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## Short term toxicity study on water melon rind extract

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### ABSTRACT

We carried out sub chronic toxicity study on water melon rind to investigate its safety for consumption and also its effect on the liver and kidney of rats.

Twenty rats were used for the experiment and were divided into four groups with five animals per group. Group A animals served as the control, whereas groups B, C and D animals were orally administered 500, 1500 and 3500 mg/kg extract of water melon rind, respectively, for 28 days. The activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined in the serum of the rats. Levels of malondialdehyde (index of lipid peroxidation), glutathione (GSH) and activities of antioxidant enzymes - superoxide dismutase (SOD) and catalase were evaluated in the liver and kidney of the rats.

There was no significant increase in the activity of ALT and AST in the serum of rats administered the extract when compared with control group. Also, there was no induction of lipid peroxidation and no significant change in glutathione concentration and activities of superoxide dismutase and catalase in rats administered the extract compared with control group.

These results show that water melon rind extract does not have adverse effect on the liver and kidney of the rats and may therefore be safe for human consumption. However there is a need for further study on the rind of water melon in order to investigate its medicinal properties and also its effect on other organs.

### Introduction

To enjoy a disease free and healthy life, it is necessary to eat fresh fruits and vegetables (Bhowmik et al., 2012). The consumption of fruits and vegetables lowers high blood pressure which reduces the risk of cardiovascular diseases, and also prevents certain types of cancers (Hobbs et al., 2012). Vegetables and fruits are recognized as natural sources of antioxidants (Huang et al., 2013; Shebis et al., 2013; Goldberg, 2012). Most vegetables and fruits possess antioxidant activities which allow them to get rid of reactive oxygen species and modulate certain cellular enzyme activities (Nimse and Pal, 2015). Antioxidants are substances that hinder cellular oxidation by reactive species. Antioxidants

act by mediating the production of reactive species via cascades of reactions in order to overcome their potentially injurious action (Rajendran et al., 2014). A number of synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are available (Carocho and Ferreira, 2013). These synthetic antioxidants are added to food to increase its shelf life, by delaying lipid peroxidation, in order to prevent food deterioration during processing and storage.

Watermelon (*Citrullus lanatus*) is a vine-like flowering plant that originated from South Africa (Koocheki et al., 2007). The watermelon fruit has a smooth exterior rind which could be green, yellow and sometimes whitish in colour. It also has an interior flesh which is juicy and sweet. The colour of the flesh is usually deep red to pink but sometimes orange, yellow and even green if not ripe

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(Koocheki et al., 2007). Watermelon is from the family Cucurbitaceae and specie *Citrullus lanatus* (Guo et al., 2013). It is rich in minerals, lycopene, and vitamins (Shahzad et al., 2014; Naz et al., 2014). It is also a source of amino acid citrulline which is present in all parts of the fruit (Collins et al., 2007). Water melon flesh is usually consumed while the rind and seeds are seen as waste and discarded by most people. However, watermelon rind is used in Chinese traditional medicine in treating some diseases such as diabetes, jaundice, kidney problems, oedema and erectile dysfunction. Most people avoid eating water melon rind due to its unpleasant flavour and fear of being toxic. The analysis of the rind of watermelon confirmed the presence of moisture, ash, fat, protein, crude fibre and carbohydrate (Erukainure et al., 2010). It has been shown by some researchers that the rind of most fruits has higher antioxidant activity than the pulp (Fatemeh et al., 2012). Watermelon rind has also been reported to be rich in antioxidants (Johnson et al., 2012); however, there is a need for toxicological evaluation of the water- melon rind for human consumption.

Therefore, in this study we carried out sub chronic toxicity study on watermelon rind o to investigate its effect on some serum markers of liver damage and markers of oxidative stress in the liver and kidney of rats.

## Materials and methods

### Source of watermelon fruit

Fresh watermelon fruits were obtained from Orita - Challenge market in the city of Ibadan, Oyo State, Nigeria.

### Preparation of rind extract

The rinds were peeled off from the whole fruit. The rind extract was prepared by grating the rind into a pulp using a grater. The crushed pulp was filtered to obtain the rind extract, while the residue was discarded. The extract was stored in the refrigerator for further use.

### Experimental animals

Male Wistar albino rats of approximately 150 - 200 g obtained from Shalom farm, Iwo road Ibadan, Oyo State, Nigeria were used for all the experiments. The animals were kept in a well-ventilated cage (12 hr light/12 hr dark cycles) and fed with a commercial pellet diet (Ladokun feeds, Mokola Ibadan) and clean drinking water *ad libitum*.

### Experimental design and animal treatment

Twenty male rats were divided into four (4) groups with five animals per group.

Group A: Rats administered distilled water orally by gavage for 28 days (Control).

Group B: Rats administered 500 mg/kg body weight watermelon rind extract orally by gavage for 28 days.

Group C: Rats administered 1500 mg/kg body weight watermelon rind extract orally by gavage for 28 days.

Group D: Rats administered 3500 mg/kg body weight watermelon rind extract orally by gavage for 28 days.

