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Aqueous Extract of Potato (*Solanum tuberosum*) Modulates Cadmium-induced Liver Damage in Female Wistar Rats

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Abstract: Cadmium (Cd) is an environmental and industrial pollutant known to be highly toxic to large number of tissues in the body. In this study, the protective effects of aqueous extract of potato (*Solanum tuberosum*) against the hepatotoxic effect of Cadmium (Cd) were investigated in a set of female Wistar rats. The rats were given oral administrations of potato extract for 3 weeks at a dose of 250 mg kg⁻¹ body weight prior to one week intraperitoneal exposure to 4 mg kg⁻¹ Cadmium (Cd) as Cadmium Chloride (CdCl₂). Liver damage were monitored using markers of hepatocellular injury such as serum (GOT, Glutamate Oxaloacetate Transaminase; GPT, Glutamate Pyruvate Transaminase; γ GT, Gamma-Glutamyl Transpeptidase and ALP, Alkaline Phosphatase). The levels of reduced glutathione (GSH), GST activity, lipid peroxidation and the expressions of HO-1 and NQO1 were also determined in order to evaluate the antioxidant status of the liver after Cd exposure. The results show that the presence of aqueous extract of *Solanum tuberosum* significantly reduced the activities of all the serum enzymes in the presence of Cd. The level of GSH and the activity of GST were also significantly increased in the presence of the extract plus Cd when compared with rats exposed to Cd alone. The extract also attenuates lipid peroxidation induced by Cd indicating decrease oxidative damage. Western blot results show additive effect of the extract and Cd on the expressions of HO-1 and NQO1. Taken together, the present work shows that aqueous extract of *Solanum tuberosum* contains active agents that are potent in preventing Cd-induced liver damage in exposed rats.

Key words: Cadmium, potato, liver, aqueous extract, lipid peroxidation

INTRODUCTION

There are considerable epidemiological evidences indicating association between diets rich in fresh fruit and vegetables and decrease risks of cancer (Block *et al.*, 1992; Steinmetz and Potter, 1996; Trock *et al.*, 1990). It is generally assumed that the active dietary constituents contributing to these protective effects are the antioxidants such as vitamins, carotenoids, polyphenols and sterols (Shi *et al.*, 2011; Miller and Ruiz-Larrea, 2002). Potato is one of about 2000 species in the family of solanaceae which includes plants such as tobacco, tomato, egg plant and pepper and it is considered a good source of antioxidants such as ascorbic acid and α -tocopherol which act synergistically (Byers and Perry, 1992). It has been reported that potato also contains flavone aglycones, a major group of plant phenols which are potent antioxidants (Ding *et al.*, 2010). It is also a good source of glutathione (Jones *et al.*, 1992) and patatin (Al-Saikhan *et al.*, 1995) both of which have high

antioxidant properties. *In vitro* (Al-Saikhan *et al.*, 1995; Mohdaly *et al.*, 2010) and *in vivo* (Singh *et al.*, 2008) studies have shown that potato extracts have potent antioxidant activities which are active against toxic chemicals.

Cadmium (Cd) is ubiquitous in the environment and it is a serious industrial and environmental pollutant. Cd has been referred to as a group 1 carcinogen (Merrill *et al.*, 2001) and chronic and acute exposure to this metal has been reported to be carcinogenic to various organs in the body (Waalkes, 2000; Waisberg *et al.*, 2003). The liver is one of the major target organs in acute and chronic cadmium exposure (Oh and Lim, 2006). The metal has no known uniform mechanism of toxicity (Fotakis *et al.*, 2005). It has been reported that one of the mechanisms by which Cd induced toxicity is by increasing the levels of reactive oxygen species within the cell (Hassoun and Stohs, 1996; Riso-de Faverney *et al.*, 2001; Galan *et al.*, 2001) resulting in oxidative damage to macromolecules, cells and tissues.

