

Tamsulosin-Induced Hepatotoxicity and Nephrotoxicity and Its Prevention by Potato Peel Extract

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Abstract: Tamsulosin, widely used in the symptomatic treatment of benign prostatic hyperplasia (BPH). The aim of this study was to investigate the toxicity of this drug by measuring its ability to induce reactive oxygen species (ROS) formation by measuring rate of NO in the serum and rate of ROS in the liver and kidney tissues, followed by damaging them by measuring the activities of serum enzymes a biomarkers which reveal the rate of liver cells damage, creatinine and urea nitrogen in the serum showing the rate of nephrotoxicity. antioxidant enzymes activities.

Key words: Tamsulosin • Potato peel extract • Reactive oxygen species • Antioxidant enzymes

INTRODUCTION

Tamsulosin, TAM, 5-[2-[2-(2-ethoxyphenoxy) ethyl]-amino]propyl]-2-methoxy benzenesulfonamide-HCl, has been reported to be an extremely potent and highly selective antagonist of α_1 -adrenoceptors, widely used in the symptomatic treatment of benign prostatic hyperplasia (BPH) [1]. It is marketed under the trade Omnic, Omnic Ocas, Curcard or Cadura α_1 -adrenoceptors can be divided into at least two pharmacologically distinct subtypes, α_{1A} - and α_{1B} -adrenoceptors. α_{1A} adrenoceptors may be coupled to the acceleration of the hydrolysis of phosphoinositide and/ or to L- type Ca^{2+} channels. The α_{1B} adrenoceptors are predominantly coupled to the acceleration of the hydrolysis of phosphoinositide and they irreversibly blocked by chlorethylclonidine, an alkylation agent [2]. In receptor-binding assays, TAM has been shown to have high affinity for α_{1A} adrenoceptor [3], the types are known to exist in myocardial cells [4].

Scientists found that using α -blocker therapy is based on the observation that prostatic smooth muscle contraction is the result of α -receptor-mediated sympathetic stimulation [5]. Administration of α -receptor antagonists results in relaxation of prostatic smooth muscles and improves prostate enlargement [6].

TAM can be enzymatically bioactivated to a reactive intermediate leading to increased formation of reactive oxygen species (ROS) [7], which can oxidize lipid, protein and DNA [8]. Oxidative stress is strictly involved in the pathogenesis of many types of liver injuries, including drug-induced hepatotoxicity, but molecular mechanisms are not really defined [9]. There is a growing body of evidence suggesting that idiosyncratic drug-induced hepatotoxicity may be mediated, at least in part, by oxidative stress, characterized by enhanced levels of ROS [10].

Biochemical features of TAM hepatotoxicity are variable but generally include abnormal serum lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), glutamyl-oxaloacetic transaminase (SGOT) and glutamyl-pyruvic transaminase (SGPT) activities [11]. These parameters reflect damage to hepatocytes and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [12, 13]. TAM is taken up into hepatocytes by multi-specific transporters [14, 15] whereas, it may induce the production of potentially harmful free radicals and their reactive oxygen species [16]. These reactants interact with lipids producing malondialdehyde (MDA) which reacts with cell cytoskeleton proteins forming protein carbonyl contents (PCC) [12,17,18]. The toxic potential of TAM might be related to its ability to generate the hydroxyl radical ($\cdot OH$) [19].

Recently, a number of natural antioxidants have been evaluated for their chemopreventive effects in various pathological states [20-22]. Potato peel extract (PPE), considered as a waste food in all over the world, but it was found by others [23-26] that it has high rate of polyphenols and proved that it has high oxygen-radical scavenging and quenching capacities and are very beneficial to living tissues to reduce the risk of adverse reaction that produced by hydroxyl radicals.

The aim of this study was to investigate the toxicity of Tamsulosin-HCl (Omnice) which used world-wide in the treatment of prostate enlargement and the role of PPE in the protection against Tamsulosin-HCl-induced liver and kidney oxidative damage in Balb/c mice.

