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Propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function: Attenuation by *Withania somnifera*

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Propoxur (2-isopropoxyphenyl N-methylcarbamate) is widely used as an acaricide in agriculture and public health programs. Studies have shown that sub-chronic exposure to propoxur can cause oxidative stress and immuno-suppression in rats. Carbamates are also known to exhibit inhibitory effect on cholinesterase activity, which is directly related to their cholinergic effects. In the present study, the effect of *Withania somnifera* (Ashwagandha), a widely used herbal drug possessing anti-stress and immuno-modulatory properties was studied on propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function in rats. Male Wistar rats were divided into four groups. Group I was treated with olive oil and served as control. Group II was administered orally with propoxur (10 mg/kg b.wt.) in olive oil, group III received a combination of propoxur (10 mg/kg b.wt.) and *W. somnifera* (100 mg/kg b.wt.) suspension and group IV *W. somnifera* (100 mg/kg b.wt.) only. All animals were treated for 30 days. Cognitive behaviour was assessed by transfer latency using elevated plus maze. Blood and brain acetylcholine esterase (AChE) activity was also assessed. Oral administration of propoxur (10 mg/kg b.wt.) resulted in a significant reduction of brain and blood AChE activity. A significant prolongation of the acquisition as well as retention transfer latency was observed in propoxur-treated rats. Oral treatment of *W. somnifera* exerts protective effect and attenuates AChE inhibition and cognitive impairment caused by sub-chronic exposure to propoxur.

Keywords: Carbamate, Propoxur, *Withania somnifera*, Behavioral toxicity, Transfer latency, Acetylcholineestrase (AChE), Cognitive function, Tissue toxicity

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Abbreviations: AChE, acetylcholine esterase; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; GABA, gamma aminobutyric acid; LPO, lipid peroxidation; NMDA, N-methyl-D-aspartic acid; SOD, superoxide dismutase.

Propoxur (2-isopropoxyphenyl N-methylcarbamate) is widely used as an acaricide in agriculture and public health programs. Brain, skeletal muscles and heart are the potential targets of carbamate which act by inhibiting enzyme acetylcholinesterase (AChE) in central and peripheral nervous system[1]. The resulting overstimulation of cholinergic system can paralyze the nervous system and produce a rapid knock-down effect that includes fasciculation, salivation, lacrimation, full body tremors and dypsnea at high doses[2]. Recent *in vitro* and *in vivo* studies from our laboratory suggest that sub-chronic exposure to propoxur can cause oxidative stress and immuno-suppression in rats[3]. There is evidence that pesticides which inhibit cholinesterase may trigger neurological and behavioral changes at very low doses. Decreased ambulation of mice, treated subcutaneously with 1/4 or 1/5 of the propoxur LD_{50} dose has been observed[4]. Diminished motor activity is reported, when there is 50% reduction of blood and brain cholinesterase activity[5].

Withania somnifera, commonly known as 'Ashwagandha' (Indian Ginseng or dunal) is widely used as an ayurvedic medicine and is known to possess anti-stress and immuno-modulatory properties[6,7]. The active principles of WS sitoindes VII-X and withaferin A (glycowithanolides) have shown an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties[8]. Its root extract exhibits therapeutic potential against protracted social isolation-induced behaviour and reserpine-induced orofacial, dyskinesia and cognitive dysfunction in rats[9-11]. In the present study, the protective effect of *W. somnifera* has been investigated on AChE activity and cognitive impairment in propoxur-treated rats.

Materials and Methods

Chemicals

Technical grade propoxur (purity 99.4%) was obtained through the courtesy of M/s Bayer AG, Monheim, Germany. *Withania somnifera* (Ashwagandha) pure root powder was procured from Himalaya Drug Company, Bangalore. All other reagents used were of analytical grade and obtained either from Sisco Research Laboratories or Qualigens Fine Chemicals, Mumbai, India.

Animals and treatment

Male Wistar albino rats (aged 10-12 weeks, weight range 200-250 g) were obtained from Central Animal House facility of the Institute. The animals were kept under standard conditions (temp. 22 ± 2°C, 80% humidity with 12 h light/dark cycle). Rodent food and water were accessible *ad libitum.* Body weights and food consumption were recorded weekly. Records were maintained to comply with the conditions as desired by Institutional Ethical Committee-Animal Research, UCMS, Delhi.

