Histomorphological Hepato-teratogenic Effects of Ethanol on the Liver Lobule in Albino Rats (*Rattus novegicus*)

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Abstract: Background: **Prenatal exposure to ethanol has been shown to have teratogenic effects to the developing fetal liver. However, the histomorphological effects of ethanol on the structural organization of the fetal liver when exposed at different gestational periods and in varied doses has not been well elucidated.**

Method: **A static-group experimental study design was adopted in this study. A sample size of 30 female albino rats dams weighing between 200 to 230gm were used as the animal experimental model. These 30 rats were further grouped into two main groups namely; the Control group (n=3) and the experimental group n=27). The 27 rats were further assigned into three study groups based on the ethanol dosage namely: LEG, MEG and HEG at trimester I, II and III each that received 2g/kgbwt, 3.5g/kgbwt and 5g/kgbwt of ethanol respectively once daily via oral gavage. The control group received food and water *ad libitum* only. All the rats were humanly sacrificed on their 20th day of gestation. A total of 90 fetuses had their liver harvested, fixed in 10% formaldehydeand processed for histological analysis. The tissue slides were mounted on BP Olympus microscope, viewed under different magnifications and a 32 megapixel digital camera was used to capture liver photomicrographs.**

Results: **This study established *v*aried histomorphological effects of ethanol on the fetal liver lobule including constriction of the central vein, dilatation of the liver sinusoids and hepatocyte disaggregation among others. In conclusion, ethanol consumption during pregnancy has a wide range of detrimental hepato-teratogenic effects throughout the three gestational periods in dose dependent manner. It is therefore recommended that expectant mothers should avoid ethanol consumption any time during pregnancy.**

***Keywords*: Ethanol, Fetal, Liver, Prenatal, Teratogenic**

I. INTRODUCTION

Ethanol, commonly referred to as ethanol has a low molecular weight of 46.069g/mol with a biochemical structure of CH_3CH_2OH (1,2). In addition, it has both polar and non-polar molecules in that the non-polarmolecules are proportionally more as compared to the polar molecules therefore making ethanol to be more water soluble but less lipid soluble therefore able to cross the blood placental barrier. Consequently, ethanol gets in to the fetal circulation leading to disturbance in the normal development of the fetal liver among other organs (1). Despite this being known there is no adequate data to show the histo-morphological effect of ethanol on the fetal liver when exposed prenatally at varied doses(3).

The most common route of ethanol administration is through the oral route(4). On the other hand, 80% of the ethanol volume consumed is absorbed from the small intestines while 20% of the ethanol volume is absorbed from the stomach(4). Via the hepatic portal vein, the absorbed ethanol gets in to the maternal liver and finally into the systemic circulation(5). Due to the low molecular weight, lipid and water solubility properties of ethanol, it finally crosses the blood placental barrier into fetal circulation(1,6). This fetal blood ethanol concentration is highly dependent on the maternal blood ethanol concentration and the mode of excretion and a counter current flow of maternal and fetal blood exist (7). Due to this ,the fetal and maternal blood ethanol concentrations attains an equilibrium and in this the fetal organs are highly exposed to ethanol (6).

Ethanol is primarily metabolized in the liver through three different enzymes namely ethanol dehydrogenase (ADH) which is the key enzyme in ethanol metabolism, cytochrome P450 2E1 (CYP 2E1), and catalase (8). Although majority of the ethanol is metabolized in the liver, 2-10% of ethanol is excreted unchanged in urine, breath and sweat (2). During ethanol metabolism, ADH catalyses' ethanol oxidation via a coenzyme known as nicotinamide adenine dinucleotide (NAD) forming acetaldehyde which is injurious to the liver cells. This NAD is reduced to nicotinamide adenine dinucleotide (NADH) (9). Subsequent oxidation of acetaldehyde by NAD forms acetic acid and more NADH and this reaction is catalyzed by aldehyde dehydrogenase (ALDH) (10). The acetaldehyde produced during ethanol metabolism leads to the formation of reactive oxygen species that are highly reactive and toxic to liver cells (10). Excessive NADH levels can inhibit glucose production (gluconeogenesis) and breakdown (oxidation) of fat molecules as well as stimulation

of fat synthesis resulting into hypoglycemia, acidosis and fatty liver (11).

