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Antioxidant effect of sodium selenite on thioacetamide-induced renal toxicity

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Abstract: Thioacetamide is an organosulfur compound and reasonably anticipated to be a human carcinogenic and toxic effect. Sodium selenite is considered to be an anticarcinogenic and antioxidant, therefore, the aim of study is to evaluate the toxic effect of thioacetamide and the role of sodium selenite on renal tissues by using rat model. 24 Albino wistar rats of male sex (b.w: 200±30gm) were divided into four groups (n = 6). Group I control (un treated), Group II received thioacetamide (200mg/kg b.w; i.p.) for 12 weeks; twice in a week, Group III received sodium selenite (1mg/kg b.w; i.p.) for 12 week; thrice in a week and Group IV received sodium selenite (1mg/kg: thrice in a week)+thioacetamide (200mg/kg: twice in a week) for 12 weeks (sodium selenite was given 30 minute prior to thioacetamide administration). Thioacetamide mediated toxic effects are measured in terms of renal functions; creatinine and urea, oxidative stress; MDA and antioxidant status; SOD, GSH and catalase levels. The significant increase in urea level and decrease GSH and MDA level in thioacetamide treated group was observed. Sodium selenite treatment restored the glutathione level in thioacetamide+sodium selenite treated group. The results describe decreased MDA levels which show the toxic effect of thioacetamide on renal tissues while the sodium selenite did not over come this toxic effect but explain its antioxidant property by increasing the GSH level in renal tissues.

Keywords: Antioxidant, sodium selenite, thioacetamide, renal toxicity.
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INTRODUCTION

Thioacetamide is an organosulphur compound that is known to be a carcinogenic[1]. Toxicity of thioamide in mammals and *Mycobacterium* spp. is dependent on metabolic activation of the compounds via sequential oxygenations of the thioamide sulfur atom by flavoprotein mono-oxygenases or cytochromes P450[2]. Free radical formation is postulated with various compounds including thioacetamide[3]. Aerobic forms of life have enzymatic antioxidant defenses; superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase. These enzymatic antioxidants operate in tandem to decompose free radicals. SOD is the known enzyme whose substrate is only free radicals[4] unlike GSH which acts on peroxides from various sources[5].

There is a balance between ROS production and its scavenger system within the cells to prevent or minimize free radicals damage, including enzymatic and non-enzymatic mechanisms. Selenium is an antioxidant and a trace element, which is incorporated into the catalytic site of antioxidant enzymes, such as GSH, and is involved in cell growth and development by protecting cells against the toxic and damaging effects of ROS[6-9].

The biological importance of selenium is at least 3-fold. First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5'-deiodinase, and thioredoxin reductase[10]. Second, sodium selenite is protective against a number of toxicants' Third, selenium excessive intake cause toxic potential[11]. Sodium selenite as an exogenous source of selenium is used for endogenous selenoprotein synthesis to scavenge the free radicals[12]. Because selenium is an essential element, sodium selenite is an ingredient in some food supplements[13]. The mode of action of selenium is unknown but may involve antioxidant defense mechanisms[14].

The kidney is highly susceptible to toxicants for two reasons. A high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones[15].

The aim of present study is to evaluate the protective role of sodium selenite against the thioacetamide induced renal and oxidative stress in rats using an experimental rat model, the present study describes plasma nitrate and renal tissue MDA levels, renal tissue enzymes SOD and catalase and plasma urea and creatinine in order to analyze antioxidant status in thioacetamide treated rats after pretreatment with sodium selenite.

MATERIALS AND METHODS

Animals and diet

Albino wistar rats of male sex (200±30 g b.w.), purchased from the animal house of ICCBS, (Karachi, Pakistan) were used for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room (23±4°C). Rats had free access to water and standard rat diet.

Study design

The animals were divided into four experimental groups; each consisting of six rats and received following treatments:

Group I: Control group, untreated.

