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***Research Article** (Open Access)*

ARSENIC INDUCED TOXICITY AND ITS MODIFICATION BY ELLAGIC ACID AND DMSA IN SWISS ALBINO MICE

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ABSTRACT
Exposure to trivalent arsenic (As III) is known for its reproductive toxicity. The present research is focused on the testicular biochemical disturbance induced by arsenic and the defense afforded by Ellagic acid alone, DMSA alone, and in combination. The study was conducted on five groups of male Swiss albino mice, keeping two control groups (I and II), which were administered intra-peritoneally (i.p) with normal saline and As III ($NaAsO_2$), respectively.
To observe the individual protective role of DMSA and Ellagic acid, 7 days post As III exposure and 10 and 30 days pre-post treatment at the dose of 50 mg/kg b.wt./day and 25 mg/kg b.wt. were administered, respectively. In the combination group, both drugs with the same dose and drug treatment duration were given along with As III i.p. administration. The biochemical changes were observed in all the groups at the autopsy intervals of 1,3,7,15, and 30 days.
Sodium arsenite exposure promoted oxidative stress with increased Lipid peroxidation, Acid, and Alkaline phosphatase activities, and decreased glutathione and lactate dehydrogenase activities. The co-administration of Ellagic acid and DMSAis responsible for higher protective effects than individual drugs in terms of considerable normalizations of all the above-mentioned biochemical parameters studied. Thus, providing helpful knowledge about using metal chelators like DMSA reduces the effective arsenic concentrationin combination with Ellagic acid and prevents oxidative stress with its anti-oxidative potential.

Keywords: *Arsenic, Sodium arsenite, mice, reproductive toxicity, DMSA, Ellagic Acid*

Abbreviations: *$NaAsO_2$, sodium arsenite; Lipid peroxidation, MDA, malondialdehyde; ROS, reactive oxygen species; LDH, lactate dehydrogenase*

INTRODUCTION
The elevated levels of arsenic in food and drinking seriously threaten the exposed population. Acute exposure has been known to cause gastroenteritis, hypotension, and renal and neurological disorders. The sub-chronic and chronic exposure may cause skin aliments like hyper-pigmentation, hyperkeratosis, hematologic, hepatic, and reproductive abnormalities, and various types of cancers(Samuel *et al.*, 2005; Kim & Kim, 2015; Kuivenhoven & Mason, 2022).
Therefore, protecting the population from these disorders is a priority issue. The currently acceptable therapeutic approach to manage metal poisoning includes chelators, which act as metal legends with variable metal binding groups forming covalent or co-ordinate links. Many chelators can bind to metals in extracellular or intracellular spaces (Flora & Pachauri, 2010).
The arsenic intoxication has long been known to be countered by chelation therapy using BAL (British Anti Lewisite; Dimercaptrol), DMPS (2, 3-dimercaptopropane sulfonic acid) and DMSA (Dimercaptosuccinic acid).DMPS and DMSA are the unithiol and dithiol derivatives of BAL. These have been experimented on animal models to protect against acute arsenic-induced toxicity if given promptly by metal binding and subsequent removal by increased excretion (Kosnett, 2013).

DMSA, its derivatives, and many chelating agents employed for arsenic detoxification have been reported in the review (Bjørklund *et al.,* 2020). The metals can be chelated very effectively in extracellular fluid if given before arsenic enters the cells and generates oxidative stress. But usually, the practical administration involves time-lapse after exposure.
Thus, supplementing antioxidants to reduce the toxic effects of arsenic can provide beneficial results. Many extracts or bioactive compounds have been tested for their protective role in a combined therapeutic approach (Bjørklund *et al.,* 2020).
Ellagic acid is a prominent bioactive compound existing in *Emblicaofficinalis* and *mentha piperita*, which has been reported in our earlier reports to afford protection against arsenic-induced toxicity(Sharma *et al.,* 2007; Sharma *et al.,* 2009)
Ellagic acid is known to protect from arsenic-caused testicular toxicity in rats owing to its anti-oxidative and anti-inflammatory potential (Hemmati *et al.,* 2018; Mehrzadi *et al.,* 2018).
These studies have provided the platform to conduct the present study, taking Ellagic acid and DMSA singly or combined to investigate their role in the defense against arsenic-caused testicular damage in mice.

