecaraman, 2014).

From the on-going, there are extensive toxicity studies presently available on ACMP. However, there is a paucity of information on the histopathological effects of ACMP abuse in organs of male Wistar rats at the cellular level. Furthermore, there is a dearth of literature regarding the prevalence of ACMP abuse in human subjects from Benin City, Nigeria. This study was to examine the histopathological effects of prolonged oral administration of ACMP in selected organs of male Wistar rats. The second goal was aimed at determining the prevalence of ACMP abuse in human subjects in Benin Metropolis, Nigeria.

MATERIALS AND METHODS

Study Design, Population, Location, and Period

A cross-sectional survey of human (male and female) subjects was espoused for the present study. The study population comprised of 1911 men and 1009 women, which is made up of a total of 2920 adults. Randomly selected volunteers, within Benin metropolis, aged (15–72 years) participated in the study. The human study was conducted in Benin City, the Capital of Edo State, Nigeria. It has a population of approximately 1.2 million people and a population density of 168 persons per km^2 (Cleen Foundation, 2014). While, data encoding, preparation, and tissue processing, were conducted at the Department of Medical Laboratory Sciences, University of Benin, Benin City, Nigeria. The study lasted for 10-month (June, 2014 to April, 2015).

Sociodemographic, Economic, and Health information

About 20 questions centered on age, sex, income, profession, regular consultation with medical practitioner, closeness/distance to the hospital, self-medication frequency, knowledge of the actual prescribed dosage for ACMP, outcome of self-medication (if known), and the types of illness that warranted ACMP intake.

Animal Grouping and Care Ethics

Inbred adult male Wistar rats from the animal holdings of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria were used. The animal studies were carried out in compliance with policies outlined in the Use and Care of Laboratory Animals (NIH Publication No. 85–23, revised 1996). The rats were housed in wire gauze cages with sawdust as beddings. Enough food (Standard Top Feed®) and water was provided *ad libitum*. Sixty male Wistar rats were assigned to 5 Groups of 6 rats per cage. They were labeled as group A_1, B_1, C_1, D_1, E_1 and A_2, B_2, C_2, D_2, E_2 (n = 6/group). E_1 and E_2 served as the untreated groups.

Empirical Measurement

The method described by Ajiboso *et al.* (2007); was used to determine body weight of experimental rats. The rats were inspected for daily gain in body weight using a digital electronic balance (Gibertini, Italy). The increase in weight was obtained from the relationship: Daily gain in weight = Final day weight − Initial day weight, while the mean weight was 228.34 g.

Physical Measurement

Behavioral signs of acute toxicity, such as: Prolonged sleep, dullness, diarrhea, watery stool, hair loss, stretching, restlessness, paw licking, salivation, and reduced activities, were assessed by a careful observation of the animals for the first few hours (4 h) of drug administration.

Drug Preparation and Administration

In Nigeria, a genuine ACMP (paracetamol) 500 mg (P) tablet has been documented to possess the actual amount of active ingredients (N-acetyl-para-aminophenol). The graded doses were diluted to appropriate concentrations using distilled water and propylene glycol to enable suspension. On each experimental day, a new ACMP dissolution was prepared and administered fresh. The untreated animals also received only the distilled water + propylene glycol; purported as a vehicle for the swift transportation and dissolution of ACMP tablet ACMP was diluted and administered orally using the orogastric tube for (21 days sub-acute) duration. The sub-acute exposure of ACMP in Groups A_1 to D_2 was terminated and sacrificed on day 22. After that, ACMP administration continued in the second phase of the experiment (acute) exposure that lasted for another 21 days at the interval of 2 days making up a total of 42 days.

Experimental Drugs and Source

ACMP tablet was purchased from a government approved pharmacy opposite University of Benin Teaching Hospital (UBTH), Benin City, Nigeria with NAFDAC number 04–0289. Manufacturer: Emzor Pharmaceuticals Industries Ltd. #10 Kolawole Shonibare Street, Ajao Estate, Lagos, Nigeria with Batch Number: L7571, Manufacturing Date: 07/14 and Expiring Date: 07/17.