Group II: Received thioacetamide (200mg/kg b.w; i.p) for 12 weeks; twice in a week.

Group III: Received sodium selenite (1mg/kg b.w; i.p.) for 12 weeks; thrice in a week.

Group IV: Received thioacetamide+sodium selenite.

Sample collection

After 48 hours of last dose of treated groups, animals were decapitated and blood was sampled from head wound in the lithium heparin coated tubes. A portion of blood was used to collect plasma. Kidneys were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer -70 ^{o}C until analyses.

Preparation of kidney homogenate

Kidney homogenates were obtained by using a tissue homogenizer, ultra taurax T-25 polytron, at 4ºC. The homogenates (1:10 w/v) were prepared by using a 100m mol KCl buffer (pH: 7) containing EDTA 0.3 m mol. All homogenates were centrifuge at 600 g for 60 minutes at 4 ºC and the supernatant marked as "sample" and used for biochemical assays.

Assessment of renal functions

Plasma samples were assayed for urea & creatinine. Urea was estimated by the monoxime method[16]. Creatinine was estimated by the Jeff's method[17].

Assessment of oxidative stress

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by Ohkawa et al., 1979 by using tissue homogenates[18].

Assessment of antioxidant status

Levels of SOD in the cell free supernatant were measured by the previously described method[19].GSH level were measured by the method described earlier[20]. Catalase activity was assayed by the method of [21].

Statistical analysis

Results are expressed as mean±SD. Statistical significance and difference from control and test values evaluated by Student's t-test. Statistical probability of *P<0.05, **P<0.01, ***P<0.001 were considered to be significant. +P< 0.005, ++P<0.01, +++P<0.001 were significant in comparison of thioacetamide (alone) group with thioacetamide and sodium selenite (combination) group. ns represent non significant result.

RESULTS

Body weight was significantly decreased in thioacetamide group (215.33±53.12, P<0.01) as compare to control. Treatment with sodium selenite increases the body weight nonsignificantly (246.125±53.12), while increased significantly in sodium selenite+thioacetamide treated group (366.33±14.57) compared with control (P<0.01) and with thioacetamide group (P<0.01) (Figure 1). Urea levels in thioacetamide and sodium selenite treated groups significantly increased (P<0.05 and P<0.001) respectively and increased nonsignificantly in sodium selenite+thioacetamide treated group (P<0.05) as compared to control. While nonsignificantly decreased in sodium selenite+ thioacetamide treated group as compared with thioacetamide group (Figure 2a). Creatinine levels increased nonsignificantly in thioacetamide treated group. While levels of creatinine decreased in sodium selenite and sodium selenite+thioacetamide treated group (P<0.05) as compared to control (Figure 2b).

Table 1: Effect on body weight, kidney weight & relative kidney weight in control, TAA, SS and SS+TAA group.

Parameters (g)	Control	Thio-acetamide (TAA)	Sodium Selenite (SS)	SS+TAA
Kidney Weight	0.294±0.043	0.302±0.046	0.404±0.024	0.441±0.069
Relative Kidney Weight10^{-3}	1.29±0.184	1.401±0.222	2.013±0.712	1.203±0.183

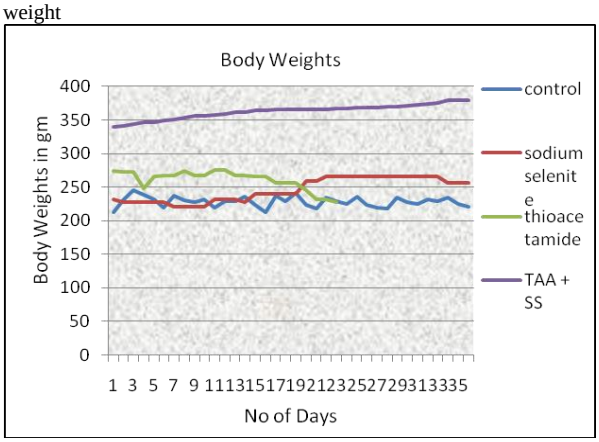
Values are mean±SD.