MATERIALS AND METHODS

Chemicals: Tamsulosin-HCl (Omnice-HCL, 0.4 mg) was obtained from local pharmacy. Fresh potato peels were obtained from local potato chips making market. All chemical used in this study were of analytical grade and purchased from Sigma Co, USA.

Preparation of Potato Peel Extract: Potato peel extract was prepared using Folin-Ciocalteu reagent according to the procedure described by Singh and Rajini [26].

Total Phenolic Compound Analysis: Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent according to the method recommended by Tezcan *et al.* [27].

Measurement of DPPH Radical Scavenging Capacity of PPE: The DPPH radical scavenging activity of PPE was determined according to the method reported by Stratil *et al.* [28].

Measurement of Reducing Power of PPE: The reducing power of the extract was quantified by the method described by Duthie and Crozier [29] using BHT as standard antioxidant.

Assessment of Toxicity: 60 Balb/c mice (about 7 weeks old and 28-30g weight each) were used in this study. Mice were obtained from the Animal House of Yarmouk University/ Jordan and kept on standard laboratory diet and tap water *ad libitum* throughout the experiments. They were assigned into four groups (15 mice each). The research was conducted in accordance with the internationally accepted guidelines for laboratory animals use and care. The experiments reported here were approved by Yarmouk University ethics committee.

First group of mice were a control (C) with mice that did not receive TAM or PPE supplementation (saline treated group) and sacrificed 30 days later; the second group received PPE supplementation (100mg/Kg day) orally for 30 days prior to sacrifice according to the method described by Singh *et al.* [23]. Group 3 of mice given TAM only (400 µg/kg daily) orally (dissolved in saline) for thirty days according to the method of Nickel *et al.* [1] then sacrificed. Group 4, mice were supplied with TAM (400 µg/kg / day) orally (dissolved in saline) and PPE (100mg/Kg day) orally for 30 days then sacrificed. All mice were sacrificed by cervical dislocation, livers and kidneys were removed immediately. Livers were perfused by Hanks buffer to remove access blood. Kidneys were excised, washed in ice-cold saline. Both tissues were homogenized separately in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) using Ultra homogenizer. Both homogenates were first centrifuged at 10,000xg for 15 min. The supernatant of each homogenate was centrifuged again at 100,000xg for 1h and the supernatants (cytosolic fraction) were recovered and the protein concentration in each sample was determined [7], aliquoted were used for the determination of enzymatic activities and lipid peroxidation as malondialdehyde (MDA) production from the thiobarbituric acid reaction in liver and kidney homogenates [20]. Blood was obtained by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10 min at 3000xg.

Activities of hepatic marker enzyme, lactate dehydrogenase (LDH) level, activities of glutamyl-oxaloacetic transaminase (SGOT) and glutamyl-pyruvic transaminase (SGPT) were determined according to the method recommended by Abdel-Zaher *et al.* [30]. Nitric oxide formation was measured in the serum by assaying nitrite according to the method described by Green *et al.* [31]. GGT activity was estimated based on the method reported before [20]. Creatinine and urea nitrogen levels (as a kidney function test) were estimated according to the method recommended by Knotek *et al.* [32].

Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels in hepatic and renal tissues by use of thiobarbituric acid reactive substances method as described by Hwang *et al.* [33] and 1,1,3,3-tetramethoxypropane was used to plot a standard reference curve. Protein carbonyl contents (PCC) were determined using a DNPH-based procedure reported by Burcham [34].

Antioxidant enzymes activities, superoxide dismutase (SOD), were determined using the method of NADH-phenazinemethosulphate-nitroblue tetrazolium formazon inhibition reaction [35]. Catalase (CAT) was assayed colorimetrically using dichromate-acetic acid reagent [36].

