Acta Scientific Biotechnology

Volume 4 Issue 5 October 2023

Research Article

Antioxidative Potential of Methanol Extract of *Citrullus lanatus* Peel in CCl_4 Induced Oxidative Stress in Albino Rats

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Received: May 03, 2023

Published: December 02, 2023

Abstract

The aim of this study was to evaluate the Antioxidative enzymes potentials of; superoxide dismutase (SOD), Glutathione peroxidase (GSH), Catalase (CAT), Lipid peroxidation (MDA), and some liver parameters such as Aspartate transaminase (AST), Alanine transaminase (ALT), Albumin, and Bilirubin level in Antioxidative potential of methanol extract from *Citrullus lanatus* peel in CCl4 induced oxidative stress in Albino rats. As well as in-vitro antioxidant parameter such as Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-2-picylydrazyl (DPPH), Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) which was both compared with Vitamin C. Twenty-five (25) wistar rats which was grouped into five rats each was used for the in-vivo experiment while crude plant extract was used for the in-vitro determination. The level of Aspartate aminotransferase (AST) in methanol extract of Citrullus lanatus on sillymarin + CCl4, *Citrullus lanatus* peel low dose + CCl_4, *Citrullus lanatus* peel high dose + CCl_4, CCl_4 only, and as well as control had 173.66 ± 44.79, 170.90 ± 4.11, 185.00 ± 10.00, 496.66 ± 25.16, 190.00 ± 31.22 respectively shows that Aspartate aminotransferase (AST) has a significant ($P > 0.05$) increase of CCl_4 negative control when compared with sillymarin + CCl_4, Citrullus lanatus peel low dose + CCl_4, *Citrullus lanatus* peel high dose + CCl_4, and as well as positive control. In ALT, 200mg/kg body weight of sillymarin + CCl_4, 200 and 400mg/kg of the extract *Citrullus lanatus* peel has a significant ($P > 0.05$) increase of ALT when compared with CCl_4 negative control. There was a dose dependent in Albumin which shows non-significant ($P > 0.05$) in group treated with sillymarin + CCl_4, 200 and 400mg/kg of Citrullus lanatus peel and as well as control when compared to negative control. The group treated with sillymarin + CCl_4 shows significantly ($P < 0.05$) decrease of Bilirubin when compared with *Citrullus lanatus* peel low dose + CCl_4, Citrullus lanatus peel high dose + CCl_4, and as well as normal control. The result gathered shows that the group treated with 400 mg/kg of Citrullus lanatus peel high dose + CCl_4 indicates non-significantly ($P > 0.05$) increase of glutathione when compared with negative and as well as normal control. The result shows that the group treated with *Citrullus lanatus* peel low dose + CCl_4 indicates non-significantly ($P > 0.05$) increase of Catalase when compared with negative control. It was observed that the methanol extract contains moderate level of flavonoids and considerably high level of phenolic content. In conclusion, the methanol extract of *Citrullus lanatus* peel was found to possess antioxidant potentials which can mob up free radical generated in the body and hence manage conditions associated with oxidative stress.

Keywords: Antioxidative Potentials; Methanol Extracts; *Citrullus lanatus*; CCl4; Oxidative stress; Albino Rats

Introduction

Carbon tetrachloride (CCl_4) is a well-known hepatotoxic widely used to induce toxic liver damage in a wide range of laboratory animals. CCl_4 induced oxidative stress damage, inflammation, fatty degeneration and fibrosis in the liver [1]. NF-KB is activated in the liver after CCl_4 administration and is believed to play a vital role in long term CCl_4 administration induced chronic liver injury and fibrosis. CCl_4 is commonly used for free radical induced liver injury [2]. CCl_4 is not the only target organ of liver but it also affects several organs of the body such as lungs, hearts, testes, kidneys and brain. It was reported from the investigation carried out on animal models of acute CCl_4 induced liver damage [3].

Free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules. Oxygen by-products are relatively unreactive but some of these can undergo metabolism within the biological system to give rise to these highly reactive oxidants [4]. Not all reactive oxygen species are harmful to the body. Some of them are useful in killing invading pathogens or microbes. However, free radicals can chemically interact with cell components such as DNA, protein or lipid and steal their electrons in order to become stabilized.

