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Argemone Oil Augmented Oxidative Stress in Discrete Areas of Rat Brain

A. Siddiqui, I. Sayeed, K. S. Zafar, F. Islam

Neurotoxicology Laboratory, Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India

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Adulteration of mustard oil with argemone oil has been reported so many times in India and other tropical countries. Consumption of such an adulterated oil leads to a clinical condition known as dropsy (Tandon et al. 1975). The toxicity of argemone oil has been attributed to its physiologically active benzophenanthridine alkaloids, sanguanarine and dihydrosanguanarine (Sarkar 1948). The acute or chronic exposure to alkaloids in general has been shown to cause toxic manifestations ranging from hepatotoxicity, carcinogenicity to teratogenicity (Dalvi and Jones 1986). Clinical manifestations of argemone oil poisoning are erythema, oedema, hepatomegaly etc. (Das and Khanna 1997). The basis for the biological activity of the alkaloid sanguinarine could be the result of its electrophilic character (Tolkachev and Vichaknova 1978). Reactive oxygen species such as singlet oxygen and hydroxyl radical produced by argemone oil intoxication caused enhancement of lipid peroxide formation and depletion of endogenous tripeptide glutathione (Upreti et al. 1989; Peeples and Dalvi 1982) in liver. Histopathological studies in argemone oil intoxicated animals showed focal necrosis in liver along with vascular proliferation and fatty acid changes (Chandra et al. 1972). Brain tissue is especially prone to radical damage as it is enriched with highly specialized lipid rich membrane, the myelin (Zia and Islam 2000). The main factors that contribute to the vulnerability of brain include high content of polyunsaturated fatty acids in brain membrane and low level of enzymatic and non-enzymatic antioxidant (Liu and Mori 1993). The vulnerability of the central nervous system (CNS) to xenobiotics is evidenced by recent finding that indicate some glutathione (GSH) conjugate formed in the liver are able to traverse the blood brain barrier and gain access in the CNS (Patel et al. 1992).

The hepatotoxicity of argemone oil and its effect on oxidative stress in liver and serum are well known but its neurotoxicity is not available in the literature. The recent dropsy in New Delhi which was due to adulteration of argemone oil in mustard oil has created our interest to investigate the toxicity of argemone oil on oxidative stress parameters in the discrete brain areas of rat.

MATERIALS AND METHODS

Glutathione (oxidised and reduced), nicotinamide adenine dinucleotide phosphate

Correspondence to: F. Islam

reduced form (NADPH), thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), 5-5'-dithio-bis-2-nitrobenzoicacid (DTNB) were purchased from Sigma-Aldrich Chemical Corporation, St Louis, MO, USA. Other chemicals were of analytical grade.

The seeds of argemone maxicana (250 g) were crushed and the oil was extracted with chloroform (1000 mL) in soxhlet apparatus. The extracted argemone oil was filtered through buchner funnel at reduced pressure. The extract was evaporated in rotary vacuum evaporator at reduced pressure and temperature. The amount of sanguinarine was 3 mg/mL in the oil sample as estimated by the method of Shinolikar et al. (1981) by performing thin layer chromatography. The chemical composition of total alkaloids in *Argemone maxicana* seeds as reported by Upreti et al. (1991) was dihydrosanguinarine, sanguinarine, berberine, protopine, cheletrythrine and coptisine.

Adult male Wistar rats (150±10 g) from Jamia Hamdard animal breeding colony were used in this study and kept on commercial pellet diet and water *ad libitum* for 12 hr light and dark cycle each. The animals were divided into four groups two control and two experimental each having six rats. Group 1 and 2 respectively received intra peritoneal (i.p) injection of saline for 3 and 15 days which served as control. The group 3 (1.5 mL/kg b wt) and group 4 (0.2 mL/kg b wt) received argemone oil through i.p. route for 3 and 15 days respectively. Thereafter, the animals were sacrificed and brains were quickly removed and carefully washed with chilled saline and placed ventrally on a rat brain matrix for coronal dissection of striatum, hypothalamus, thalamus and hippocampus in the light of rat brain atlas (Pellegrino et al. 1981). The homogenate was used for lipid peroxidation (LPO) and post mitochondrial supernatant was used for the enzymes assay and reduced glutathione content. The method of Utley et al. (1967) was slightly modified for small brain parts. Each brain part was weighed and homogenized in 2.5 mL of phosphate buffer, pH 7.4. Thereafter, from each homogenate 1 mL was pipetted out in 20 mL RIA vials and placed at 37 °C in a metabolic shaker for 1 hr continuous shaking and another 1 ml was pipetted out in a centrifuge tube and kept at 0 °C for 1 hr. After that 1 mL of 10% of TCA and 1 mL of 0.67% of TBA were added to each set and centrifuged at 3000 rpm for 15 minutes. The supernatant was heated in a boiling water bath for 10 minutes. Samples were allow to cool under tap water and O.D. was taken at 535 nm.