Ethanol has been associated with raising cases of mortality and morbidity, for instance cases of maternal ethanol consumption as well as liver related deaths emanating from excessive ethanol consumption have been reported to be on the increase globally (12,13)Further, 50% mortality rate in the global population has been associated with acute ethanol hepatitis among expectant mothers and this has become a major public health problem (14,15). On the other hand, the prevalence of ethanol related liver conditions that includes alcoholic hepatitis, liver cirrhosis, steatosis, steato-hepatitis among others during pregnancy has been estimated to be 10% globally and between 3.4%-20.5% in East Africa (13) .Studies have also projected that with the current rates of ethanol consumption, cases of liver failure may continue to increase globally. Though studies have shown that ethanol has injurious effects to the developing fetal liver, there is lack of adequate data on its histo-morphological effect of ethanol on the fetal liver when exposed prenatally at varied doses. Data on the most vulnerable periods and the critical doses of ethanol teratogenicity to the fetal liver is also generally lacking.

II. MATERIAL AND METHODS

2.1 Study subjects

Healthy albino dams weighing between 200 $\pm$ 30 grams were obtained from Small Animal Facility for Research and Innovation of JKUAT for the experimentation. This rats were preferred due to the following existing facts: (a) They have low incidence of spontaneously occurring congenital defects, (b) a relatively short gestational span, (c) a large litter size, (d) low cost of maintaining the rats, (e) and considerable amount of the reproductive data on this laboratory rats is already available(16).

The young female rats usually attain their sexual maturity at around 40 - 60 days of age (approximately 5 - 6 weeks). The female albino rats used in the current study were colony from the third series pure breeds, aged between 5-6 weeks. This was considered in order to avoid genetic differences(16). Their gestation period is 21 - 23 daysand they usually gives birth to an average of 6-8 pups (16). All rats in trimester 1(TM_1) study groups received alcohol treatment from gestational day (GD) one all through to gestational day 20 (GD 1-20), those in TM_2 study category received alcohol treatment from GD 7-20 while those in TM_3 received alcohol treatment from GD 14-20.On the other hand, the male rats attains sexual maturity at 40 - 60 days of age(18,19). In the current study a colony of healthy males from second series breeds were used in mating in order to get a pure breed and they were between postnatal days 45-50.These animals were kept in groups of six rats per cage and were *a*cclimatization to laboratory conditions for a period of one week before administration of ethanol. The animals were also fed on rodent pellets and water *ad libitum.*

2.2 Mating and pregnancy determination: Two female rats were introduced into a cage with one male rat at 2:00 P.M. to 09:00am. The following morning, pregnancy was confirmed by light microscopic observation of large cornified cells, many neutrophils and scattered epithelial cells following a vaginal swab. The animal were assigned to either the control or experimental study groups.

2.3 Grouping of the animals: Upon confirmation of pregnancy**,** the animals were non-randomly assigned into ten subgroups (the control group, low dose ethanol group (LEG) at trimester I, II, III, medium ethanol group (MEG) at trimester I, II, III and high ethanol group (HEG) at trimester I, II, III comprising of three rats in each of the ten subgroups. The first subgroup (control) was give tap water while the LEG, MEG and HEG received 2g/kg, 3.5g/kg and 5g/kg body weight of 30% ethanol respectively once daily via gavage needle at different gestational periods. All trimester I animals received ethanol from GD1-GD20, trimester II received ethanol from GD7-GD20 while trimester III animals received ethanol from GD 14-GD20.

Ethanol used in this study was acquired from chemo quip pharmaceutical company, Nairobi with butch number 1614105017 and concentration of 98%. This ethanol was reconstituted with deionized water to a concentration of 30% which was then administered to the experimental animals. All the rats were humanely sacrificed on their 20^{th} day of gestation and a total of 90 fetuses had their liver harvested, fixed in 10% formaldehydeand processed for histological analysis. Ethical approval was sought from animal ethical and research committee of JKUAT and all procedures were performed according to the guidelines for the care of laboratory animals.

