Effect of toluene exposure on the antioxidant status and apoptotic pathway in organs of the rat

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ABSTRACT

The chronic abuse of the solvent toluene results in structural and functional impairment of various organs. However, the pathophysiological mechanisms that cause these impairments of function are not clearly understood. This study aims to assess the effect of chronic toluene exposure (15, 30 and 45 days) on the oxidative stress and antioxidant status of different organs in the rat. Also, cyclooxygenase-2 and caspase-3 activities (a marker of apoptosis) are studied. Forty male albino rats were used and divided into four groups: controls (group I) and three other groups receiving a single daily dose of toluene (650 mg/kg) for 15 days (group II), 30 days (group III) and 45 days (group IV). The animals were then sacrificed and the brain cortex, cerebellum, liver, kidney and testis were separated for the determination of thiobarbituric acid reactive substance (TBARS), GSH, glutathione disulphide (GSSG) and glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), cyclooxygenase-2 (COX-2) and caspase-3 activity. Results showed a significant and time-dependent increase in the levels of TBARS, GSSG and in GST, SOD, COX-2 and caspase-3 activity, while GSH, GR and GPx showed a marked decline in most tissues. The brain (cortex and cerebellum) was the most affected organ, showing the greatest increase in one apoptotic marker (caspase-3), while the testis and kidneys were least affected. In conclusion, oxidative stress and derangement of the GSH:GSSG ratio, induced chronic inflammatory change and apoptosis may play an essential role in toluene toxicity

KEY WORDS: Antioxidants.
Apoptosis.
Cyclooxygenases.
Oxidative stress.
Toluene.
Toxicity.

Introduction

Toluene is an organic solvent that is produced worldwide in large quantities for use in a variety of industrial and commercial applications. For example, it is used widely in paints, varnishes, inks, adhesives and plastics.[1] Toluene has a high potential for abuse, primarily through inhaling products such as glue or spray paints.[2,3] Also, it has addictive properties, so exposure to toluene-based organic solvents is an important public health problem.[4]

It is well-recognised that solvent abuse can result in sudden death and can produce pathological changes in the liver, kidney, brain, heart and lungs.[5,6] Moreover, its influence on neurons and the central nervous system has been reported.[7–10] Recently, it has been documented that brief repeated prenatal exposure to toluene causes growth restriction, malformation and impairment of behavioural development in rats.[3] In addition, the male reproductive system is affected.[11] However, the pathophysiological mechanisms responsible for impairment of organ function are not clearly understood.[1,12–14]

This study is designated to assess the effect of chronic toluene exposure (15, 30 and 45 days) on the oxidative stress and antioxidant status of different organs in the rat. In addition, cyclooxygenase-2 and caspase-3 activities (as a marker of apoptosis) are studied.

Materials and methods

The study was conducted on 40 adult male albino rats (100–140 g) kept in an air-conditioned room (23±2°C) under a 12-h light/12-h dark cycle. Food and tap water were available *ad libitum*. Rats were divided randomly into four groups of 10 rats, comprising a control group (group I) and three further groups each receiving a single daily dose of toluene (650 mg/kg) for 15 days (group II), 30 days (group III) and 45 days (group IV). This level of exposure aimed to produce blood toluene concentrations equivalent to those obtained by inhalation of toluene for 3h at > 4100 ppm.[15] The animals were then sacrificed and the liver, kidney, testis and brain (cortex and cerebellum) were separated.

The organs were washed in phosphate-buffered saline and homogenised in ice-cold 20 mmol/L Tris-HCl-buffered saline (pH 7.4) to produce a 1 in 10 (w/v) homogenate. Homogenates were centrifuged at 10,000 x*g* for 10 min at 4°C and the supernatant were used to determine the end products of lipid peroxidation by the thiobarbituric acid reactive substance (TBARS) assay.[16] In this assay, the malondialdehyde is heated with thiobarbituric acid (TBA) at low pH to produce a pink chromogen with a maximum absorbance at 532 nm.