Therefore, in this study we tried to examine the protective effects of aqueous extract of potato (*Solanum tuberosum*) on Cd induced toxicity in the liver of exposed female rats in order to establish the efficacy of this extract in preventing oxidative damage.

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (UK). Antibodies were purchased from Santa Cruz Biotech. Inc (Santa Cruz, CA, USA). Goat anti-rabbit antibody (horseradish peroxidase conjugated) was obtained from Bio-Rad (UK). Nitocellulose membranes were purchased from Amersham Biosciences and Acrylamide was obtained from Severn Biotech Ltd (UK).

Plant material: Fresh Potato (*Solanum tuberosum*) tubers were obtained locally from Abadina, University of Ibadan, Nigeria.

Preparation of potato extracts: The potato extracts were prepared according to the method of Al-Saikhan *et al.* (1995). Extract was stored in capped tubes at -40°C until when required for use. The concentration of the extract was determined before use.

Animals: Forty female wistar rats weighing between 160-240 g were used in this study. The animals were purchased from physiology department, University of Ibadan and were maintained and housed under controlled conditions of ambient temperature (25°C). Food and water were provided *ad libitum*.

Animal treatment and sample collection: Animals were divided into 4 experimental groups (n = 10) with free access to food and water. The first group received only the vehicle (0.9% saline). The second group received an oral administration of potato extract at a dose of 250 mg kg⁻¹ body weight every other day for 3 weeks and 0.9% saline at the fourth week. The third group received an intraperitoneal injection (ip) of cadmium as cadmium chloride in 0.9% saline at a dose of 4 mg kg⁻¹ body weight every other day for one week at the fourth week of treatment. The fourth group received both an oral administration of potato extract (250 mg kg⁻¹ body weight) every other day for 3 weeks and intraperitoneal injection of cadmium chloride (4 mg kg⁻¹ body weight) every other day for 1 week at the fourth week of treatment. The animals were given anaesthesia 24 h after the last

treatment and blood were collected from the heart into the non-heparinized tubes. The blood was left to stand at room temperature for 20 min after which the tubes were centrifuged at 6,000 xg for 1 min. The supernatants were transferred into new 15 mL conical tubes and re-centrifuged at 4,000 xg for 10 min. The serum was transferred into a new tubes and store at -20°C for further use. The animals were afterward sacrificed by decapitation and the livers were dissected and store in eppendorf tubes at -80°C.

Enzymes assay: The activity of serum GPT was determined as described by Reitman and Frankel (1957). GPT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitro phenylhydrazine at 546 nm. Serum GOT activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546 nm according to the method Reitman and Frankel (1957). The activity of serum GGT was determined as described by Szaz (1976) and (1974). GGT activity was measured by monitoring the increase in absorbance of p-nitroaniline formed by the reaction between L-Gamma-g-Glutamyl-p-Nitroanilide and glycylglycine at 405 nm. The activity of serum ALP was determined as described by Roy (1970). GST activity in the liver was assessed spectrophotometrically according to the method of Habig *et al.* (1974). The method was based on the conjugation of 1-chloro-2 4-dinitrobenzene (CDNB) with reduced Glutathione (GSH) in a reaction catalyzed by GST. Increase in absorbance was monitored for 3 min at 30 sec intervals at wavelength of 340 nm. Results were expressed as $\mu\text{mol}/\text{min}/\text{mL}/\text{mg}$ protein.

Assay for tissue glutathione: Reduced glutathione was assayed in the liver homogenate using Ellman's reagent according to the method of Jollow *et al.* (1974). The method was based on the formation of 5-Thio-2-Nitrobenzoic Acid (TNB) (a stable yellow coloured compound) in a reaction between Ellman reagent (5, 5'-dithiobis (-2-nitrobenzoic acid) (DTNB) and GSH. The amount of GSH in the sample was extrapolated from a standard GSH curve and result was expressed as μg mL homogenate.