Reduced glutathione was measured using the method of Jollow et al. (1974). The activity of glutathione peroxidase was measured in terms of nmol of NADP reduced min^{-1} mg^{-1} protein according to the procedure described by Mohandas et al. (1984). Glutathione reductase activity was expressed in terms of nmol of NADP reduced min^{-1} mg^{-1} protein by the method of Carlberg and Mannervik (1975) as modified by Mohandas et al. (1984). Glutathione-S-tansferase activity was estimated in terms of nmol of CDNB conjugate formed min^{-1} mg^{-1} protein by the method of Habig et al. (1974). Protein was measured by the method of Lowry et al. (1951).

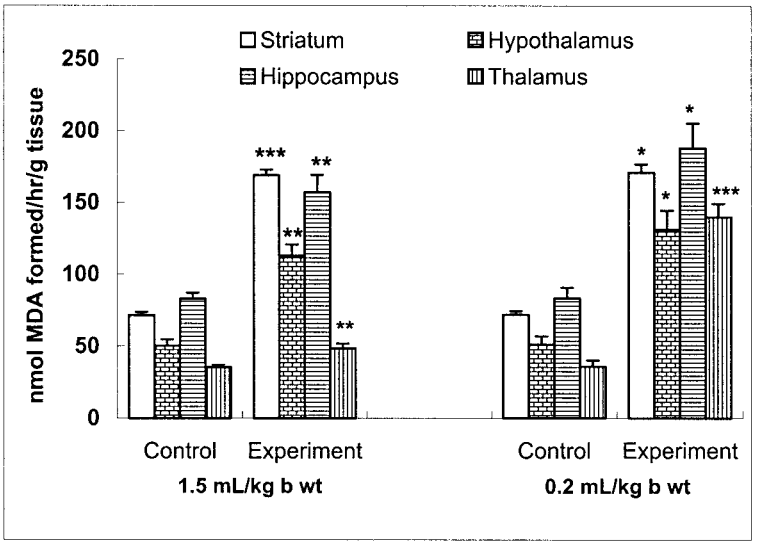

Figure 1. Effect of argemone oil (1.5 mL/kg b wt for 3 days and 0.2 mL/kg b wt for 15 days) on lipid peroxidation in discrete brain areas of rat. Values are expressed as mean ± SE of six animals. The significance are presented as *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Data were statistically analysed using Student's 't' test to assess the significant differences between experimental and control groups, the significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of 1.5 and 0.2 mL/kg b wt of argemone oil on lipid peroxidation in discrete brain areas. After 3 and 15 days of intoxication the lipid peroxidation was significantly increased in all four regions of the brain as compared to the saline treated control but the elevation of lipid peroxide formation was more marked with the low dose and long duration. Fig. 2 shows the effect of 1.5 and 0.2 mL/kg dose of argemone oil on the glutathione content in striatum, hypothalamus, hippocampus and thalamus of the brain. The content of reduced glutathione was depleted significantly with both the doses of argemone oil but the depletion was more marked with the low dose and long duration i.e. 0.2 mL/kg for 15 days.

Reactive oxygen species are involved during argemone oil intoxication which propagate the initial attack on lipid rich membrane to cause lipid peroxidation (Das et al. 1997). The enhanced elevation of LPO was correlated with marked depletion in the content of GSH, which acts as one of the guarding factors against oxidative stress (Levine 1982). Thus, GSH inactivation in argemone oil treated rats may be correlated with increased susceptibility of plasma membrane towards peroxide attack.

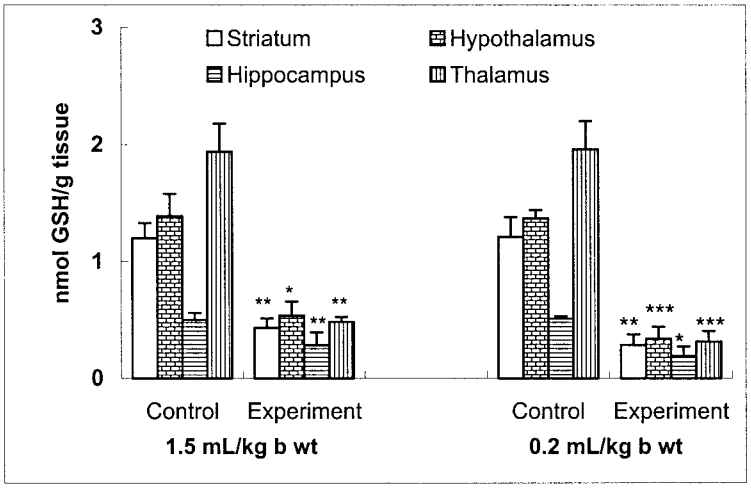

Figure 2. Effect of argemone oil (1.5 mL/kg b wt for 3 days and 0.2 mL/kg b wt for 15 days) on glutathione level in discrete brain areas of rat. Values are expressed as mean ± SE of six animals. The significance are presented as *p<0.05, **p<0.01, ***p<0.001.