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Glutathione and glutathione disulphide (GSSG) were assayed using the method of Griffith,[17] which depends on the oxidation of GSH by 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to yield GSSG and 5-thio-2-nitrobenzoic acid (TNB). Oxidised GSSG is reduced enzymatically by the action of glutathione reductase and NADPH to regenerate GSH. The rate of TNB formation is monitored at 412 nm and is proportional to the sum of GSH and GSSG present in the sample. The GSSG content is determined by the same assay used for total glutathione, but the reduced glutathione is bound by 2-vinylpyridine.

Glutathione reductase (GR) activity was assayed using the method of Smith *et al.*,[18] which is based on the reduction of DTNB by GSH to produce GSSG followed by the release of TNB monitored at 412 nm. Glutathione-S-transferase (GST) activity was determined by the method modified by Carmagnol *et al.*,[19] which is based on GST-catalysed conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), measured at 340 nm.

Glutathione peroxidase activity was determined by the method of Flohe and Gunzler.[20] This method is based on monitoring the generation of GSH from GSSG by the action of glutathione reductase in the presence of NADPH. Total GPx activity was measured using cumene hydroperoxide as the substrate.

Superoxide dismutase (SOD) activity was determined by the pyrogallol method of Marklund and Marklund.[21] This depends on the spontaneous autoxidation of pyrogallol at alkaline pH, resulting in the production of superoxide anion radicals ($O^{\cdot 2-}$). These radicals enhance the autoxidation of pyrogallol, which manifests itself by an increase in absorbance at 420 nm. The presence of SOD in the reaction leads to the removal of superoxide anion radicals and the inhibition of pyrogallol autoxidation.

Activity of the proinflammatory marker cyclooxygenase-2 was assayed by a kit obtained from the Cayman Chemical Company (Ann Arbor, Michigan, USA). It measures the peroxidase activity of cyclooxygenase. Peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidised N,N,N′,N′,-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.[22] Activity of the apoptosis marker caspase-3[23] was assayed using a substrate (Ac-DEVD) labelled with the chromophore *p*-nitroaniline (*p*NA), which is released from the substrate when cleaved by caspase-3. Free *p*NA produces a yellow colour that is monitored at 405 nm.

All data are presented as mean±SD. All analyses were carried out using the SPSS software. Student's *t* test and ANOVA were used to assess differences. $P<0.05$ was considered to be statistically significant.

Results

The results of the study of lipid peroxidation end products, measured as TBARS, are summarised in Figure 1, which shows a time-dependent increase in all the organs following toluene exposure.

Reduced glutathione in the liver, kidney, testis and cerebellum showed a time-dependent decline following toluene exposure, with the lowest levels observed after 45 days (Fig. 2). However, depletion of GSH in the brain cortex reached its lowest level after 15 days of toluene

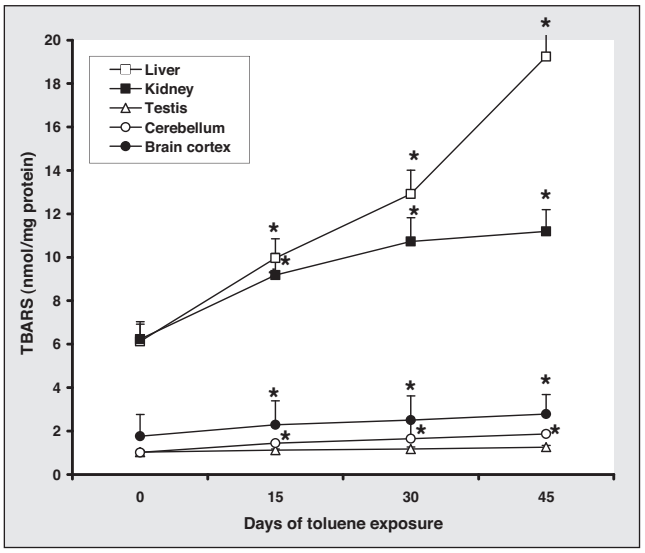

Fig. 1. Change in level of TBARS in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