Experimental Design

The rat in groups A_1, B_1, C_1, D_1 (sub-acute exposure for 21 days) and A_2, B_2, C_2, D_2, (acute exposure for 42 days) were treated with 400, 800, 1200, and 1600 mg/kg body weight. It was done with regards to the no observed adverse effect level of 1000 mg/kg ACMP in Wistar rats (Venkatesan and Deecaraman, 2014). Untreated rats in Group E_1 and E_2 were served as the control without an experimental dose.

Pattern of Sacrifice of the Animals

At the termination of the experiment, all rats were sacrificed by cervical dislocation (sub-acute exposure on day 22 and the acute exposure on day 43). A midline incision was made through the anterior abdominal wall of the rats. Organs of interest were excised (liver, kidney, heart, pancreas, urinary bladder, spleen, lungs, muscles, and gastrointestinal tract). They were fixed in neutral buffered formal saline for 24 h.

Histopathology Samples

The standard histological method with improved modification in histochemical techniques was used for animal studies (Avwioro, 2010). Gross examination was carried out on each organ of interest. The tissues were cut at 3–5 mm after grossing. The cut tissues were processed in an automatic tissue processor (Hestion-ATP7000 tissue processor-Germany) for dehydration, clearing, and impregnation. Embedding using molten paraffin wax was prepared with the aid of the embedding machine (Sakura Tissue-Tek 5). Sections were obtained at 3–5 microns using the digital rotary microtome (Hestion ERM 4000 Germany) to produce serial ribbons. Staining was according to Hematoxylin and Eosin method and the periodic acid-Schiff (Avwioro, 2010). Two or more histopathologist reviewed histology slides in UBTH, Benin City, Nigeria.

Microscopy and Photomicrography

The sections were examined using Swift® binocular microscope with an inbuilt lighting system and white films with an Olympus photomicroscope (Opticshot-2; Nikon, Tokyo, Japan) at ×10 and ×40 magnification.

Ethical Approval

An authorization for this study was approved by the Ethics and Research Committee of the UBTH, Benin City, Nigeria, before the commencement of the experimental study and the cross-sectional survey. The actual intention of the study was adequately described to the participant while secrecy was assured. Informed consent by writing was mandatory by the participants before the questionnaire was given out.

Statistical Analysis

The data were analyzed using Chi-square (χ^2) test, with the statistical software INSTAT + version 3.3(Informer Technologies, Inc, United States). Values were presented in means ± standard deviation. Statistical significance was set at $P < 0.05$.

RESULTS

Human Subjects

Out of the 2920 male and female respondents in this study, 2851 (97.6%) agreed to the indiscriminate use of ACMP without a doctor's prescription. The survey further revealed that the average age of 44.6 years for ACMP intake was without a prescription. The mean number of years of literate respondents was 43.5 years; the median was 43.8 years and range was 15–72 years. The result showed that about 38% had no formal education, 42% were mostly artisans, 36% were civil servants, and 32% were students both secondary and university [Table 1]. Overall, the prevalence (65.7%) was significantly higher in young men and women (youths) ranging from ages 15 to 24 years ($P < 0.05$) [Table 1].

Physical Examination

Result from animal studies showed that upon physical examination of experimental rats, behavioral signs of acute toxicity were observed at high-dose 1200 and 1600 mg/kg (acute exposure), respectively. Prolonged sleep, dullness, and reduced activities were the major behavioral signs observed in the latter days (acute stage) of the experiment. Empirical measurements revealed weight loss in the high-dose treated rats (1200 and 1600 mg/kg in C_1, D_1, C_2, and D_2). There were marked severity in the acute exposure of ACMP for 42 days of treatment (C_2 and D_2) [Table 2]. Gross examination showed renal and hepatic necrosis in organs of the high-dose treated rats (C_2 and D_2 acute exposure) and was marked with variation in color and consistency. There was no significant statistical difference ($P > 0.05$) in the absolute organ weight of all the animals (A_1 to D_2) when compared to the control (E_1 and E_2). Although, relative organ weight of all the animals were not considered for the different groups of animals in this study. ACMP treated organs from the sub-acute exposure (21 days duration) were normal.