Gpx was assayed according to the method reported before [37] based on the reaction between glutathione remaining after the action of GPx and DTNB to form a complex that absorbs maximally at 412nm. Hepatic and renal GSH levels were estimated colorimetrically using Ellmans reagent as described by Bukowska [38].

Determination of ROS in Mice Liver and Kidney Cells:

By using oxidant sensitive probe dihydrorhodamine 123 (DHR), concentration of ROS was detected in both liver and kidney homogenates. As an oxidative sensitive indicator, DHR can be oxidized to the positive charged rhodamine 123 localized in mitochondria and therefore has been suggested to be effective probe to measure ROS production [39].

Statistical Analysis: Results were expressed as mean \pm S.E.M. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukeys test. Statistical significance was considered at $P \leq 0.05$. The statistical analysis was done using Sigma state statistical software version 3.5.

RESULTS

The present results revealed no significant alterations in the body weight among the various treated groups. MAT treated mice liver showed a slight increase in the body mass index ratio due to intra-hepatic hemorrhage and pooling in the liver, making the liver appear slightly darker in colour when compared with control group. Also, these results showed no any change in kidney weight or colour of MAT treated group.

The results of the Folin-Ciocalteu total polyphenolic assay of PPE were found to be 4.41 mg/ g powder.

Total DPPH scavenging potential of the PPE at varying concentrations was measured and the results are depicted in Table 1. The scavenging effect increased with increasing PPE concentration up to certain value (4.0 mg) then levelled off.

Table 1: Scavenging effect of PPE. Values are means \pm SD of three determinations

Concentration of PPE (mg)	Scavenging effect %
0.5	12
1.0	32
1.5	47
2.0	62
2.5	79
3.0	82
3.5	89
4.0	90
4.5	90

Table 2: Reducing power of PPE and BHT. Values are means \pm SD of three determinations

Mg	Absorbance at 700 nm	Mg	Absorbance at 700 nm
1.00	0.06	1.00	0.06
2.00	0.18	2.00	0.20
3.00	0.33	3.00	0.46
4.00	0.46	4.00	0.57
5.00	0.55	5.00	0.62

Table 2 shows the reducing power of both PPE and BHT (a known standard antioxidant).

Serum values of ALT, LDH, SGOT and SGPT were utilized to evaluate liver injury. TAM administration increased serum values of ALT (about 2.8 fold), LDH (about 2.1 fold), SGOT (about 3 fold) and SGPT (about 3 fold) compared with the control. Treatment of mice with PPE, significantly ($P \leq 0.05$) inhibits the rise of these enzymes in the serum induced by MAT as shown in Table 3. The results presented in Table 3, illustrate that nitrite levels increased into two times in the sera of mice treated with TAM only when compared with control and dropped to about the normal in group supplemented with TAM and PPE.

Mice group received MAT only showed high level of GGT in serum when compared with control (about 2.1 fold) and this level was diminished in the group treated with TAM and PPE as shown in Table 3, which improve that

Table 3: Effect of PPE supplementation (100 mg /kg/day for 30 days) on the serum parameters after received single orally dose of Tamsulosin (400 μ g/kg/day) for 30 days

Parameters	CON	PPE	TAM	TAM+ PPE
LDH (U/mg)	327.8 \pm 3.60	321.2 \pm 3.04	711.8 \pm 4.22	304.2 \pm 2.07
SGOT U/L	10.88 \pm 1.007	10.1 \pm 0.91	32.6 \pm 0.07	13.2 \pm 1.002
SGPT U/L	10.61 \pm 1.001	9.2 \pm 0.99	33.2 \pm 1.044	14.7 \pm 1.002
NO (μ mol/l)	4.4 \pm 0.22	4.2 \pm 0.21	7.88 \pm 0.42	4.0 \pm 0.27
GGT (U/mg)	19.8 \pm 2.01	19.0 \pm 2.01	40.4 \pm 4.04	27.3 \pm 2.88
Creatinine mg/dl	0.44 \pm 0.003	0.34 \pm 0.002	1.21 \pm 0.007	0.66 \pm 0.008
Urea nitrogen mg/dl	30.1 \pm 2.7	30.0 \pm 2.1	84.8 \pm 8.1	38.7 \pm 3.8