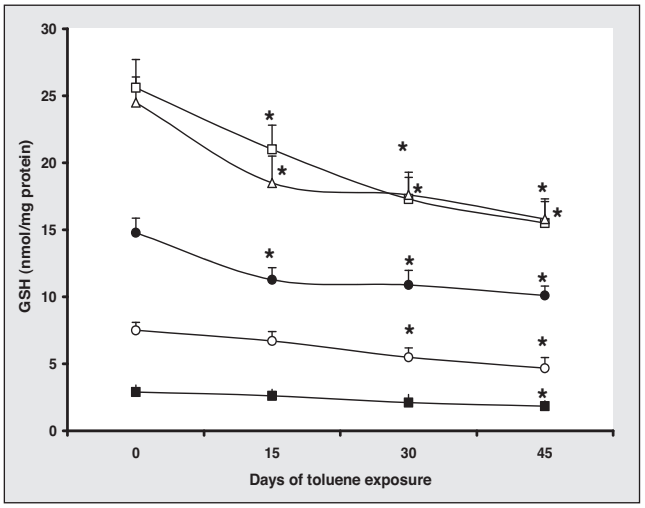

Fig. 2. Change in level of reduced glutathione in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

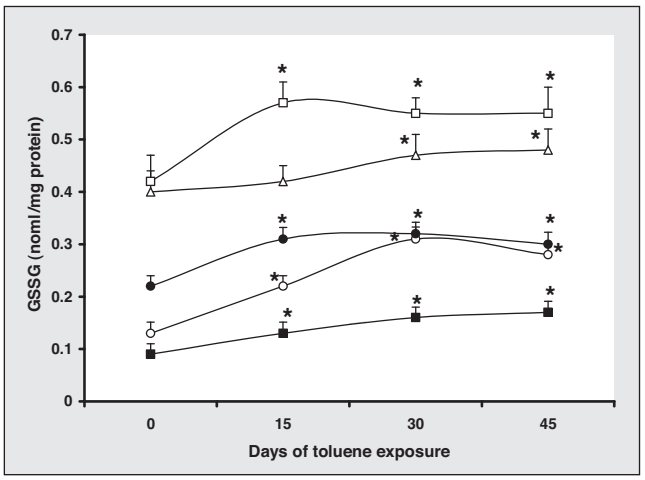

Fig. 3. Change in level of GSSG in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

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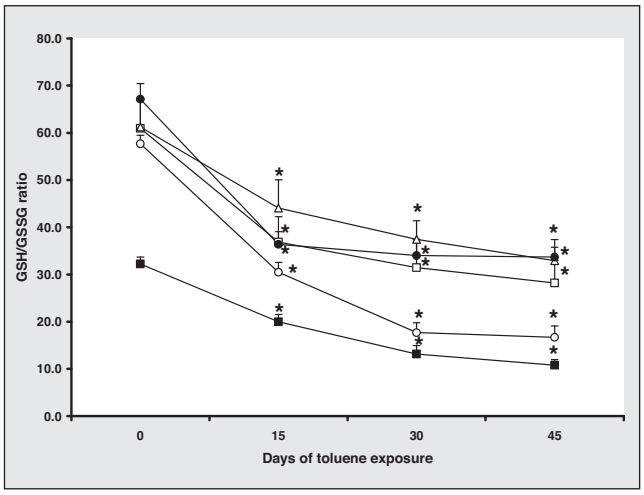

Fig. 4. Change in level of GSH:GSSG ratio in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