Rats were randomly divided into four groups (eight animals each) and received the treatment orally using syringe and 20 gauge Ryle's tube. Group I (control) received olive oil. Propoxur was dissolved in olive oil and administered to Group II animals at a dose of 10 mg/kg body wt/day[2]. Group III received daily a dose of both propoxur (10 mg/kg) and freshly prepared *W. somnifera* (100 mg/kg) aqueous suspension[12]. Group IV was administered with *W. somnifera* (100 mg/kg) alone. The rats were treated with vehicle or test chemicals for 30 days. At the end of the study, animals were fasted overnight and blood was collected by puncture of the venous retroorbital plexus, altering the left and right eyes in heparin tubes. Each animal was euthanized by cervical dislocation and their brains were harvested, weighed and processed for estimation of AChE activity.

Transfer latency on elevated plus maze

Cognitive behaviour was assessed by using elevated plus maze learning task, which measures spatial long-term memory. The elevated plus-maze consisted of two open arms (50 × 10 cm) and two closed arm (50 × 10 × 40 cm) with an open roof. The maze was elevated to a height of 50 cm from the floor. Transfer latency (TL), the time in which animal moves from the open arm to closed arm was utilized as an index of learning and memory process. The animals were trained 24 h prior to testing. On re-testing, the time taken to enter the closed arm was taken as transfer latency. A time of 180 s was taken as cut-off and animals not entering the closed arm in this period were assigned the transfer latency of 180 s[13,14]. Transfer latency test was conducted at the end of the experimental period.

Measurement of AChE activity

Brain and blood AChE activity was assessed within 60 min after sampling in all rats. Brain was weighed and homogenized in 0.1 M phosphate buffer (pH 8.0) at a concentration of 5 mg tissue/ml of buffer. AChE activity in blood and brain homogenate was determined spectrophotometrically at 412 nm with 0.01 M dithio-bis-nitrobenzoic acid and 0.075 M acetylthiocholineiodide as substrate at 25°C, according to Ellman and co-workers[15].

Statistical analysis

Values were expressed as the mean ± SD. Statistical analysis was performed using ANOVA (SPSS version12). One-way-analysis of variance was used to determine significant difference among groups. Least significance difference (LSD) post-hoc test was used for pair-wise comparisons after analysis of variance. Statistical significance was accepted at $p<0.05$.

Results and Discussion

Propoxur is a carbamate and is known to cause psychopharmacological increase in lipid peroxidation (LPO) in brain regions, accompanied by decreased levels of glutathione and increase in the glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase (SOD)[3,16]. Although propoxur is known to produce neurotoxicity, the exact mechanism by which this effect is mediated is still unknown. AChE is a key component of cholinergic brain synapses and neuromuscular junctions. The major biological role of this enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine. Therefore, the present study was designed to explore the correlation, if any, between AChE activity and behavioral changes. The propoxur-induced neurotoxicity was studied in terms of derangement of cognitive functions, as measured by transfer latency in plus maze in rats. Efforts were also made to investigate whether *W. somnifera*, a commonly used herbal drug could exert protective effect on propoxur-induced modulation of cognitive function.

The oral administration of $1/10^{th}$ of the LD_{50} of propoxur (10 mg/kg) resulted in a significant prolongation ($p<0.05$) of both the acquisition (Fig. 1A) as well as retention transfer latency (Fig. 1B) during the same time period of treatment as compared to control. *W. somnifera* produced a significant reversal of propoxur-induced dysfunction of transfer latency on plus maze, when administered 1 h prior to propoxur treatment and caused a significant decrease in acquisition as well as retention.