MATERIALS AND METHODS

2.1. Test animals: Mature male Swiss albino mice (6-8 weeks old) procured from IVRI, Izatnagar, India, were used in the study. These animals were maintained under controlled conditions as per standards.

2.2. Test chemical

Analytical grade chemicals were used in all the experimentations [Arsenic ($NaAsO_2$; Himedia), Ellagic acid, and DMSA (MP Biomedicals)]. The doses and duration of each of these agents have been described in Table 1.
Experimental design: - Five groups with five animals in each were made and given treatment as given in the table1

Table 1. Treatment, doses, and groups of experimentations

Type	Groups	Normal saline	As III	DMSA	Ellagic acid
Dissolved in		DDW	0.9% NaCl	$NaHCO_3$	DDW
Dose		0.9% NaCl	4 mg/kg b.wt	50 mg/kg b.wt	25 mg/kg b.wt
Route of exposure		i.p.	i.p.	Orally	Orally
Control	I	Once i.p.	-	-	-
	II	-	Once	-	-
Experimental	III	-	once	7 days Post As III treatment	-
	IV	-	Once	--	10 days pre and 30 days post As III administration
	V	-	once	7 days Post As III treatment	10 days pre and 30 days post As III administration

Autopsy Intervals: One animal from each group was sacrificed at 1, 3, 7, 15, and 30 days of experimentation. Their testes were removed and prepared for the biochemical tests.

Biochemical studies:

- **Alkaline phosphatase:** Fiske &Subbarow (1925) method was adopted for ALP estimation. The blue color of ANSA with phosphomolybdic acid formed by TCA precipitation of protein with tissue phosphate was read at 410nm (Uv-Vis spectrophotometer (Systronics). The intensity of the blue color is a measure of liberated phosphate due to the activity of alkaline phosphatase. The optical density was converted to tissue alkaline phosphatase activity by placing the values of standard and sample in Equation 1**Molybdate + Inorganic Phosphate (tissue) →Phosphomolybdate**
Phosphomolybdate + ANSA → Blue color

$$\frac{Readingofunknown}{ReadingofStandard} \times 0.04 \times \frac{3}{2} \times \frac{1000}{tissuetaken} \times 2 = mgPi/gm/hr \quad \textbf{Eq.1}$$

Where 0.04 = concentration of standard;3/2 = volume of solution taken; Pi=inorganic phosphate

- **Acid phosphatase:-** The liberated phosphate due to the activity of tissue Acid phosphatase activity was estimated by the method given by Fiske &Subbarow (1925) at pH -5 with buffered acid phosphatase substrate .
- **Lactate dehydrogenase (LDH):-**The testicular LDH activities were measured by standard protocol [Wroblewski (1967) and Sharma et al.(2007)].
- **Reduced Glutathione (GSH):-** The testicular GSH levels were measured by the method given by Moron *et al.,* (1979) and compared with the standard curve of metaphosphoric acid (Sharma *et al.,* 2007)
- **Lipid Peroxidation (LPO):**-The testicular LPO levels were measured using the procedure given by Ohkawa et al. (1979). Teramethoxy propane was used to prepare the standard curve. LPO levels of tissue samples were obtained by reading the pink-colored complex developed at pH 3.5 by reaction of malondialdehyde, which is a lipid peroxide product with Thiobarbituric acid to give a pink-colored complex (MDA-TBA $_2$)at 532nm and comparing with the standard values.

Statistical analysis

The results of triplicate experimentation were expressed as mean± standard error. All experimental group results were compared with control group results by the Student's 't' test to estimate the significance of the difference between them. Significance levels were set at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