2.4 Tissue processing for histology: After fixing the liver in formaldehyde solution for 24 hours, the nine fetal livers per sub group were each placed in a tissue cassette, dehydrated in an ascending concentration of alcohol (50%, 70%, 80%, 90%, 95% and 100%(absolute) each for one hour and cleared with xylene for 30 minutes. The sections were then infiltrated with paraplast wax for 12 hours and embedded in paraffin wax. Leitz sledge microtome was used to cut longitudinal thin liver sections which were floated in water at 37^0 then stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper. The researcher was blinded by coding the tissue slides differently to avoid researcher biasness.

In each subgroup 20-25 slides were selected through systematic uniform random sampling, dried in an oven at 37^0 for 24 hours then stained with haematoxylin and eosin (19) to demonstrate the general features of fetal liver components

2.5 Histomorphological analysis: The liver tissue slides were mounted on the light microscope, photographs were taken at different magnifications of x4, x10, x40, and x100 in four systematically selected random fields to show the different histological changes. The photomicrographs were uploaded in adobe fireworks software for labeling and outlining of the

identified histological changes. Un-coding was done after complete analysis of the results.

III. RESULTS

3.1 The TM_1 comparative histomorphological features of the classical liver lobule between LEG, MEG and HEG against the control

At TM_1, it was observed that the classical liver lobule (hexagonal area enclosed in black in photomicrographs A-D), were well developed in the control group (Photomicrograph A) with an even distribution of the hepatocytes, well outlines central vein as well as un-dilated sinusoids. On the other hand, the classical liver lobules in the ethanol treated groups as per the photomicrographs B for the LEG, photomicrograph C for the MEG, and photomicrograph D for the HEG, shown sparse distribution of hepatocytes in dose dependent manner. In addition varying degrees of sinusoidal dilation directly correlated to dose and time of ethanol exposure across the three trimesters. Lastly, the central vein **(**CV**)** in the classical liver lobule of the ethanol treated groups were constricted with cellular aggregation around them in dose dependent manner (Figure 3.1).