Oxidative stress is a pathological state that arises when free radicals (collectively known of as reactive oxygen species or ROS) chemically interact with living system and damage biological molecules. The process of oxidation happens as our bodies metabolize (or process) the oxygen that we breathe and our cells produce energy from it. This process also produces free radicals which interact with the molecules within our cells resulting in damage (or stress) to nearby cells, mitochondria, and DNA (our genes) [5].

Free radicals are normal and necessary to some degree. In addition to causing some damage, they also stimulate repair. It is only when the number of free radicals produced overwhelms the repair processes that it becomes an issue. That is what we call oxidative stress [4].

Oxidative stress research has largely focused on the role and effects of antioxidants in protecting these molecules from damage.

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals [6]. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer. Examples of antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), vitamins C, E, and other substances. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species [7].

The targeted plant fruit peel for this study *Citrullus lanatus*. (*Citrullus lanatus*) of family Cucurbitaceous is a flowering plant. It is mainly originated from southern Africa [8]. Its fruit is called pepo by botanist. It is also known as watermelon, pepo is berry like fruit, the exocarp is the thick rind, the outer surface of the fruit and mesocarp and endocarp are the freshly coloured center of the fruit [9].

The inference from the above suggests that oxidative stress is caused by liver damage and since *Citrullus lanatus* comprises of so many pharmacological properties and anti-nutrients, it can be used to scavenge free radicals.

Materials and Methods

Plant materials

Citrullus lanatus are available throughout the country during the summer season. The watermelons were collected from Ahiaeke i market Umuahia, Abia state. The rind of *Citrullus lanatus* were identified and authenticated in the Department of Plant and Biotechnology, College of Natural Sciences Michael Okpara University of Agriculture, Umudike, Abia State.

Preparation of plant extract

- The outer most skin of the exocarp of samples was peeled with a peeler.
- The rinds were pieced into small pieces.
- Then the rind pieces were taking into the hot oven and the oven was set at 50 degrees centigrade (50°C) for proper drying. After proper drying it was grounded into powdery using analytic grander
- 150g of the powdered *Citrullus lanatus* was soaked in1000ml of Methanol (95%v/v) for 48 hours under room temperature and was occasionally stirred using orbital shaker.
- The extract was filtered and concentrated to dryness using retro-evaporator. A known volume of extracts was orally administered to the animals using gavage.

Animals

Twenty-five (25) male albino rats were used. They were caged and fed on commercial pellet diet (vital growers mash by grand cereals and oil mills, Nigeria) and water were given to the animals adlibitum.

Experimental design

The animals were allowed to acclimatize for two weeks and were grouped into five (5) groups of rats per cage.

Group one was given 200mg/kg of body weight of silymarin drug, group two and three rats were given 200 and 400 mg/kg body weight of *Citrullus lanatus peel and* CCl_4 for twenty days followed by CCl_4 for three days in a seven days interval. Group four were serves as negative control (only CCl_4), and group five serves as positive control (normal without CCl_4) animals of this group were fed with pellets and water ad libitum for 21 days. All animals were sacrificed by cervical dislocation at the end of twenty first day of the treatment after being fasted overnight.

Statistical analysis

All the grouped data were statistically evaluated using SPSS/11.5 software package. The values were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test. All the results were expressed as mean ± SD for each group. (P values <0.05) were considered significant. Independent sample analysis was also used to compare scavenging/inhibition capacities of the extract for free radicals in comparison with of the standard antioxidants at the various determined concentrations.

Results

The result of this research showed antioxidant potentials of methanol extract of *Citrullus lanatus* of carbon tetrachloride (CCl_4) induced oxidative stress on male wistar albino rats. From the result and interpretation above, it was seen that the level of antioxidant activity of Superoxide dismutase (SOD) of the level of Superoxide dismutase (SOD) in methanol extract of *Citrullus lanatus* on sillymarin + CCl_4, *Citrullus lanatus* peel low dose + CCl_4, *Citrullus lanatus* peel high dose + CCl_4, negative control shows no significance (P > 0.05) decrease when compared with the normal. This shows that the effect of methanol extract of *Citrullus lanatus* on SOD activity was insignificant. This could also be attributed to equal SOD present in the entire sample group since they are synthesized in the body and not from diet.