Significant difference between control, thioacetamide, sodium selenite and sodium selenite+thioacetamide treated rats was *p<0.05.

MDA levels in thioacetamide, sodium selenite and thioacetamide+sodium selenite treated groups were significantly decreased (P<0.05) as compared to control (Figure 3).

SOD levels significantly decreased in sodium selenite (P<0.001) and sodium selenite+thioacetamide treated group (P<0.01) as compared to control. The SOD levels significantly decreased in sodium selenite+thioacetamide treated group (P<0.01) as compared to thioacetamide group (Figure 4a). Significantly decreased GSH levels were observed in thioacetamide treated group (P<0.01). GSH significantly increased in sodium selenite treated group (P<0.01) as compared to control. The GSH level significantly increased in sodium selenite+thioacetamide treated group (P<0.05) as compared to Thioacetamide group (Figure 4b).

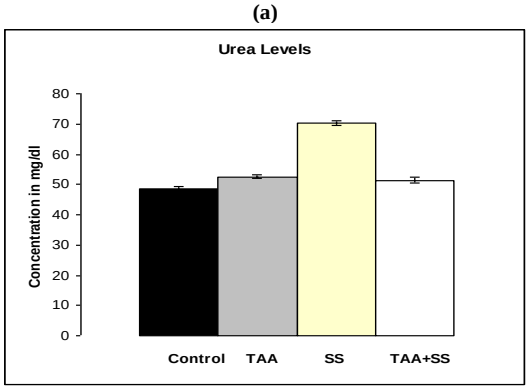
Figure 1: Effect of sodium selenite and thioacetamide on body weight



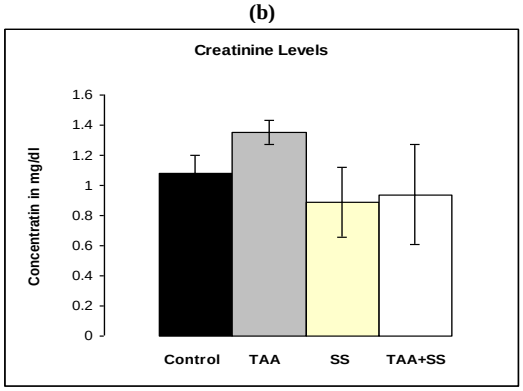
Values are mean±SD.
Significant difference between control, thioacetamide, sodium selenite and sodium selenite+thioacetamide treated rats was *P<0.05 and ***P<0.001.

Figure 2: Effect of sodium selenite and thioacetamide on renal functions (a) urea (b) creatinine

(a)



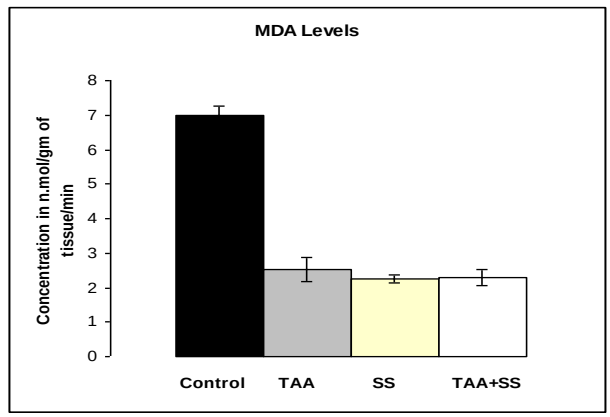
(b)



Values are mean±SD.
Significant difference between control, thioacetamide, sodium selenite and sodium selenite+thioacetamide treated rats was *P<0.05 and ***P<0.001.