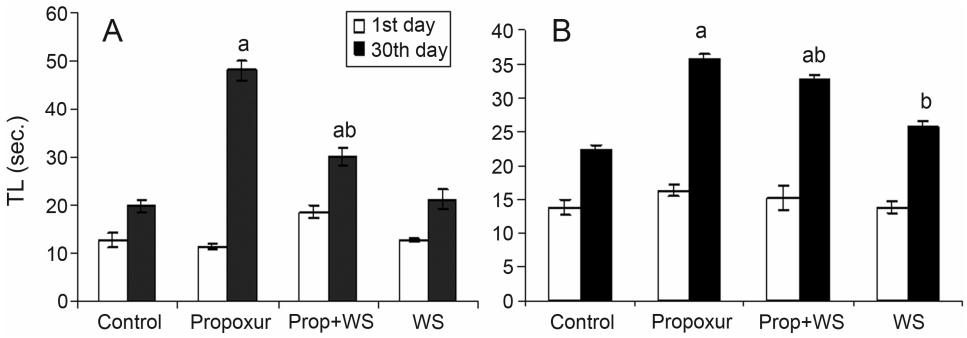

Fig. 1—Effect of propoxur (10 mg/kg) and *W. somnifera* (100 mg/kg) on acquisition **(A)** and retention transfer latency (**B**) [Values are expressed as mean ± SD. n = 8 animals in each group. Significantly different from (a) control (b) propoxur ($p<0.05$)]

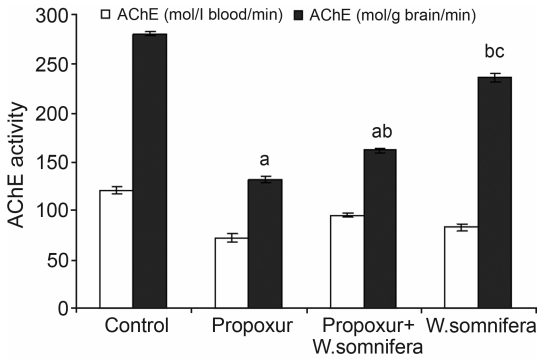

Fig. 2—Effect of propoxur (10 mg/kg) and *W. somnifera* (100 mg/kg) on AChE activity in blood and brain [Values are expressed as mean ± SD. n = 8 animals in each group. Significantly different from (a) control (b) propoxur (c) propoxur + *W. somnifera* ($p<0.05$)]

Oral administration of propoxur (10 mg/kg) resulted in a significant reduction of brain and blood AChE activity (Fig. 2). The aqueous suspension of *W. somnifera* alone and when administered along with propoxur significantly increased the AChE activity.

According to the cholinergic theory of memory, recalling and forgetting are consequences of time-dependent cholinergic dysfunction at the synaptic levels[17]. It is well accepted that acetylcholine is not the sole neurotransmitter in learning and memory, a cascade of biochemical changes underlies learning performances. In different brain regions, GABA, acetylcholine and norepinepherine initially modulate the process, while late modulation relies on dopamine, norepinephrine, serotonin and glutamate AMPA receptor-mediated process. Memory consolidation depends on glutamate NMDA receptor[18]. In summary, any changes of equilibrium may pose serious consequences on behaviour. In conjunction, decreased choline-esterase activity and increased acetyl choline levels induced by propoxur could be speculated to disrupt the biochemical sequences that are necessary for memory formation. The observation of the present study of propoxur-induced disruption of cognitive function was in accordance with the earlier findings, where the effect of administration of this pesticide was observed on both non-working as well as working memory procedure[19].

Sub-chronic exposure to propoxur triggers cognitive impairment and inhibits the activity of AChE both in blood and brain. Propoxur-induced increase in transfer latency suggested a relationship between behavioral changes and decreased blood and brain AChE activity. Several authors have reported anti-AChE agent-induced alterations in motor activity, learning and memory with various substances, confirming the deleterious effects of excessive cholinergic activity on behaviour[5,20]. From the present study, it might be inferred that cholinergic hyperactivity hindered the learning acquisition process.

W. somnifera is used in Indian traditional medicine Ayurveda and is believed to have a variety of health promoting effects[21]. The plant exhibits varying degrees of therapeutic values and is useful in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, dyspepsia[22]. Our study revealed that it's exhibited protective effect against propoxur-induced cognitive dysfunction and AChE inhibition in experimental animals. It may be suggested that *W. somnifera* has a neuroprotective effect. The detection of behavioral changes induced by a low dose of propoxur suggested the need to analyze several other behavioral parameters that might be altered by such pesticides.

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