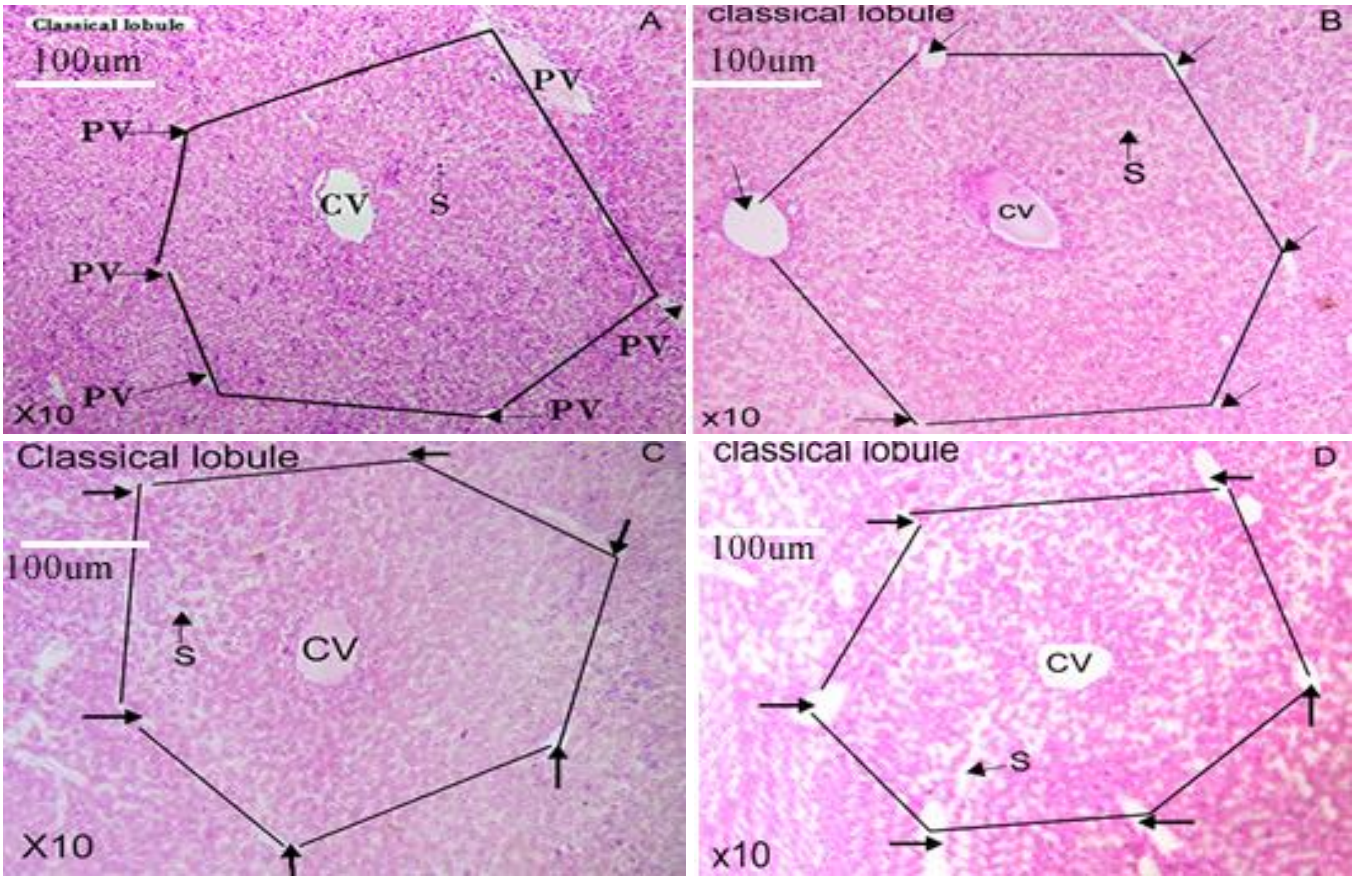

Figure 3.1: The TM_1Photomicrograph of comparative histomorphological features of the classical liver lobule in:- (A) control; (B) LEG; (C) MEG; and (D) HEG

3.2 The TM_2 comparative histomorphological features of the classical liver lobule between LEG, MEG and HEG against the control

From figure 3.2 below it can be observed that when alcohol was administered at TM_2, the classical liver lobule (i.e the hexagonal area enclosed in black) in the photomicrographs A-D, the lobules were well developed with an even distribution of the hepatocytes in the control (Photomicrograph A). On the other hand, the alcohol treated groups (photomicrographs B, C and D), the classical liver lobules shown sparse distribution of hepatocytes with moderate dilation of the sinusoids being in the HAG followed by the medium alcohol group and the least effects in LAG. The central vein **(CV)** in the Photomicrograph B, C and D were moderately dilated probably due to the vasodilative effects of ethanol with high aggregation of cells around it depicting presences of inflammation. The hepatic sinusoids **(S)** also appeared moderately dilated while some hepatic portal veins (**PV**) as shown by arrows (↗) at the corner of the classical lobule shows moderate dilatation. The cellular densities of the hepatocytes in the alcohol treated groups were relatively fewer and sparsely distributed.