Catalase activity was increased nonsignificantly in thioacetamide treated group as compared to control while significantly decreased in sodium selenite and sodium selenite+thioacetamide treated group (P<0.05). The sodium selenite+thioacetamide group were also decreased catalase activity (P<0.05) when compare with thioacetamide treated group (Figure 4c).

Figure 3: Effect of sodium selenite and thioacetamide on oxidative stress (MDA).



Values are mean±SD.
Significant difference between control, thioacetamide, sodium selenite and sodium selenite+thioacetamide treated rats was **p<0.01.

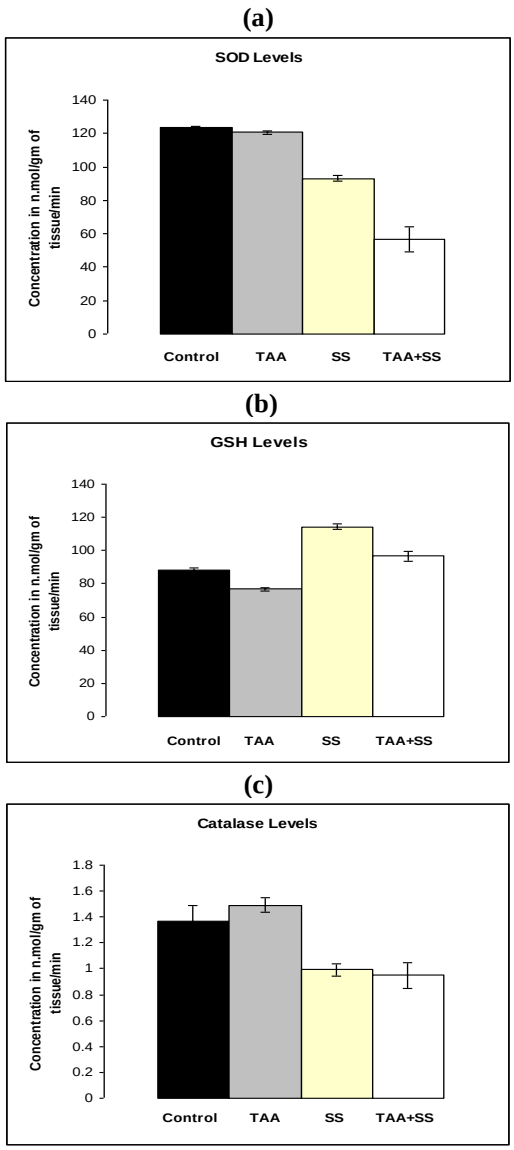
DISSCUSSION

Thioacetamide is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. Thioacetamide given orally to rats produces centrolobular hepatic necrosis and also causes death of the cells in the terminal portion of the proximal renal tubule[1].

In the present study TAA administration (200mg/kg) to rats for 12 weeks has been observed to cause renal toxicity as assessed biochemical parameters these were renal function (urea and creatinine), oxidative stress (MDA) and antioxidant status (SOD, GSH and Catalase). Free radicals are believed to play a major role in the development of TAA-induced toxicity[22]. Exacerbation of lipid peroxidation and depletion of antioxidants have been reported by various investigators[23,24]. Indeed in the present study the prooxidant–antioxidant balance has been observed to change of oxidation, as also found in the previous study[25]. TAA administration results in increased urea and creatinine levels (Figure: 4a and b). The increased levels of urea and cretinine show insufficiency of renal function. Studies in animals have established that tubular injury plays a central role in the reduction of glomerular filtration rate in acute tubular necrosis. Two major tubular abnormalities could be involved in the decrease in glomerular function in TAA treated rats: obstruction and back leak of glomerular filtrate. The alterations

in glomerular function in TAA treated rats may also be secondary to ROS (reactive oxygen species) as described earlier[26] which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate[27].

Figure 4: Effect of sodium selenite and thioacetamide on antioxidant status: (a) SOD (b) GSH and (c) Catalase Levels.