Table 1: Summary of the contributing factors to ACMP self-medication

Factors	Description of factor and percentage of respondent
Age group	Majorly 15-24 years of the study population regarded as youths use ACMP without prescription, and it accounts for 65.7% of the respondent (X^2=2.342, P<0.05)
Income	48% of the respondents are low income while 52% are high income respondent. Therefore, the high income earners are probably more prone to ACMP abuse (X^2=2.142, P<0.05)
Profession	Artisans accounts for 42% of the respondent thereby signaling that this category of workers mostly indulge in ACMP abuse (X^2=2.324, P<0.05). Another group of respondents is the civil servants (36%). It implies that working environment may play a significant role in livelihood
Nature of illness	Fever is widely reported by a vast majority of the respondents (96.8%) as the reason for ACMP abuse, while (89.4%) headache. This further suggests that fever is on the increase than headaches (X^2=6.402, P<0.05)
Closeness to the hospital	48% of the respondent discloses that the nearest hospital is within 500-1000m away. Therefore, distance to the hospital may predispose respondents to ACMP abuse (X^2=0.864).
Frequency of self-medication	97.6% takes ACMP indiscriminately which subsequently leads to abuse of ACMP consumption (X^2=1.021, P<0.05)
Knowledge of ACMP dosage	Fortunately, 99.3% of the respondent irrespective of age, sex, and level of education, knows the standard dosage of ACMP for an average adult of about 40kg and above (X^2=1.017, P<0.05

ACMP - Acetaminophen

Histopathological Examination

Histopathology results showed more features of toxic reactions in the liver and kidney sections than were seen in the renal and hepatocellular injuries in C_2 and D_2, respectively [Figures 1 and 2]. Other organs examined such as the heart, urinary bladder, spleen, lungs, muscles, and gastrointestinal tracts showed a normal histology of the organs.

DISCUSSIONS

The reports on ACMP show effective mild analgesic, antipyretic agent, and probably the most widely used of all drugs in the world (Al-Belooshia *et al*., 2010). In many countries, it is fashionable to misuse over-the-counter analgesics for self-poisoning (Al-Belooshia *et al*., 2010). Although, self-prescription is extensively practiced all over the world, it could aid in the management of trivial and petty illnesses like cold, headaches, and fever. Nonetheless, the practice must be founded on the appropriate therapeutic measures. Else, undifferentiating use of medications could bring about a severe health risks, multiple drug resistance to pathogens, and adverse drug reaction in human (Banerjee and Bhadury, 2012).

From our study, greater histopathological effects were evident as cellular changes that were observed more in the high-dose treated rats with longer duration of exposure in the liver and partially in the kidney. These observations agreed with those of Venkatesan and Deecaraman (2014) by which the animal model indicated hepatocellular toxicity. Deleterious effects observed

Table 2: Sub-acute/acute analysis of ACMP administration in male wistar rats

Cages/ groups	Dose in mg/kg	Initial mean weight before drug administration	Final mean weight after drug administration	Empirical measurement (weight loss/or gain)	Physical examination (activities/or dullness)
A_1	400	216.40±1.4	216.32±1.1	↓	±
B_1	800	228.88±1.6	228.16±1.2	↓	±
C_1	1200	242.58±2.2	240.23±1.4	↓	±
D_1	1600	250.60±2.6	248.10±2.2	↓	+
E_1	-	208.48±1.4	219.66±1.2	↑	-
A_2	400	218.26±1.6	215.12±1.4	↓	+
B_2	800	238.80±2.4	231.24±1.2	↓↓	+
C_2	1200	242.65±2.2	231.12±1.2	↓↓	++
D_2	1600	249.28±3.4	229.14±1.2	↓↓	++
E_2	-	200.44±2.2	219.66±2.6	↑	-

Key: O - Oral, S - Subcutaneous, R - Right kidney, L - Left kidney, ↑ - Slight increase in weight, ↓↑- Marked increase in weight, ↓- Slight weight loss, ↓↓- Severe weight loss, + - Presence of features, ± - Intermediate features, ++ - Marked presence of features, → - Absence of features

