Table 3: Effect on antioxidant status in hippocampus and frontal cortex

Treatment	Catalase		SOD		GSH	
	HC	FC	HC	FC	HC	FC
Vehicle control	4.37±1.05	3.43±0.57	27.62±0.41	39.16±1.54	0.0123±0.0004	0.0125±0.0005
DOX control	0.92±0.18$^{\#}$	1.86±0.28	37.25±2.34	27.99±1.05#	0.102±0.0002	0.0092±0.0002$^{\#\#}$
DOX + SA (100 mg/kg)	0.15±0.97	6.31±1.36*	41.30±7.02	31.56±0.75	0.0150±0.0013	0.0105±0.0002
DOX + SA (200 mg/kg)	7.17±2.03***	4.73±2.15	45.39±5.70	29.09±2.88	00.0168±0.0020*	0.0090±0.0032
	TBARS		**Nitrite**		**Percentage of MPO**	
	HC	FC	HC	FC	HC	FC
	842.63±83.8	691.8±87.8	9.6±1.2	7.2±1.45	-	-
	1410.55±107.44$^{\#\#\#}$	1115.8±132.7$^{\#\#}$	25.84±6.36$^{\#\#\#}$	14.01±1.48$^{\#}$	62.09±0.59	55.48±1.52
	958.32±45.61***	719.3±23.9**	9.57±1.29**	5.88±2.10**	36.38±2.43*	32.49±3.65**
	1001.05±48.35**	606.2±41.2***	14.52±1.95*	2.79±1.14***	28.95±8.85*	31.70±4.86**

Effect of DOX (2.5 mg/kg) and DOX + SA treatments (SA 100, 200 mg/kg) on antioxidant systems. Catalase activity (μM of hydrogen peroxide decomposed/min/mg of protein), SOD activity (units of SOD (1 unit=50% adrenochrome formed)/min/mg of protein), GSH levels (μmol of GSH/mg of protein), TBARS (MDA formed nM/mg of protein), nitrite (nM/mg of protein), percentage increase in the MPO levels. All the values are mean±SEM of six animals. $^{\#}P<0.05$ versus vehicle group, $^{\#\#}P<0.001$ versus vehicle group, $^{***}P<0.001$ versus DOX group, $^{**}P<0.01$ versus DOX group $^{*}P<0.05$ versus DOX group. HC: Hippocampus; FC: Frontal cortex; TBARS: Thiobarbituric acid reacting substances; DOX: Doxorubicin; SA: Saraca asoca; SOD: Superoxide dismutase; GSH: Reduced glutathione; MDA: Malondialdehyde; MPO: Myeloperoxidase; SEM; Standard error of mean

phenotypic differentiation and formation of neurite extensions when treated with ATRA.[32,33] Induction with ATRA manifested formation of neurite extensions. SA bark extract at 60 μg/ml along with DOX at 1.5 μg/ml showed comparative retention in neurite length in comparison to the DOX alone treated well. The presence of apoptotic cell bodies in treatment wells, however, less when compared to DOX alone, could be due to DOX treatment. A similar course was observed in studies conducted to evaluate neuroprotective potential against DOX.[30] Comparatively fewer cell bodies and presence of more neurite growth extensions purport the extract's protective effect ($P < 0.001$). In addition, the treatment showed significant reversal ($P < 0.01$) against glutamate-induced neurotoxicity. DOX mediates TNF-alpha induced upregulation of the excess release of glutamate from hemichannels leading to glutamate-induced excitotoxicity causing neurodegeneration.[4] The conducted *in vitro* study confirmed its neuroprotective effect of the extract against glutamate, demonstrating its possible role in preventing glutamate-induced excitotoxicity. Furthermore, this can be proposed for molecular evaluation studies to identify the possible protective mechanism.