Lipid peroxidation assay: Thiobarbituric Acid Reactive Species (TBARS) assay (Varshney and Kale, 1990) was used to measure the level of Malondialdehyde (MDA) formed as a result of membrane lipid damage. The assay was based on the formation of a pink coloured complex in

a reaction between malondialdehyde and Thiobarbituric Acid (TBA). The complex formed has a characteristic absorbance at 532 nm. The amount of MDA formed was extrapolated from a standard MDA curve and result was expressed as nmole MDA mL⁻¹ homogenate.

Gel preparation and Western blot analysis: Western blot analysis was performed on the liver homogenate. Protein concentration of the homogenate was performed using Bradford (1976) method and normalization of the protein loading on the SDS-PAGE was done using GAPDH antibody. Image reader LAS 3000 was used to quantified protein expression and band intensities were quantified by image J software.

Statistical analysis: Results were analysed using the unpaired student's t-test (comparison within group). Statistical analysis was carried out using GraphPad Prism software.

RESULTS

Potato extract attenuates the toxic effects of Cd in rat liver: Liver was chosen as a model to study the toxic effect of Cd in rats because is the most target organ of Cd toxicity. Evaluation of serum enzymes in rats exposed only to 4 mg kg⁻¹ body weight Cd and rats pre-treated with 250 mg kg⁻¹ potato extract (ST) shows that there were significant reductions in the levels of all the serum enzymes in the presence of ST (Fig. 1). The results show that pre-treatment with 250 mg kg⁻¹ ST produced a significant reduction in the serum levels of GOT (Fig. 1a), GPT (Fig. 1b), θ GT (Fig. 1c) and ALP (Fig. 1d) by 2.19, 1.33, 2.18 and 1.82-fold, respectively. The presence of 250 mg kg⁻¹ ST alone also caused a significant reduction of 1.67, 1.26 and 1.53-fold in serum GOT, GPT and θ GT levels, respectively when compared with rats exposed to vehicles alone (Fig. 1a-c). These results show that ST attenuates the toxic effects of Cd in exposed rats and that the presence of ST can enhance the integrity, viability and functionality of liver.

Potato extract enhanced the reducing power of rat liver exposed to Cd: The ability of a cell or tissue to maintain its integrity is a function of the levels of the reducing agents present when compared to the level of the oxidants. The balance between these two determines the susceptibility of the cell or tissue to free radicals attack and oxidative stress. The results obtained from this study show that ST pre-treatment significantly increased the level of reduced glutathione, GSH (Fig. 2a) and activity of GST (Fig. 2b) by

6.29 and 2.3-fold, respectively in the liver when compared with rats given only intraperitoneal dose of 4 mg kg⁻¹ Cd. ST alone also increased the liver GSH levels (1.94-fold) and GST activity (1.7-fold) when compared to the control rats. These results clearly show that ST protects liver from Cd toxicity by enhancing the GSH levels and by increasing microsomal GST activity. These two parameters are involved in the protection of cells from drugs and xenobiotics attack.

Potato extract attenuates rat liver membrane damage caused by Cd exposure: In order to evaluate the protective influence of ST in liver exposed to Cd, Malondialdehyde (MDA) levels were monitored in the liver homogenate after intraperitoneal exposure to 4 mg kg⁻¹ Cd. The results show a significant reduction of 1.51-fold in the MDA levels of ST pre-treated rats when compared with rats exposed to only Cd (Fig. 3). No significant difference was seen between rats exposed only to ST and the control (Fig. 3). These data confirm an association between the levels of GSH, GST and lipid peroxidation in ST pre-treated rats when compared with rats exposed only to Cd.

Potato extract enhanced the expression of protective enzymes in the liver of rat exposed to Cd: In order to evaluate the role of Nrf2-dependent enzymes in the protection of liver against Cd toxicity in the presence and absence of ST western blots were carried out on the liver homogenate using antibodies to the previously well characterized Cd-inducible enzymes NAD (P) H:quinine oxidoreductase (NQO1) and heme oxygenase (HO-1) (He *et al.*, 2008). The results from the western blots show inductions in both enzymes in the presence of 250 mg kg⁻¹ ST, 4 mg kg⁻¹ Cd or both when compared with control (Fig. 4). However, the quantification of the bands by image J shows that ST has an addictive effect on Cd at inducing HO-1 (Fig. 4b) and NQO1 (Fig. 4c) expressions. These results show that the presence of ST enhanced the protective response of rat liver in the presence of Cd and this addictive response may account for the enhanced protection observed in ST pre-treated rats.