Table 4: Effect of PPE (100mg/kg/day) on the levels of LPO (MDA), antioxidant enzymes, GSH, Protein carbonyl contents and rate of ROS production in liver and kidney tissues

Parameters	Liver				Kidney			
	CON	PPE	TAM	TAM+PPE	CON	PPE	TAM	TAM+PPE
MDA (μM)	0.067 \pm 0.01	0.055 \pm 0.01	1.01 \pm 0.21	0.22 \pm 0.11	0.058 \pm 0.02	0.051 \pm 0.01	0.76 \pm 0.01	0.12 \pm 0.01
SOD (U/mg)	48.8 \pm 1.7	48.1 \pm 3.3	36.6 \pm 2.7	44.4 \pm 3.4	42.2 \pm 2.5	42.7 \pm 3.1	39.3 \pm 2.6	41.6 \pm 2.4
CAT U/min/mg)	28.7 \pm 2.2	30.8 \pm 2.8	21.4 \pm 2.1	24.8 \pm 3.3	26.1 \pm 2.1	26.7 \pm 2.1	20 \pm 1.8	24.6 \pm 1.9
Gpx ($\mu\text{mol}/\text{min}/\text{mg}$)	1.9 \pm 0.01	2.1 \pm 0.01	0.9 \pm 0.01	1.4 \pm 0.02	1.7 \pm 0.01	1.8 \pm 0.01	0.7 \pm 0.01	1.2 \pm 0.01
GSH (nmol/mg/protein)	4.9 \pm 0.6	5.2 \pm 0.3	3.1 \pm 0.2	4.4 \pm 0.2	4.1 \pm 0.3	4.7 \pm 0.4	3.3 \pm 0.2	3.9 \pm 0.2
PCC (nmols/mg/protein)	0.66 \pm 0.06	0.61 \pm 0.08	1.01 \pm 0.04	0.82 \pm 0.01	0.61 \pm 0.06	0.60 \pm 0.05	0.80 \pm 0.01	0.71 \pm 0.03
ROS % of control	100	88	143	122	100	71	131	118

PPE inhibit the increase of GGT in the serum. A significant ($P \leq 0.05$) increase in serum creatinine and urea nitrogen levels were observed in mice treated with TAM only and the elevation was inhibited in group supplemented with PPE (Table 3).

Antioxidant enzymes (SOD, CAT and Gpx) activities were decreased significantly in mice treated with TAM only, while they were about the normal in group of mice supplemented PPE with TAM (Table 4). Administration of TAM alone diminished hepatic and kidney cells GSH by 12% and 9% respectively. These values were attenuated by using PPE which protected GSH depletion induced by TAM.

MDA is a product of oxidative damage to lipids and in this study the concentration of MDA in liver and kidney homogenates is considered as a biomarker of TAM toxicity. Results in Table 4, show that homogenates of liver and kidney of mice exposed to TAM contained higher levels of MDA (about 6 fold increase in both tissues) when compared with control values. These levels decreased significantly ($P \leq 0.05$) in liver and kidney homogenates of mice supplemented with PPE. This table also, shows the increment of the level of protein carbonyl contents by 2.2 and 2 fold in the liver and kidney homogenates of mice treated with TAM only respectively, while these values were decreased significantly ($P \leq 0.05$) in the homogenates of mice supplemented with TAM and PPE. The level of protein carbonyls was measured as an indicator of protein oxidative damage.

ROS is known to be an important element in the induction of tissue damage. They were detected spectrophotometrically using the fluorescent probe DCFH-DA, which readily diffuses through the cell membrane and hydrolyzed by intracellular esterases to non-fluorescent 2,7-dichlorofluorescein, which will rapidly oxidized to highly fluorescent 2,7-dichlorofluorescein in the presence of ROS. It was observed that PPE significantly reduced TAM-induced ROS levels from 138.3 (liver) and 122.7 (kidney) into 88.4 and 67.2 respectively.