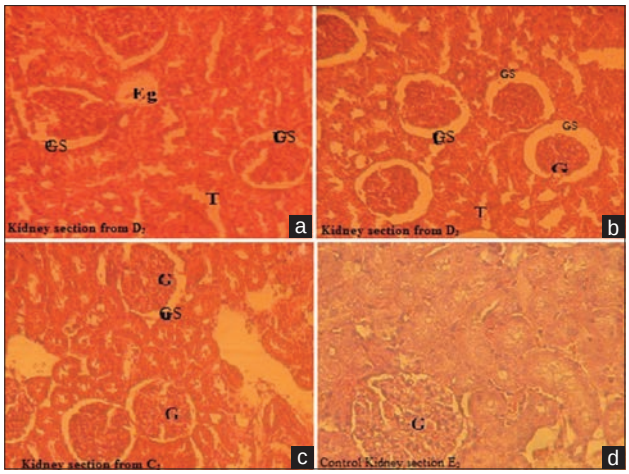

Figure 1: Kidney sections showing (a) Increase in glomerular space (GS), tubular expansion (T), and complete erosion of the glomerulus (Eg). (b) Marked increase in glomerular space (GS), tubular extension (T), and congestive glomerulus (G). (c) A slight increase in glomerular space (GS). (d) The control kidney section E2 with a normal glomerulus (G). Stain uptake: Control-periodic acid-Schiff, Others-Mayer's H and E, ×400

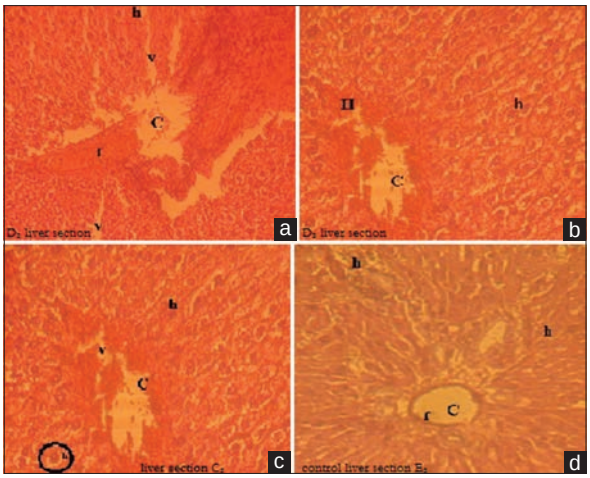

Figure 2: Liver sections showing (a) Hepatocellular degeneration (h), vacuolation of the portal triand (v), presence of hematoma in the central canal (C), and presence of Frank red blood cells (f). (b) Marked presence of hematoma in the central canal (C), evidence of inflammatory cell infiltration (H), hepatocellular degeneration (h). (c) Group C2 showed hepatocellular degeneration and vacuolation of portal triad (v), while (d) Control liver section from E2 with a typical central canal (C). Stain uptake: Control-periodic acid-Schiff, Others-Mayer's H and E, ×400

in the high-dose kidney section may not support the opinion raised by Venkatesan and Deecaraman, (2014), which showed that the renal effects of ACMP overdose was less commonly seen than the hepatic effects. Payasi *et al*., (2010) suggested that ACMP was safe even at maximum dose level. Even when we recalled that it was the ACMP (paracetamol tablet) that was used in the present study as against the infusion used by Payasi *et al*. (2010). The difference in the experimental observations may be due in part to the difference in the route of the drug (ACMP) administration between Payasi *et al*. (2010) and the present study. There was a marked decrease in body weights of high-dose treated rats which was an indication of acute toxicity in an animal study. There were no observable signs of behavioral changes observed in the low-dose treatment animals (400 and 800 mg/kg) during the study period. The result of the present study was in agreement with the studies by Payasi *et al*. (2010) in this regard.

It has been established that some strains of rat with high concentrations of microsomal cytochrome P-450 in their kidneys develops severe tubular necrosis, following a single nonlethal amount of ACMP dosage (Norman and Campbell, 1992). The earlier observation suggests that a condition associated with glutathione depletion or increased activity of P-450 microsomal oxidase enzymes enhanced ACMP toxicity even at the therapeutic dosages (Norman and Campbell, 1992). Examples include chronic alcohol use, starvation, fasting, or ingestion of drugs that induce these enzymes, such as anticonvulsants (Payasi *et al*., 2010). Importantly, the proximal tubules are the target of ACMP toxicity because of their active absorptive and secretory activities (Payasi *et al*., 2010; Seham *et al*., 2008). Furthermore, Theodore *et al*. (1996) reported that studies in humans and animals showed an overall minimal incidence of acute renal failure by ACMP toxicity. The deleterious cellular effects shown in the high-dose kidney sections in this study are evidence of renal toxicity. It was, therefore, a clear indication that our findings corroborate with earlier reports of Payasi *et al*., (2010); Seham *et al*. (2008), and Theodore *et al*. (1996). Furthermore, the present study strongly agreed with the work by Sathish *et al*. (2012), who reported that renal impairment may be more characteristics of late than previously recognized.