DISCUSSION

The protective effects of water soluble compounds present in potato against Cd toxicity were examined in this study. In agreement with previous studies we have shown that Cd-induced liver damage in exposed female rats (Lupo *et al.*, 1986) and that this may be due to depletion in cellular thiol (SH) levels (Muller, 1986).

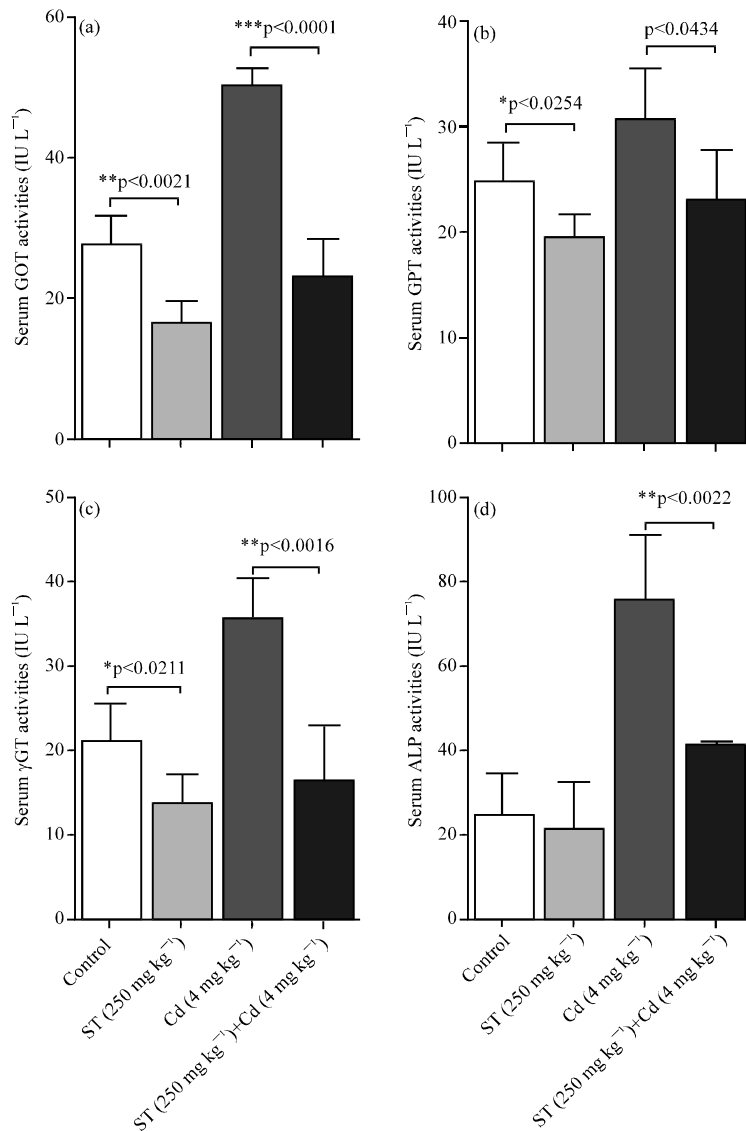


Fig. 1(a-d): Effects of potato (*Solanum tuberosum*, ST) pre-treatment on Cadmium (Cd)-induced liver damage. Wistar rats were treated with oral dose of 250 mg kg⁻¹ ST every other day for 3 weeks before intraperitoneal treatment with 4 mg kg⁻¹ Cd every other day for 1 week. After 24 h treatment with the last dose of Cd, the rats were euthanized and blood were drawn from the heart into an heparinised tubes and centrifuged. The activities of serum enzymes (a) GOT, (b) GPT, (c) γGT and (d) ALP were determined. Data represent the mean value (n = 10 rats, in triplicate) relative to control ±SD. Data were analyzed using unpaired student's t-test