The effects of high dose (1.5 mL/kg) of argemone oil for short duration and low dose for long duration on the activity of glutathione peroxidase, glutathione reductase and glutathione-S-transferase in striatum, hypothalamus, hippocampus and thalamus are shown in Table 1 and 2. Intoxication with high dose of argemone oil for 3 days resulted a significant depletion in the activity of glutathione peroxidase, glutathione reductase and glutathione-S-transferase in striatum, hypothalamus, hippocampus and thalamus. While the intoxication of rats with low dose for 15 days resulted in a profound decrease in the activity of glutathione peroxidase, glutathione reductase and glutathione-S-transferase in all the studied regions in rat brain.

The inhibition of the activity of glutathione peroxidase, glutathione reductase and glutathione-S-transferase was correlated with the marked depletion in the content of GSH. Hepatic cytosolic glutathione-S-transferase responsible for the catalysis of the conjugation reaction for the biotransformation of toxic chemicals was found to be inhibited as a result of reactive intermediate or lipid peroxide formation by argemone oil intoxication (Upreti et al. 1991). Our previous study has shown that glutathione peroxidase plays a predominant role in removing excess of some free radicals of hydro peroxides, a major defence system against oxidative stress in the brain (Imam et al. 2000). The activity of the enzymes was inhibited due to the reactivity of the iminium bond of sanguinarine on nucleophilic sites, which are present at the active sites of the enzymes (Sarkar1948). A possible explanation for the peroxidation potential of argemone oil is the production of reactive oxygen species *in vivo* by redox cycling of argemone alkaloid, sanguanarine, which may deplete glutathione and its metabolising antioxidant enzymes and thereby suppressing their antioxidant

Table 1. Effect of argemone oil (1.5 mL/kg b wt) in discrete areas of rat brain on glutathione dependent enzymes.

Brain Region	Groups	Glutathione peroxidase	Glutathione reductase	Glutathione-S-transferase
Striatum	Control	34.1±2.1	22.3±1.0	32.1±2.1
	Treated	19.8±2.0** (42%)	18.8±1.0* (16%)	18.7±1.7** (44%)
Hypothalamus	Control	29.5±1.6	17.3±2.1	20.2±1.1
	Treated	18.1±1.4** (39%)	12.2±1.3* (28%)	12.9±0.9** (36%)
Hippocampus	Control	35.1±3.0	23.4±1.0	14.4±0.8
	Treated	26.5±1.2** (26%)	17.4±1.0** (26%)	6.6±0.6*** (54%)
Thalamus	Control	19.8±1.8	26.6±1.1	17.1±1.2
	Treated	13.7±1.1* (31%)	16.9±1.7** (36%)	12.6±1.2* (26%)

(Values in parentheses indicate the percent change in the enzymes activity compared to control. The values are expressed as mean ± SE of six animals and significance are presented as *p<0.05, **p<0.01, ***p<0.001).

Table 2. Effect of argemone oil (0.2 mL/kg b wt) in discrete areas of rat brain on glutathione dependent enzymes.

Brain Region	Groups	Glutathione peroxidase	Glutathione reductase	Glutathione-S-transferase
Striatum	Control	33.0±1.9	23.2±1.1	33.0±2.9
	Treated	15.0±2.0** (55%)	11.1±1.2*** (52%)	15.0±1.0** (55%)
Hypothalamus	Control	29.8±2.2	16.2±2.0	19.2±1.7
	Treated	17.4±1.7** (42%)	6.0±1.2** (63%)	8.3±1.0** (57%)
Hippocampus	Control	33.6±4.2	22.9±3.2	15.4±1.0
	Treated	21.9±1.9* (35%)	13.7±0.1** (40%)	5.6±1.1** (64%)
Thalamus	Control	19.4±1.9	27.0±1.1	16.5±1.1
	Treated	10.1±1.3** (48%)	11.3±1.8*** (58%)	9.6±1.2** (42%)

(Values in parentheses indicate the percent change in the enzymes activity compared to control. The values are expressed as mean ± SE of six animals and significance are presented as *p<0.05, **p<0.01, ***p<0.001).

potential (Upreti et al. 1991).

The sulfhydryl group is essential component of all cellular structure in the brain and play a vital role in the cellular detoxification against free radical mediated damage (Chio and Tappel 1969). Our previous finding revealed that the cell deficient with thiol group undergo fast lipid peroxidation resulting its accumulation in various regions of the brain (Zia and Islam 2000). Thus the effect of acute or sub acute doses of argemone oil on lipid peroxidation might be mediated by sanguanarine, which induce diminution of endogenous bio-antioxidants responsible for cellular defence. These results suggest that the neurotoxic effect might be due to the production of reactive oxygen species by argemone oil.

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