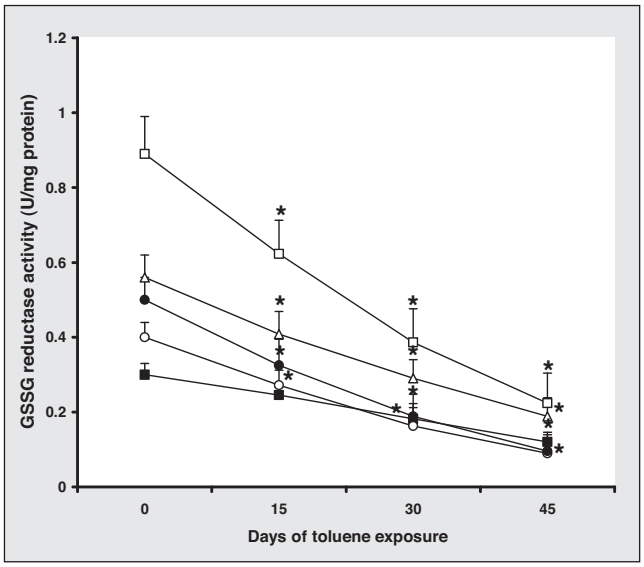

Fig. 5. Change in glutathione reductase activity in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

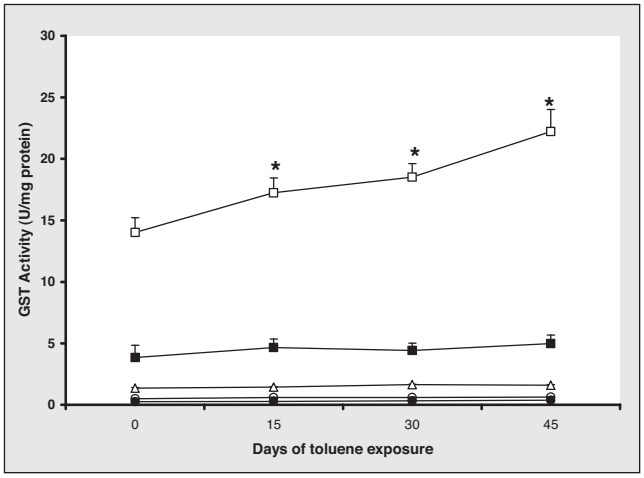

Fig. 6. Change in GST activity in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

exposure, and no further change was seen thereafter. In contrast to GSH, GSSG levels showed a time-course increase in all the organs studied (Fig. 3).

The redox state, as measured by the GSH:GSSG ratio, showed severe derangement following toluene exposure (Fig 4), with a time-dependent decline (i.e., shift towards an oxidising state) following toluene exposure in all organs.

Toluene exposure resulted in change to the activities of glutathione metabolising enzymes. Liver, brain cortex and cerebellum showed a prominent inhibition of GR activity. (Fig. 5), while GST activity showed a small gradual increase with duration of toluene exposure, especially in the liver (Fig. 6). The liver and kidney showed no significant changes in GPx activity following toluene exposure, while the testis, brain cortex and cerebellum showed only gradual inhibition (Fig. 7).

Activity of the antioxidant enzyme SOD showed no significant change in the liver, kidney and testis across the entire exposure period (Fig. 8). In contrast, brain cortex and cerebellum showed a significant, time-dependent elevation in SOD activity. Finally, with the exception of activity in the rat testis, COX-2 displayed no significant change following toluene exposure (Fig. 9).

The apoptotic marker caspase-3 activity was greatly increased in the brain cortex and cerebellum (Fig 10), particularly after 15 days' exposure to toluene. Liver was the only other tissue that showed an increase in caspase-3 activity, and this was apparent only after 45 days' exposure to toluene.

Discussion

There is evidence to suggest that organic solvents express their toxicity through ROS-induced cell damage.[7,8] The present study confirms the role of oxidative stress and an altered redox state in toluene-induced toxicity, as indicated by elevated lipid peroxidation, depletion of GSH, a derangement in antioxidant enzymes and an increase in apoptosis in different organs of the rat.