Values are mean±SD.
Significant difference between control, thioacetamide, sodium selenite and sodium selenite+thioacetamide treated rats was *p<0.05.

Decreased antioxidant enzymes levels (SOD, GSH) were observed in the present study after thioacetamide administration (Figure: 4a and b). Decreased SOD level (p<0.001) may indicate an imbalance in free radical levels and hence increase in cellular damage. There is an imbalance between the amount of free radicals generated and the antioxidant present in the cell in favor of the oxidation. As the toxicity produce SOD was not synthesized by cells hence, present in low amount[28]. The significant decrease of GSH level observed in thioacetamide treated group (p<0.01). GSH is a scavenger of hydroxyl radicals and singlet oxygen. It is capable of either directly scavenging free radicals or enzymatically via glutathione peroxidase. In addition, GSH is crucial to the maintenance of enzymes and other cellular components in a reduced state[29]. Decreased level of GSH during the present study indicates that toxic effect is produced by thioacetamide in renal tissues.

There is increased in catalase activity in thioacetamide treated group as compared to control (p<0.05) (Figure 4c) which indicates increased antioxidant status. Catalase and glutathione peroxidase seek out hydrogen peroxide and convert it to water and diatomic oxygen. An increase in the production of SOD without a subsequent elevation of catalase leads to the accumulation of hydrogen peroxide, which gets converted into the hydroxyl radical. Low level of the activity of total antioxidant status, SOD and GSH indicate the distortion of oxidant-antioxidant balance and decreased organism antioxidant system efficiency[29].

Although the exact mechanism of thioacetamide induced nephrotoxicity is not well understood, several studies suggested the involvement of free radicals. Oxidative stress develops when the disturbances between reactive oxygen forms are produced in excess and the factors preventing their harmful effect occur[30].

Thioacetamide induced nephrotoxicity is associated with decrease level of MDA (p<0.01) (Figure 4). MDA is the end product of ω_3 and ω_6 polyunsaturated fatty acids produced by decomposition[31]. Due to thioacetamide administration, thioamide S,S-dioxides formed via sequential oxygenations of the thioamide sulfur atom by flavoprotein monooygenases or cytochrome P450[32], which have not been isolated or further oxidized species exert the observed toxic effects. This activity results in elimination of the thioamide sulfur and formation of nitrile or amide derivatives[33], by renal cells and stabilized by intracellular GSH for several hours. In case of intracellular GSH depletion the complexes undergo the rapid transformation to receive metabolites. This depletion seems to be the prime factor that permits lipid peroxidation and impair antioxidant enzymes[27].

Nephroprotection by the exogenous selenite might be directly related to its antioxidant activity.

Selenite, an important exogenous source for endogenous selenium compounds, is known to be extensively biotransformed[34]. It undergoes glutathione (GSH)-dependent reductions to form, consecutively, GS-Se-SG, GS-SeH, and hydrogen selenide (HSeH). The last is methylated sequentially, resulting in production of methylselenol (CH_3SeH), then the volatile and expirable dimethyl selenide (CH_3SeCH_3), and finally trimethylselenonium ion [$(CH_3)_3Se^+$], which is excreted in urine. Selenide is also the precursor for selenocysteine and thus is needed for synthesis of selenoproteins. Additionally, selenide is also a toxic metabolite of selenite[27], whereas methylselenol is thought to be involved in both toxicity and anticarcinogenic activity[31] of selenite and it proved by increased urea levels and decreased creatinine levels after administration of sodium selenite (1mg/kg) in present study.

In present study, decreased SOD and catalase levels observed in sodium selenite treated group. Which represent that SOD and Catalase act as a cellular defense element against potentially harmful effects of superoxide ions by catalyzing the dismutation of these ions[28].

Our results demonstrate that counter effect of sodium selenite not overcome the thioacetamide - induce renal toxicity but showed no significant result with MDA. Elevated levels of GSH in this study exhibited the antioxidant property of sodium selenite[35].

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