An *in vivo* study was conducted to evaluate the effect of extract in counteracting effect DOX systemic toxicity mainly by cognitive decline mediated by oxidative and nitrergic stress. The study was conducted using 50 days treatment including 10 cycles, given every 5 days at a dose of 2.5 mg/kg. The chronic exposure of DOX for 57 days produced a reduction in body weight, comparable to the vehicle although it was not significant. This was significantly reversed by treatment with the SA (100 mg/kg) treatment.

Episodic memory is mainly associated with the H and FC. Most of the conscious learning is performed by the declarative memory function in the H and FC, which is said to be temporally dated conscious recollection of past experiences and events. The animals' ability to recollect past experiences (episodic memory) is evaluated by NORT in which the animals were allowed to explore two familiar objects in the familiarization trial followed by choice trial after anITI. In the choice trial, one object was replaced with a novel object and the ability to explore and discriminate the objects was evaluated.[34] The animals irrespective of the treatment tend to explore the two similar objects for almost equal time during the familiarization phase. However, the animals which were able to remember the familiar object were able to discriminate the novel object and spent significantly more time exploring a novel object. In DOX study, animals were treated for 57 days, after which the episodic memory was assessed. An ITIof 4 h was given which was standardized in our laboratory. DOX-treated animals showed insignificant difference in exploration time of the novel or familiar object. This signifies that the animal failed to recognize the previously encountered familiar objects. Vehicle-treated animals were able to discriminate the novel object from the familiar one. Treatment with extract at 100 mg/kg p. o. showed significantly more exploration time toward the novel object ($P < 0.001$) compared to familiar one and reversed the impairment caused due to DOX. This suggests the beneficial effect of SA against DOX-induced cognitive impairment. However, the higher dose of extract, DOX + SA 200 mg/kg did not reverse the impairment caused due to DOX as the animals failed to recognize the novel object over the familiar object.

Cholinergic system in the brain is mainly responsible for learning and memory function, which contributes to the cognitive ability. Acetylcholinesterase is an enzyme responsible for the degradation of acetylcholine terminating the cholinergic transmission. DOX causes neurodegeneration leading to impairment in the cognitive domain[1] which makes it necessary to evaluate the acetylcholinesterase activity in the brain. Chronic exposure of the toxicant may attribute to the

decreased acetylcholine levels in the body due to a decrease in the choline uptake due to neurodegeneration in the H and FC. According to Agamy *et al*. suggested a possible mechanistic role of DOX-induced oxidative and nitrosative stress which might abnormally increase AchE levels implying an increased degradation of acetylcholine which could be one of the pathways of DOX-induced neurotoxicity.[35] *Ex vivo* acetylcholinesterase inhibition activity study signified potential degradation of substrate acetylthiocholine iodide when treated with SA, determined by intense yellow-colored formed byproduct, thionitrobenzoic acid. This entails a possible acetylcholinesterase inhibition potential of SA. After chronic exposure of DOX, a similar activity was shown. *S. acosa* had significantly reduced the AchE activity in the H which could be due to its potent anti-oxidant activity indicating the restoration of cognitive function.

Oxidative stress is one of the major pathways for the neurodegenerative disorders such as Alzheimer's disease. Most of the cancer chemotherapeutic drugs including DOX produce cognitive decline by producing oxidative stress and nitrergic stress. DOX will undergo redox cycling and absorbed into the body not only affects the cancer cells but also the normal cells. This effect in the brain leads to neuroinflammation and mitochondrial dysfunction leading to apoptosis and neurodegeneration. In the present study, oxidative stress markers such as catalase, SOD, GSH, MDA and inflammatory markers, nitrite, and MPO levels were evaluated. DOX increased the oxidative and nitrosative stress which was significantly ameliorated by SA.

CONCLUSION

SA bark extract showed an improvement in episodic memory which was impaired by DOX treatment. The neuroprotective and antioxidant potential of the test extract possibly underlies the efficacy to reverse cognitive deficits associated with the DOX-induced chemobrain like condition. SA extract at 100 mg/kg p.o. showed better chemoprotective activity against extract at 200 mg/kg, p.o.

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Conflicts of interest

There are no conflicts of interest.

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