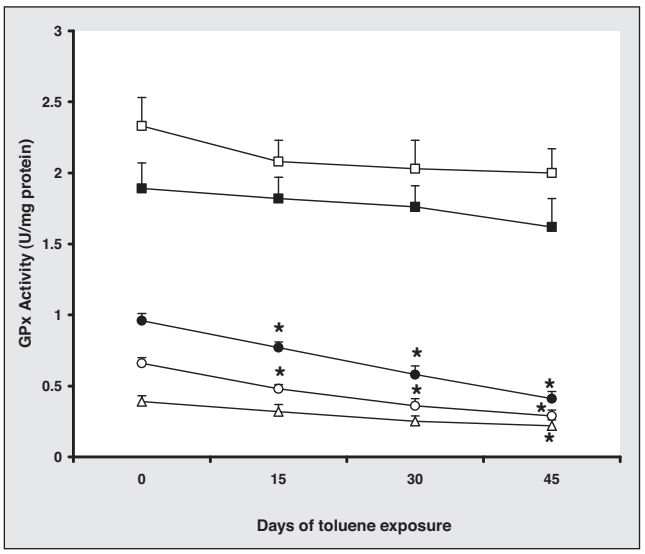

Fig. 7. Change in glutathione peroxidise activity in different organs of the rat in response to different durations of toluene exposure. *Significant difference from control values ($P<0.05$).

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It is well documented that *in vivo* and *in vitro* exposure to toluene leads to ROS formation in rat brain, liver, kidney and lung.[24] In line with the present results, it has been reported that toluene-containing thinners increase lipid peroxidation in different brain regions in the rat.[7] A human study also showed increased malondialdehyde levels in the serum of people working with paint thinner.[25]

Considerable evidence has accumulated to show that agents which alter GSH concentration affect the activity and transcription of detoxification enzymes, cell proliferation and apoptosis.[26,27] In principle, GSH, GSSG or the redox state of the GSH:GSSG couple provide a mechanistic control or signal for these phenotypic changes. In line with this, the present study clearly shows that toluene exposure resulted in time-dependent depletion of GSH and elevation of GSSG in various rat organs. These changes are associated with increased GST activity, especially in the liver and brain.

The derangement in redox state of the GSH:GSSG couple resulted in a shift towards an oxidising state, which drives the cells along an apoptotic pathway. In the present study, the highest rate of apoptosis, as indicated by caspase-3 activation, was found in the brain cortex and cerebellum. These results suggest that brain cells are more sensitive to toluene toxicity or toluene-induced apoptosis.

It is reported that apoptosis is not dependent on GSH but is dependent on the GSH:GSSG ratio,[28] which confirms the findings of the present study. The observed increase in apoptosis in different regions of the brain may explain the effect of toluene exposure in documented central nervous system conditions such as cerebellar ataxia, diffuse cortical atrophy, tremors, convulsions, memory function deterioration and dementia.[1,29–32]

The observed increase in SOD activity, together with a reduction in GPx activity, may result in the accumulation of hydrogen peroxide (H_2O_2) in different organs. This effect was more prominent in the brain cortex and cerebellum, and increased ROS levels may also explain the increased rate of apoptosis.

The highest level of TBARS was observed in the liver. This can be explained partially by increased oxidative stress due to toluene toxicity and also by the metabolic load on the liver to eliminate toluene by excretion as benzyl mercapturic acid through oxidation by CYP2EL and conjugation with GSH.[33]

With the exception of COX-2 activity, the testis showed only minor changes in the studied parameters, which is consistent with other reports.[34] Increased COX-2 activity in the tests conducted here may indicate a state of inflammation, particularly in the brain cortex in response to increased ROS generation following toluene exposure.