In-vitro antioxidant activities of methanol extract of (watermelon) *Citrullus lanatus* peel using Ferric reducing antioxidant power (FRAP).

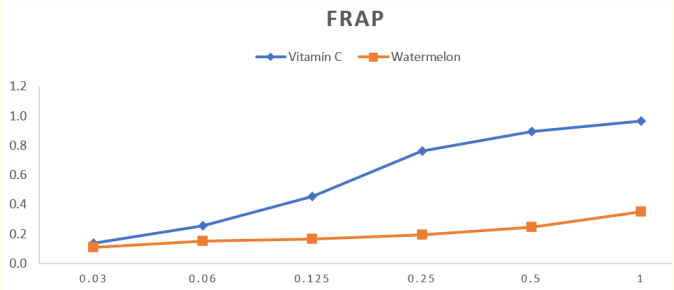

Figure 1: The data in figure above showed the ferric reducing antioxidant power of methanol extract of (watermelon) *Citrullus lanatus* peel and Vitamin C (Ascorbic acid) a standard antioxidant species.

From the graph above, it was observed that both Vitamin C and methanol extract of *Citrullus lanatus* peel exhibited low ferric reducing antioxidant at low concentrations 0.0313 – 0.25mg/ml. However, at increased concentration of 0.5 -1mg/ml both vitamin C and methanol extract of *Citrullus lanatus* showed higher ferric reducing antioxidant power with that of methanol extract of *Citrullus lanatus* peel been significant (P < 0.05) lower when compare with Vitamin C.

In-vitro antioxidant activities of methanol extract of (watermelon) *Citrullus lanatus* peel using 2,2-diphenyl-2- picrylhydrazyl (DPPH).

In-vitro antioxidant activities of methanol extract of (watermelon) *Citrullus lanatus* peel using Flavonoids and phenolic content.

Discussion

The result gathered shows that the group treated with *Citrullus lanatus* peel high dose + CCl_4 indicates non-significantly (P > 0.05) increase of glutathione peroxide when compared with sillymarin + CCl_4, *Citrullus lanatus* peel low dose + CCl_4, CCl_4 only, and as well

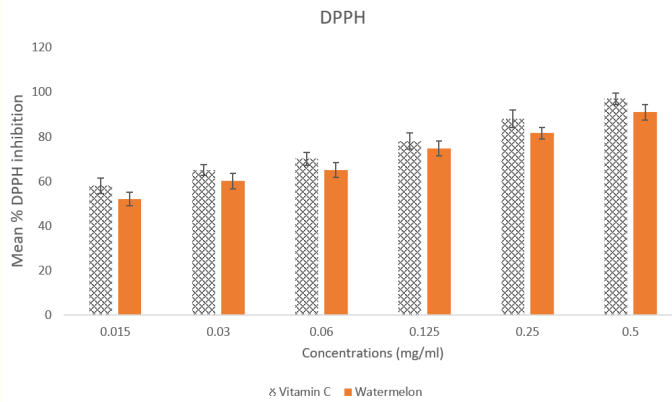

Figure 2: The bar chart above showing the DPPH free radical scavenging activities of methanol extract of (watermelon) *Citrullus lanatus* peel and vitamin C. This figure indicates a dose dependent increase in DPPH free radical scavenging of the extract and vitamin C respectively. Both methanol extract of *Citrullus lanatus* peel and vitamin C had the least and highest DPPH inhibition at 0.015 mg/ml and 0.5 mg/ml respectively. The EC_{50} of the methanol extract and vitamin C was found to be 0.0134 mg/ml and 0.0129 mg/ml respectively. The methanol extract exhibited no significant ($P > 0.05$) increase in DPPH free radical scavenging activity at all concentrations tested when compared to vitamin C concentrations.