Cd is a known hepatotoxic compound and the liver has been reported as the main target organ in Cd toxicity (Oh and Lim, 2006). The increase in serum enzymes levels is an indicator of hepatotoxicity and Cd has been reported to elevate these serum enzymes in female rats (Chapatwala *et al.*, 1982). The results from this present study was in agreement with these previous studies as Cd was seen to cause significant increase in the serum levels of these enzymes. Study has shown that potato peel

extract protects against carbon tetrachloride induced liver damage in rat resulting in reduced activities of serum enzymes, lower MDA levels and higher GSH levels (Singh *et al.*, 2008). The results from this work were in agreement with this previous study as the extract of potato prevents this Cd-induced increase in serum enzyme levels.

Cd has been shown in several studies to elevate ROS in cells (Liu *et al.*, 2009; Lawal and Ellis, 2010) and has

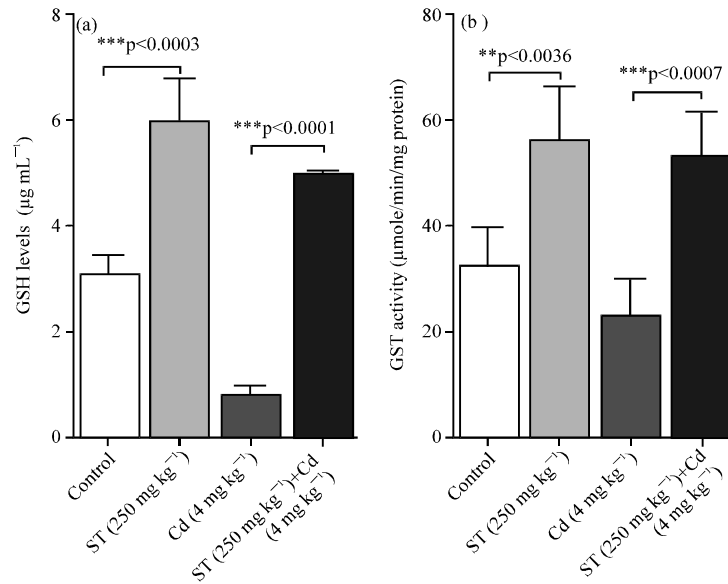


Fig. 2(a-b): Effects of Potato (*Solanum tuberosum*) pre-treatment on Cadmium (Cd)-induced alteration in redox status in exposed rat liver. Wistar rats were treated with oral dose of 250 mg kg⁻¹ ST every other day for 3 weeks before intraperitoneal treatment with 4 mg kg⁻¹ Cd every other day for 1 week. After 24 h treatment with the last dose of Cd, the rats were euthanized and liver was excised and homogenized in 0.1 M phosphate buffer (pH 7.4). (a) Tissue GSH level and (b) GST activity were determined as described in the Material and Method. Data represent the mean value (n = 10 rats, in triplicate) relative to control ±SD. Data were analyzed using unpaired student's t-test