### Serum and tissue preparation

At the end of the 28 days administration of the extract, the blood was collected from each rat via the retro orbital sinus of the eye by ocular puncture and put into plain bottles. The rats were then sacrificed by cervical dislocation and the liver and kidney harvested. The serum was prepared by centrifugation of the blood for 10 minutes at 3000 rpm using a table centrifuge. After centrifugation, the supernatant (the serum) was carefully removed and stored at -4 °C. The livers and kidneys were homogenized with ice cold Tris-HCL buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 4 °C at 12500 rpm for 10 minutes and the resultant supernatant was used for different biochemical assays.

### Biochemical analysis

Protein concentration was determined by the Biuret method as described by (Gornall et al., 1949). Catalase activity was estimated according to the method of Sinha (1972). The glutathione-S-transferase activity was determined according to the procedure of (Habig et al., 1974). Super oxide dismutase activity was estimated by the method of (Misra and Fridovich, 1972). Lipid peroxidation was estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of (Varshney and Kale, 1990).

### Statistical analysis

Results are expressed as means  $\pm$  SEM of 5 rats. The significant difference between groups was determined by using the one-way analysis of variance (ANOVA) followed by the t-test. Statistical test was done using prism graph pad, version 6.0.

## Results

### *The effect of different doses of watermelon rind extract on AST and ALT activity in Wistar rats*

There was no significant difference in the activity of AST and ALT in rats given graded doses (500, 1500 and 3500 mg/kg body weight) of the extract compared with control. Results are shown in Table 1.

### *The effect of different doses of watermelon rind extract on reduced glutathione (GSH) concentration in rat tissues*

The effect of different doses of watermelon rind extract on reduced glutathione (GSH) concentration in the experimental animals is presented in Table 2. There was no significant change in GSH level in livers and kidneys of rats given graded doses of watermelon rind extract compared with the control group.

### *The effect of different doses of watermelon rind extract on superoxide dismutase (SOD) activity*

Superoxide dismutase activity in livers and kidneys of the rats is shown in Table 3. Compared with the control group, oral administration of watermelon rind extract at the doses of 500, 1500 and 3500 mg/kg body weight did not significantly affect SOD activity in the liver and kidney of the experimental animals.

### *The effects of different doses of watermelon rind extract on catalase activity in rat tissues*

Catalase activity in livers and kidneys of the experimental animals is shown in Table 4. There was no significant difference in catalase activity in rats administered oral doses of watermelon rind extract at 500, 1500 and 3500 mg/kg body weight compared with control.

**Table 1.** The effect of different doses of watermelon rind extract on AST and ALT activity

Experimental Group	AST(U/I)	ALT(U/I)
Control	18.20 ± 2.01	14.14 ± 2.31
500 mg/kg body weight	18.00 ± 2.68	16.33 ± 0.81
1500 mg/kg body weight	19.30 ± 1.77	14.50 ± 1.90
3500 mg/kg body weight	9.80 ± 3.63	15.26 ± 0.54

**Table 2.** The effect of different doses of watermelon rind extract on reduced glutathione (GSH) concentration in livers and kidneys of rats

Experimental Group	Liver GSH (µmol/ml)	Kidney GSH (µmol/ml)
Control	57.60 ± 2.07	57.00 ± 1.41
500 mg/kg body weight	55.67 ± 0.58	58.00 ± 0.00
1500 mg/kg body weight	56.20 ± 1.09	58.00 ± 0.00
3500 mg/kg body weight	56.00 ± 0.50	50.75 ± 1.89

**Table 3.** The effect of different doses of watermelon rind extract on superoxide dismutase (SOD) activity in livers and kidneys of rat

Experimental Group	Liver SOD (U/g tissue)	Kidney SOD (U/g tissue)
Control	1.52 ± 0.59	1.23 ± 0.07
500 mg/kg body weight	1.40 ± 0.28	1.43 ± 0.02
1500 mg/kg body weight	1.36 ± 0.21	1.56 ± 0.01
3500 mg/kg body weight	1.33 ± 0.46	1.27 ± 0.11

**Table 4.** The effects of different doses of watermelon rind extract on catalase activity in livers and kidneys of rats

Experimental Group	Liver Catalase (µmol/mg protein)	Kidney Catalase (µmol/mg protein)
Control	29.50±1.71	21.00 ± 1.58
500 mg/kg body weight	23.00 ± 1.08	16.55 ± 1.25
1500 mg/kg body weight	25.50 ± 0.65	20.25 ± 0.48
3500 mg/kg body weight	24.75 ± 0.85	18.75 ± 1.32

### *The effect of different doses of watermelon rind extract on tissue protein of Wistar rats*

Protein concentration in livers and kidneys of the rats are shown in Fig 1. Compared to the control group, oral administration of watermelon rind extract at the doses of 500, 1500 and 3500 mg/kg body weight did not significantly affect the level of protein in livers and kidneys of the experimental animals.

### *The effects of different doses of watermelon rind extract on MDA level in rat tissues*

Figure 2 presents the effect of different doses of watermelon rind on MDA level (an index of lipid peroxidation) in livers and kidneys of Wistar rats. There was a decrease in MDA level in livers of rats given graded doses of the extract compared with control, though the decrease was not statistically significant while there was no difference in MDA level in kidneys of rats given the extract compared with control.