Theodore *et al*. (1996), stressed that the overall incidence of acute renal failure in patients with ACMP poisoning was <2%, while Benjamin *et al*. (2002), suggested that ACMP poisoning may be due to cases of ACMP overdoses. However, a considerable amount of ACMP is metabolized by oxidation because of saturation of the sulfate conjugation pathway (Pajoumand *et al*., 2003; Benjamin *et al*., 2002). To buttress the above views, Venkatesa and Deecaraman (2014) suggested that once the protective intracellular glutathione stores are in depletion, hepatic and renal damage may ensue. The above statement may be valid to some extent and may have played a vital role in our study. It may also account for the hepatic and renal damages observed in parts in the present study.

Previous studies concluded that the rates of self-medication are relatively high and alarming. The prevalence in the present study (97.6%), agreed to a similar survey from other authors that have earlier been reported. Up to 76% in Karachi-Pakistan, 94% in Hong Kong, and 80% in U.S-Mexico border (Zafar *et al*., 2008; Chang and

Trivedi, 2003; Casner and Guerra, 1992). In countries where drug purchase is regulated like Portugal, a reduced prevalence of 26.2% was reported (Martins *et al*., 2002). However, in the former, the studies were vague in scope (over-the-counter) medications which include ACMP. While, the present study focused majorly on ACMP abuse excluding other nonprescription medications. Our study again recorded fever (96.8%), closely followed by headache (89.4%), as the major conditions that contributed to ACMP abuse. These contributing factors have been reported, while ACMP is the major class of drugs for self-medication, according to reports by Gutema *et al*. (2011); Abay and Amelo, (2010).

Burak and Damico, (2000) reported that the indiscriminate consumptive nature of nonprescription drugs is more common among the youth, and it might relate to pharmaceutical advertorial approaches. This report agrees with our study in that 55% of the respondents who consume ACMP indiscriminately fall within the age range (15–24 years) and are regarded as youths. High prevalence of the youth as the most affected in self-medication and ACMP abuse has previously been in the report (Agbor and Azodo, 2011). However, some studies revealed that there was no association between age and self-medication (Afolabi, 2008). Our report, therefore, disagreed with Afolabi (2008) of which age and other contributing factors to ACMP abuse in this study were significantly affected.

Our study also suggested that the type of profession engaged in by an individual highly influences the rate of ACMP abuse. This study recorded a prevalence of 42% of artisans who responded that they consume ACMP indiscriminately. It may, however, result from an overwhelming work-induced stress. Sometimes as a result of lack of effectiveness of the average standard required dosage for consumption. Therefore, an individual from this category may turn to the indiscriminate use or abuse of ACMP. The nature or type of work/profession as a contributing factor to self-medication, or drug abuse has been in the report from different regions of the world (Banerjee and Bhadury, 2012; Gutema *et al*., 2011; Ritu *et al*., 2011; Souza *et al*., 2011). Our study, therefore, corroborates with these reports in like manner (Banerjee and Bhadury, 2012; Gutema *et al*., 2011; Ritu *et al*., 2011; Souza *et al*., 2011).

CONCLUSION

The present study reiterates the previously recognized information on ACMP harmful effects in humans. Our data suggest that oral administration of ACMP between 1200 and 1600 mg/kg body weight in male Wistar rats showed marked level of adverse effects at the cellular level. There was a high incidence of ACMP abuse from the respondents in human subjects within the study area (Benin City, Nigeria). Therefore, prolonged oral consumption of ACMP results in hepatocellular and renal effects in animals and may act similarly in humans.

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Conflicts of Interest

There are no conflicts of interest.

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