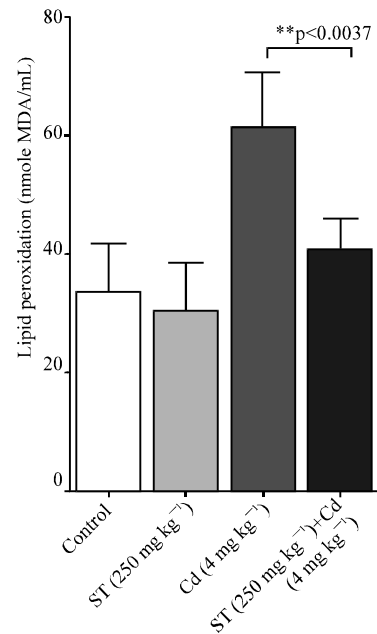


Fig. 3: Effects of potato (*Solanum tuberosum*) pre-treatment on Cadmium (Cd)-induced Lipid peroxidation in rat liver. Wistar rats were treated with oral dose of 250 mg kg⁻¹ ST every other day for 3 weeks before intraperitoneal treatment with 4 mg kg⁻¹ Cd every other day for 1 week. After 24 h treatment with the last dose of Cd, the rats were euthanized and liver was excised and homogenized in 0.1M phosphate buffer (pH 7.4). Membrane Lipid peroxidation was assessed by Malondiadehyde levels as described in the Materials and Methods. Data represent the mean value (n = 10 rats, in triplicate) relative to control ±SD. Data were analyzed using unpaired student's t-test

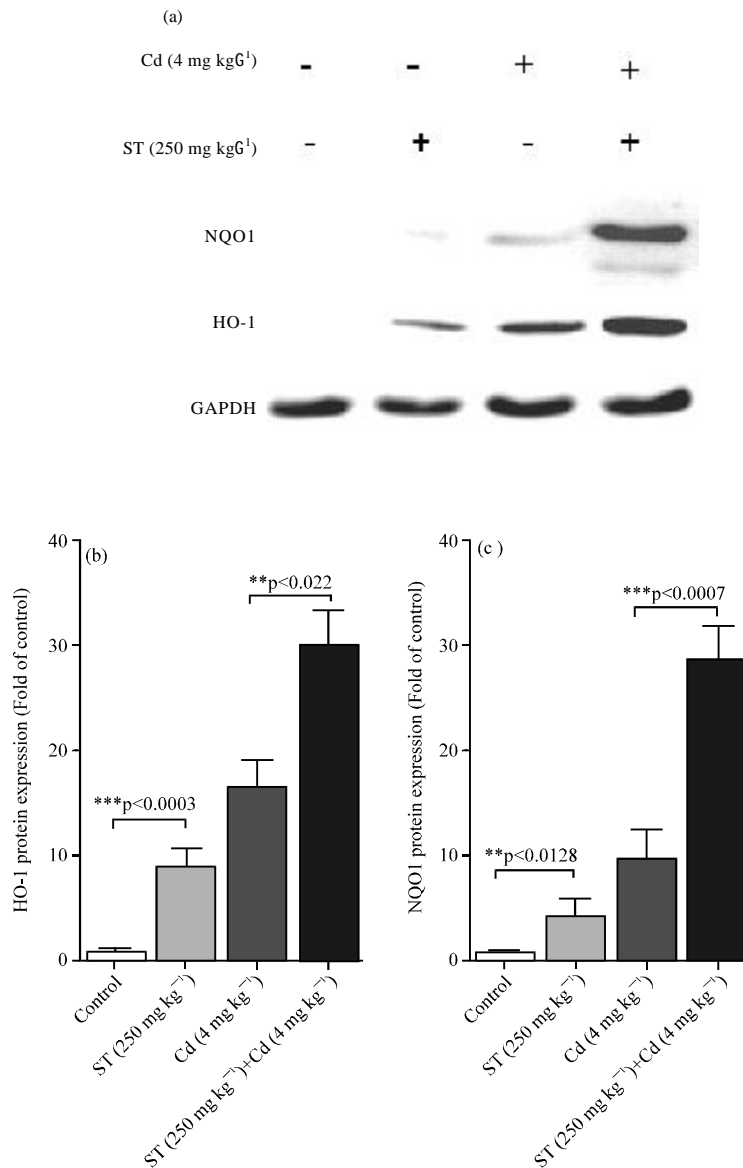


Fig. 4: Effects of Potato (*Solanum tuberosum*) pre-treatment on the expression of ARE-dependent cytoprotective enzymes after Cadmium (Cd) exposure. Wistar rats were treated with oral dose of 250 mg kg⁻¹ ST every other day for 3 weeks before intraperitoneal treatment with 4 mg kg⁻¹ Cd every other day for 1 week. After 24 h treatment with the last dose of Cd, the rats were euthanized and liver was excised and homogenized in 0.1M phosphate buffer (pH 7.4). (a) Homogenate were diluted in 2 lysis buffer as described in Materials and Methods and 20 µg of total protein was loaded on SDS-PAGE and transferred to nitrocellulose membranes by Western blotting and detected using specific HO1 and NQO1 antibodies. GAPDH was used as loading control. (b, c) values represent mean ±SD. Data were analyzed using unpaired student's t-test