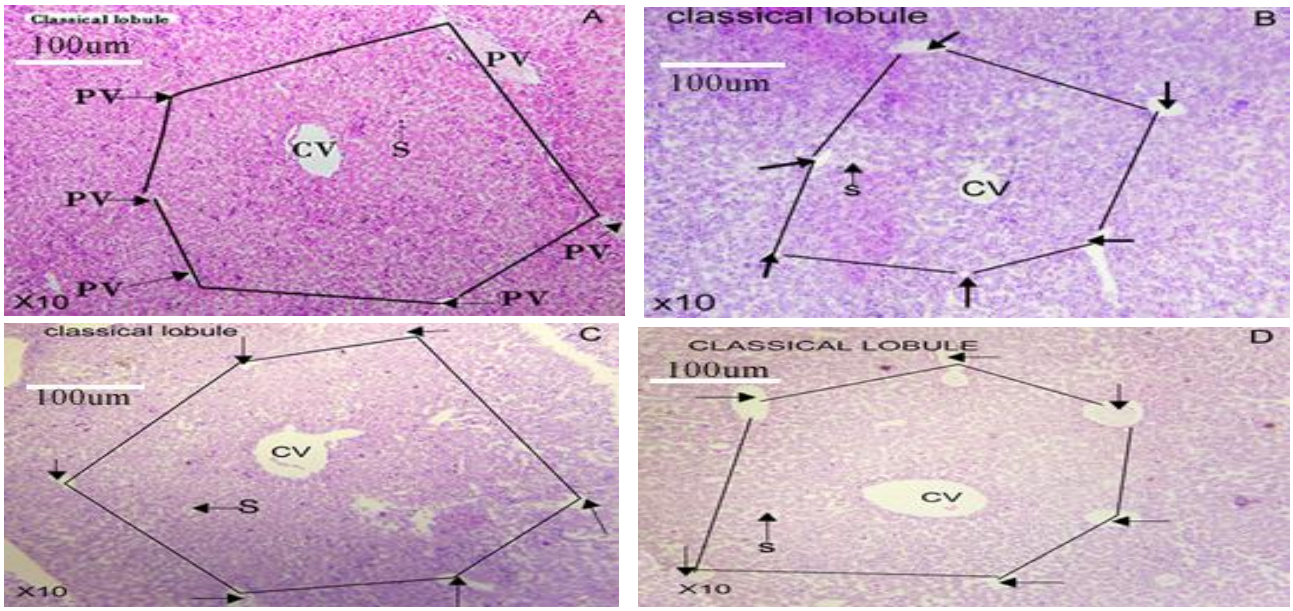

Figure 3.2: The TM_2Photomicrograph of comparative histomorphological features of the classical liver lobule in:- (A) control; (B) LEG; (C) MEG; and (D) HEG

3.3 The TM_3 comparative histomorphological features of the classical liver lobule between LEG, MEG and HEG against the control

When alcohol was administered at TM_3, the hexagonal area occupied by the classical liver lobules shown sparse distribution of hepatocytes with mild dilation of the sinusoids being in the HAG while no sinusoidal dilatation was observed in the MAG and LAG. The central vein **(CV)** in the classical liver lobule in the alcohol treated groups (i.e Photomicrograph B, C and D) were constricted, varying in size with high aggregation of cells around it depicting presences of inflammation. The hepatic portal vein appeared less dilated in the HAG while some hepatic portal veins (**PV**) as shown by arrows (↗) at the corner of the classical lobule shows no dilatation. The cellular densities of the hepatocytes in the alcohol treated groups were normally distributed in the LAG, MAG except the HAG similar to the control group (Photomicrograph A) as shown below (Figure 3.3)