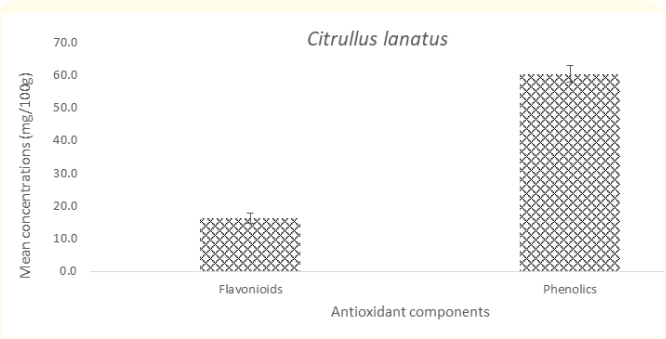

Figure 3: The data in the figure above showed Flavonoid and Phenolic content of methanol extract of *Citrullus lanatus* peel. It was observed that the methanol extract contains moderate level of flavonoids and considerably high level of phenolics.

as control could be attributed to the extract possessing a hepatoprotective activity which was able to suppress and prevent the excess peroxide radical generated by CCl_4. The enzyme GPx catalyzes the reduction of hydrogen peroxide using GSH, thereby protecting mammalian cells against oxidative damaged. In fact, metabolism of GPx is one of the most essential antioxidant defense mechanisms according to [10,11].

Moreover, the result obtained above shows that the group treated with silymarin + CCl_4 and *Citrullus lanatus* peel high dose+CCl_4 indicate significantly ($P < 0.05$) decrease of Malondialdehyde (lipid peroxidation) when compared with *Citrullus lanatus* peel low dose + CCl_4, CCl_4 only, and as well as control. This shows the level of lipid peroxidation in group treated with *Citrullus lanatus* peel high dose+CCl_4 and control was higher than the *Citrullus lanatus* low dose. The significant increase of MDA level against the group administered with low dose of *Citrullus lanatus* could be due to the monocyclic peroxides formed from fatty acids with three or more double bonds which might be formed or arises in high dose of *Citrullus lanatus* when induced with CCl_4, this could serve as a precursor to increase MDA level as well as resulting to toxic effect in the body through various tissue damages. It could also be in line with that foods high in poly unsaturated fats like vegetables oil, soybeans oils etc which are fatty acids that contains more than two carbon-carbon bonds undergoes oxidative deterioration, which is lipid peroxidation that has MDA as its end products.

The result in (figure 2) above shows that sillymarin + CCl_4 and CCl_4 has a significant ($P < 0.05$) decrease of Alanine transferase (ALT) when compared with other groups *Citrullus lanatus* peel low dose + CCl_4, *Citrullus lanatus* peel high dose + CCl_4, well as control. This significant showed by ALT on standard sillymarin drug compare with group treated with *Citrullus lanatus* extract suggest that sillymarin drug may be less toxic to animal, also the significant increase on the standard drug sillymarin could be attributed to the standard drug possessing sufficient antioxidant potential which was able to scavenge the free radicals generated as a result CCl_4 induction. This increase may also be attributed to the standard drug stabilizing the antioxidant enzyme system which invariably led to the increase in the antioxidant activity, this is in agreement with the

finding of (Peter, 2015) which report that sillymarin contribute to the antioxidant defense system by direct free radical scavenging and by preventing free radical formation by inhibition of specific enzyme responsible for free radical generation.

It was observed that the methanol extract contains moderate level of flavonoids and considerably high level of phenolic.

Protective effect of *citrullus lanatus* is probably due to the counteraction of free radicals by its antioxidant nature, as evidenced from the histological observation of the pancreas, which shows regeneration in the pancreatic islet of Langerhans cells. The slide for normal control group rats shows rounded and oval shaped islets cells which are evenly distributed throughout the cytoplasm. The effect of the extract is consistent with the work of who also reported regeneration of pancreatic beta-cell with basophilic cytoplasm and no inflammatory cells seen on treating streptozotocin-nicotinamide induced oxidative stress rats with *Hippophaerhamnoides* L. for about 3 weeks. The present study thus confirms that pancreatic islet cells get destroyed by CCl_4 and *Citrullus lanatus* extract could bring about its partial or total regeneration.

Conclusion

The methanol extract of *Citrullus lanatus* was found to possess anti-oxidative potentials which can mob up free radicals generated in the body and hence manage conditions associated with oxidative stress. Phytochemical investigations of the extract have of course demonstrated the presence of flavonoids and phenolic compounds as the main active principles having potent antioxidant activities. Pancreatic regeneration studies of the methanol extract of *Citrullus lanatus* treated rats showed nesidioblastosis. It can thus be concluded that the factors causing regeneration of the islet cells are present within the pancreas.

Conflicts of Interest

There was no conflict of interest by the authors.

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