also been shown to elevate ROS in rat liver cells (Liu *et al.*, 2009; Qu *et al.*, 2005) leading to decrease GSH/GSSG ratio. This present study was in agreement with these previous studies as decrease GSH level was

observed in the presence of Cd. It is possible that the ROS-mediated oxidation of cysteines in GSH may be responsible for the decrease in GSH levels observed in the presence of Cd in this study. Also Cd is known to bind to

thiol (SH) groups directly thereby modifying the GSH molecules (Irato *et al.*, 2001) resulting in enhanced oxidative stress.

Several studies using animal model have shown that the use of phytochemicals from plants extracts were protective against the oxidative stress induced by many toxic agents mostly by modulating the GSH and GST levels (Shanmugarajan *et al.*, 2008; Nandave *et al.*, 2007; Amin, 2008; Sarhan *et al.*, 2007). GST catalysis the reaction between the thiol (SH) group of GSH and potential alkylating agents, such as Cd, thereby neutralizing the electrophilic sites and rendering them more water soluble. This enzyme is therefore a major component of the GSH redox cycle. The activity of this enzyme is a crucial factor in determining the sensitivity of cells to a broad range of toxic chemicals. The present study was in agreement with these previous studies as the presence of potato extract elevates GSH and GST levels in the presence and absence of Cd and this may be responsible for the protective effect of the extract against Cd toxicity.

Lipid peroxidation is a free radical mediated chain reaction which can be initiated by hydroxyl radical and attack polyunsaturated fatty acids in membranes resulting in oxidative damage. It has been shown that Cd interacts with cell membranes resulting in lipid peroxidation (Stacey *et al.*, 1980). Gaurav *et al.* (2010) also show that Cd elevates lipid peroxidation in rat liver exposed to chronic Cd toxicity and this was attributed to decrease antioxidant activities. Previous studies have shown increase malondialdehyde levels in tissues of animals exposed to toxic agents and this effect was attenuated by the use of various plants extracts (Guldur *et al.*, 2010; Al-Rejaie, 2009; Nur Azlina *et al.*, 2009; Mtgapor and Fazlina, 2006; Iyawe *et al.*, 2006). The results obtained from this present study correlates with these previous findings. This study shows that pre-treatment with potato extract attenuates MDA levels both in the presence and absence of Cd. This reduction in MDA levels may be as a result of increase GSH and GST activity in the presence of the extract.

Nrf2 is a transcription factor that regulates the expression of antioxidant enzymes such as heme oxygenase 1 (HO-1) and NAD(P)H: Quinone oxidoreductase 1 (NQO1) and plays a vital role in protection against oxidative stress. The induction in the expressions of the antioxidant enzymes observed in this work may be an adaptive response to Cd toxicity and this response was boosted additively in the presence of the extract. This work was in agreement with previous studies that show increase in Nrf2 and Nrf2-dependent enzymes

in both *in vitro* and *in vivo* models after Cd exposure (Lawal and Ellis, 2011; Alam *et al.*, 1999; He *et al.*, 2008). The extract may contains phytochemicals that may serve as signal for the translocation of Nrf2 from the cytosol into the nucleus for the transcription of the antioxidant enzymes.

In conclusion, we have been able to establish the hepatotoxicity of Cd in female rat liver and also we have been able to show that the aqueous extract of sweet potato may protect against this toxicity by several mechanisms involving the elevation of GSH and enhancement of antioxidant enzymes expressions.

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