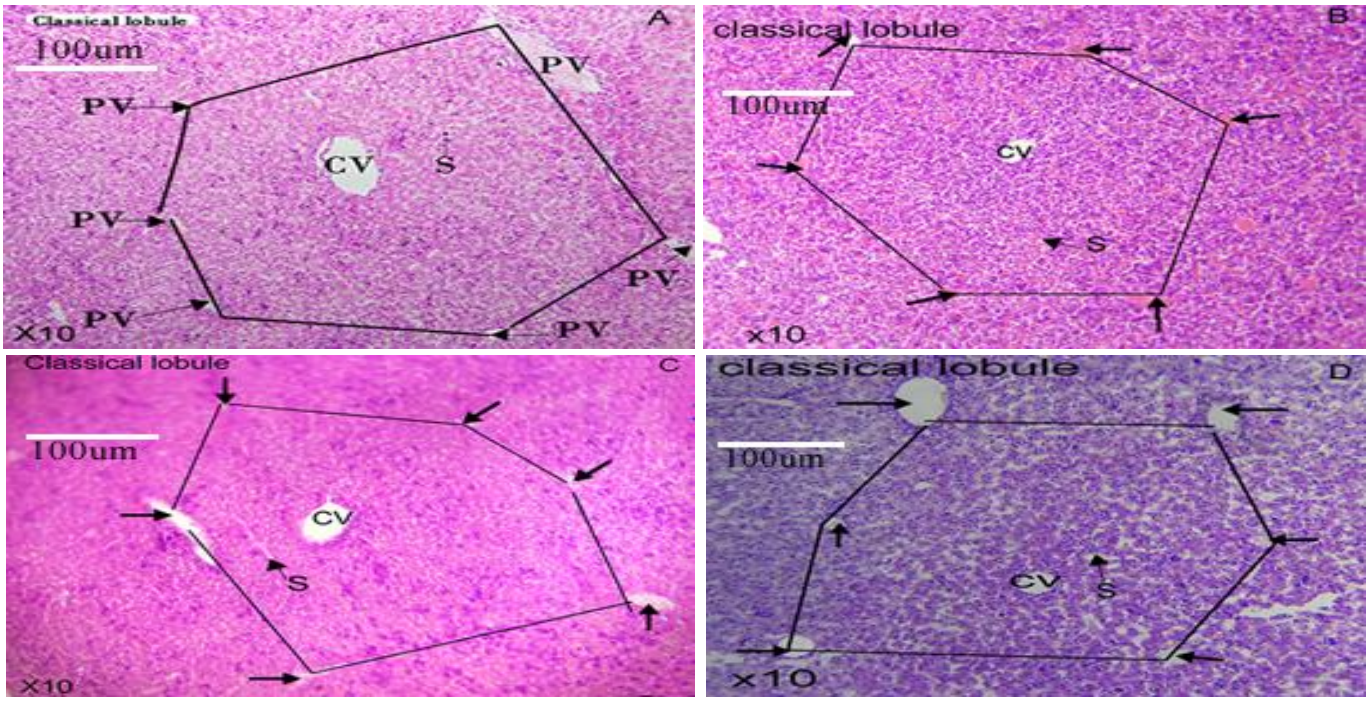

Figure 3.3: The TM_3Photomicrograph of comparative histomorphological features of the classical liver lobule in:- (A) control; (B) LEG; (C) MEG; and (D) HEG

IV. DISCUSSION

In the current study, it was observed that the rats that received high doses (5g/kg/bwt) of ethanol showed severe hepato-teratogenic effects compared to those that received 3.5g/kg/bwt and 2g/kg/bwt of ethanol (Figure 1). In addition, the animals that received ethanol from the first trimester showed more injurious effects of the liver parenchyma as compared to the 2nd and 3rd trimester. The classical liver lobule histomorphological changes observed in the current study which include constriction of the central vein as well as sinusoidal dilatation, inflammatory changes among others were prominent in the classical liver lobule of rats fed on high dose ethanol throughout the gestation (Figure 3.1, 3.2 and 3.3). This agrees with a study which was done by Saeed (2016) that showed important changes in hepatic trabecular structure and increased hepatocytes with cytoplasmic vacuoles (20). This study is in accordance with the previous studies in that administration of low, medium and high doses of ethanol affected rat fetal liver histomorphology as was shown by the cellular disorganization, dilated sinusoids, hepatocyte diaggregation among others.

The classical liver lobule changes observed in the current study could be associated with oxidative stress which induces fat deposition in the hepatocyte cytoplasm (21). A study by Stainer *et al*, (2017) shown that the byproducts of ethanol metabolism (oxygen free radicles) react with unsaturated membrane lipids that initiates self-perpetuated peroxidase process (22).This reaction produces loss of membrane function and ultimately cell death (23). A study by Chen *et al* 2016 showed that glutathione (antioxidant), which is a natural scavenger involved in elimination of the oxygen free radicles is usually present in the liver cells (24). Unfortunately, when ethanol depletes this antioxidants, oxidative stress occurs. In this study, ethanol administered at different gestational period could have yielded free oxygen radicle during its metabolism in the maternalliver. This oxygen free radicles may have crossed the blood placental barrier in to the developing fetal liver and this might have depleted the naturally occurring antioxidants. This consequently might have led to the injury of hepatocytes manifested by dilatation of the sinusoids degenerating and disorganised hepatocytes.

In addition, ethanol and its metabolites has been shown to cause reduction in the activity of ethanol dehydrogenase and cytochrome p-450 which are hormones involved in ethanol metabolism by the hepatocytes (11,25).As a result of reduced activity of this enzymes, ethanol accumulates in the liver which subsequently leads to further hepatocellular death as well as dilatation of the sinusoids due to its toxic effects which could have been the case in the present study.

Ethanol has also been found to cause leaky gut in which endotoxins from the gut end up causing liver cell injury as well as activating the Kupffer cells (liver resident macrophages) among others, that play crucial roles in the inflammatory responses of the liver (12,13) and this could have been the cause of the inflammatory cells around the central vein (Figure 3.1).

V. CONCLUSION AND RECOMMENDATION

Ethanol consumption during pregnancy is associated with the distortion of the normal fetal liver morphology especially the liver lobule in time and dose dependent manner with the most critical period being the first trimester at a dose of 5g/kgbwt. Expectant mothers therefore, need to abstain from ethanol consumption any time during pregnancy

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CONFLICT OF INTEREST

None

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