DISCUSSION

Tamsulosin - HCl, 5-[2-[2-(2-ethoxyphenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide-HCl, is widely used in the symptomatic treatment of benign prostatic hyperplasia (BPH) [40, 41]. It has been reported that TAM is a selective α_{1A} -adrenoceptor antagonist on the basis of its ability to discriminate between α_{1A} - and α_{1B} -adrenoceptor recognition sites [42]. It was found that TAM is a superior to placebo in providing symptomatic relief of BPH [43]. Recently, it has received much attention concerning its toxicity [44]. In this investigation, a study of the biochemical profile of changes in certain important enzyme activities was undertaken to access the TAM toxicity in chosen tissues [45]. The level of serum LDH, SGOT and SGPT activities reflect damage to hepatocytes and indicates the increased cellular permeability [30] and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [14]. Also, the increment of creatinin and urea nitrogen in the serum reflects kidney proximal tubule lesions [46]. Our results have shown that PPE attenuate all these alterations.

Our results indicate that the hepatotoxicity and nephrotoxicity of TAM in Balb/c mice, increased the production of NO, which might play an important role in the pathogenesis of TAM -induced hepatic and renal damage [30]. This was correlated with expression of iNOS protein and nitration of tyrosine in these tissues [47].

It is of interest that production of NO by TAM toxicity was associated with a marked reduction in the SOD, CAT and GSHpx activities, intracellular GSH level and increment of Protein carbonyl contents and lipid peroxidation levels in liver and kidney. Our results is in accordance with the findings of others [7] whom using fluorescent probe and showed that toxins increased ROS formation. The major biological process leading to oxygen -derived generation of reactive species is the electrone transport system associated with mitochondrial

membranes [9]. However, it is not yet known whether the electron transport chain is the major source of the increased ROS level induced by TAM. As the results showed that PPE attenuate the formation of ROS might PPE acts as specific iron chelator which, by depletion of intracellular iron (Fe^{3+}/Fe^{2+}), prevents the generation of $\cdot OH$ through the Fenton reaction and is as such an effective inhibitor of oxidative damage [10].

The elevation of MDA levels, which is one of the end products of lipid peroxidation in the liver and kidney cells are indicators of oxidative stress in TAM treated mice. The recovery in LPO could be one of the protective mechanisms of PPE against the toxicity. As found by others [13] that the carbonyl contents of membrane-associated proteins were higher than that of hydrophilic proteins from the same tissue. This suggest that these proteins sustain greater adduction by membrane-derived lipid peroxidation. These alterations were by ameliorated by the antioxidant potency of PPE.

To our knowledge the potential protective effect of PPE on TAM-induced hepatotoxicity and nephrotoxicity has not yet been evaluated. Unfortunately, potato peel consider as a wast and then not used as a food. Earlier authors [9, 23, 24] and in this study have shown that extract derived from potato peel was rich in polyphenols and possess strong antioxidant activity both *in vitro* and *in vivo*. Our data in the reducing power of PPE suggest that it is likely to contribute significantly towards the observed antioxidant effect. The antioxidant activity of PPE discernible in the DPPH radical assay, which primarily evaluates proton radical-scavenging ability. DPPH is one of the compounds that possesses a proton free radical with a characteristic absorption which decreases significantly on exposure to proton radical scavengers [21]. Further, it is well accepted that the DPPH free radical-scavenging by antioxidants is due to their hydrogen-donating ability [9].

It was concluded from our results that Tamsulosin, widely used all over the world as drug has low toxicity rate and PPE which unfortunately consider as a food waste, comprises of reasonable level of polyphenols act as potent antioxidant against the toxicity of Tamsulosin.

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