## Discussion

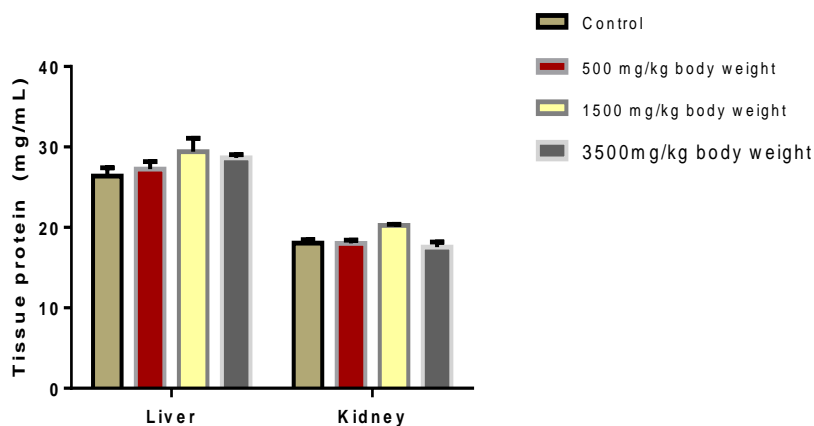
The determination of serum activities of a number of biochemical markers such as serum activity of AST and ALT is often used to determine the extent of liver damage (Kim et al., 2008). These enzymes are normally present in the liver at high concentration but they are released from the liver cells due to certain disease conditions and their levels in the blood are therefore increased (Giannini et al., 2005). The present study reveals no significant increase in the activity of AST (aspartate amino transferase) and ALT (alanine amino transferase) in rats given graded doses of the extract compared with the control group. The experimental animals in group D (animals given 3500 mg of watermelon rind extract per kg body

weight) even showed a decrease in AST activity compared to the control group. This suggests that the extract may have hepatoprotective potential.

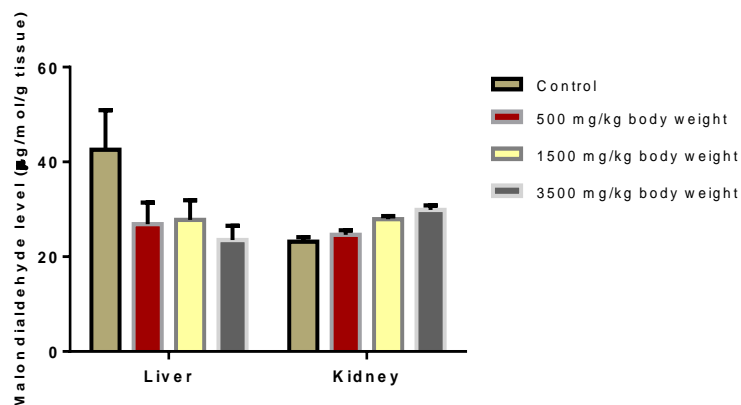
Malondialdehyde (MDA) is one of the marker indices of lipid peroxidation. Hence, the higher the MDA level in tissues, the higher/ bigger the degree of lipid peroxidation (Pandey et al., 2012). In the present study, there was no significant increase in MDA level in livers and kidneys of rats given the extracts when compared to control. This is an indication that the extract did not cause lipid peroxidation in the organs. In fact, there was a decrease in MDA level in livers of rats given graded doses of the extract compared with control; this confirms as well that the extract might have hepatoprotective potential.

Glutathione (GSH) is an important antioxidant capable of preventing damage to important cellular components caused by reactive oxygen species (Sharma et al., 2012). Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical into either ordinary molecular oxygen ( $O_2$ ) or hydrogen peroxide ( $H_2O_2$ ) (Fridovich, 2011). In the present study, there was no significant difference in GSH concentration and SOD activity in livers and kidneys of rats given oral administration of watermelon rind when compared to the control group.

Catalase is an essential component of the cellular antioxidant defence system. It catalyses the breakdown of hydrogen peroxide which results from SOD activity and other normal body metabolism. It also detoxifies other toxic substances including phenols and alcohols (Mates, 2000). As with SOD and GSH, the present study shows no significant difference in catalase activity in livers and kidneys of rats given oral administration of watermelon rind extract when compared to the control group.



**Fig. 1.** The effect of different doses of watermelon rind extract on tissue protein in livers and kidneys of rats



**Fig. 2.** The effects of different doses of watermelon rind extract on malondialdehyde level in livers and kidneys of rats

## Conclusion

Overall, the results of this study show that there was no significant increase in the levels/activities of the tested biochemical markers of toxicity in livers and kidneys of rats administered watermelon rind extract when compared to control group. Hence, it could be concluded that watermelon rind does not have adverse effect on the livers and kidneys of the rats and may therefore be safe for consumption. However, there is need for further study on the rind of watermelon to further elucidate its medicinal properties and investigate its effect on other organs apart from the liver and kidney.

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