From the present study, it is possible to conclude that oxidative stress, derangement of the GSH:GSSG couple, induced chronic inflammatory change and induced apoptosis may play an essential role in toluene toxicity. □

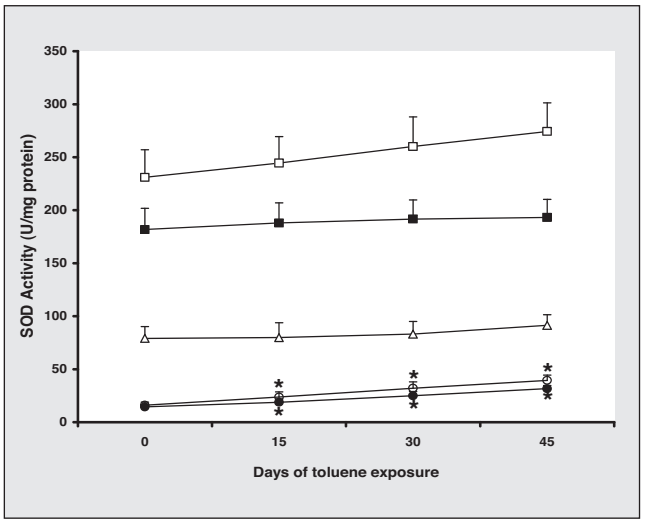

Fig. 8. Change in superoxide dismutase activity in different organs of rats in response to different durations of toluene.
*Significantly different from control values ($P<0.05$).

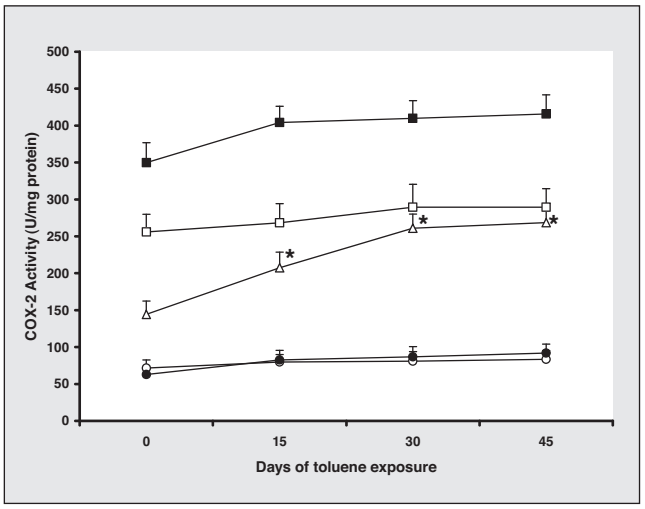

Fig. 9. Change in COX-2 activity in different organs of rats in response to different durations of toluene.
*Significantly different from control values ($P<0.05$).

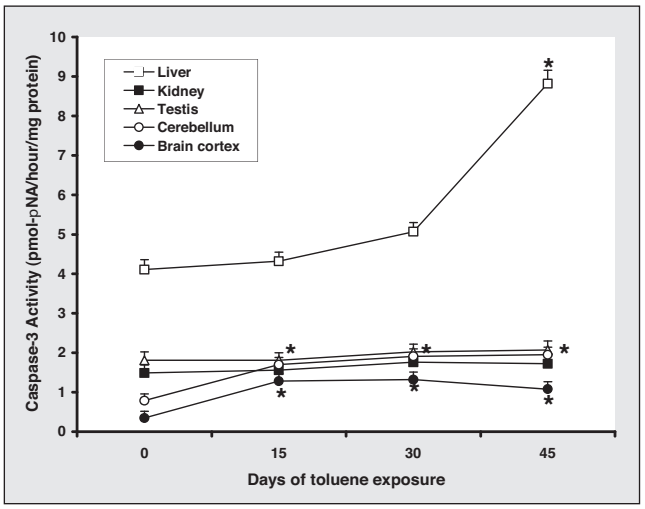

Fig. 10. Change in caspase-3 activity in different organs of rats in response to different durations of toluene.
*Significantly different from control values ($P<0.05$).

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