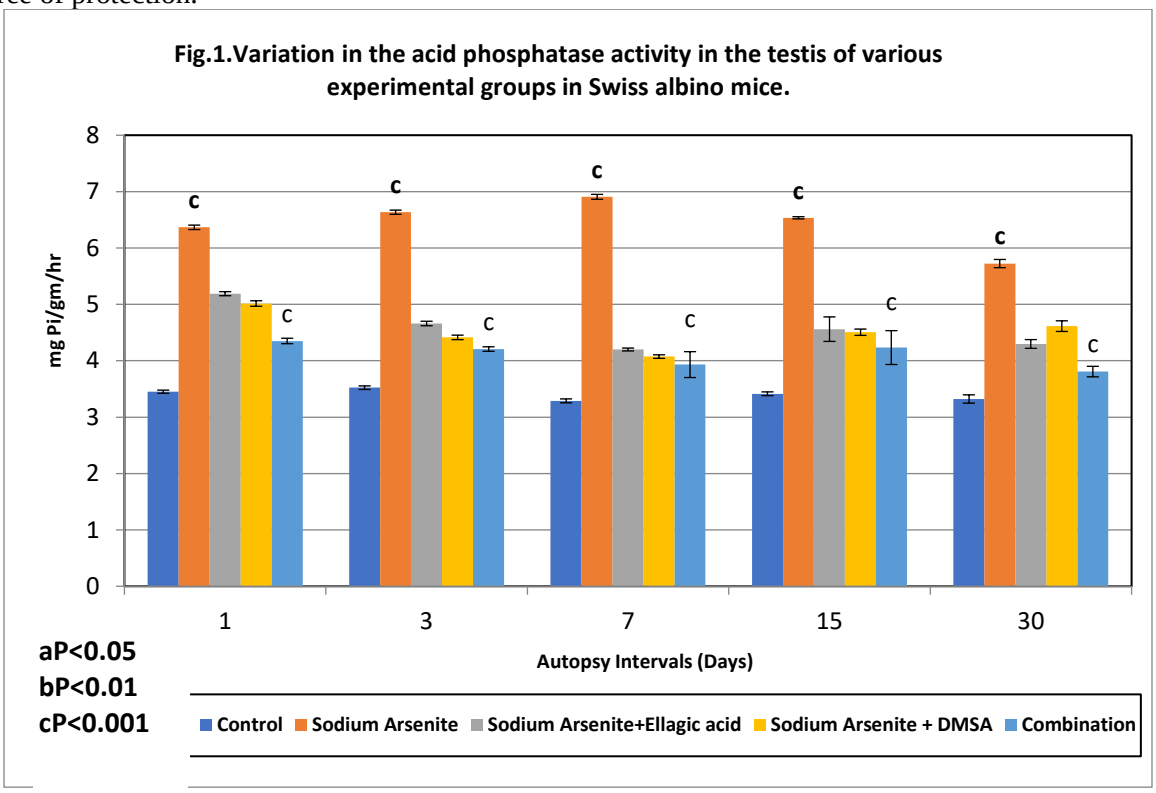
RESULTS AND DISCUSSION

The results of the control group-I give us an idea about the expected levels of the biochemical parameters as these animals were administered only the normal saline. Elevated toxicity indicators (LPO, ALP, and ACP) reduced protective parameters (GSH, Lactate dehydrogenase)at all the autopsy intervals in control group-II reflected AsIII induced damages. A similar pattern of tissue damage indicators has been stated in our earlier studies due to arsenic exposure in mice (Sharma *et al.,* 2007).

The protective role of DMSA and Ellagic acid on arsenic-induced testicular damage is evident by the biochemical comparison of groups III, IV, and V with control (group II).The enzymatic activity of lactate dehydrogenase is mainly responsible for converting Lactate to pyruvate and thus helps complete anaerobic glycolysis. In testes, LDH-C4 isotypes are reported to be present in the spermatids and spermatozoa, which provide the required ATP in mammals (Blanco &Zinkham, 1963; Odet *et al.,* 2011).In the As III treated group(control II), the decline in LDH levels indicates reduced ATP production and sperm mobility. The LDH levels have been observed to recover with DMSA and Ellagic Acid treatment(groups III and IV), with the most significant($p<0.001$) recovery in the combination group V reaching near expected values.

Exposure to As III in male rats at the dose of 5mg/kg body weight/day has been reported to significantly elevate testicular ACP and ALP levels, indicative of cellular toxicity and the subsequent degenerative effect (Jana *et al.,* 2006). Thus, restoring these damages is possible with the near normal values of ACP and ALP, which is evident in the combination group with highly significant ($p<0.001$) difference of ACP and ALP

levels at all the intervals. The individual drug administration (groups III and IV) also provides a certain degree of protection.



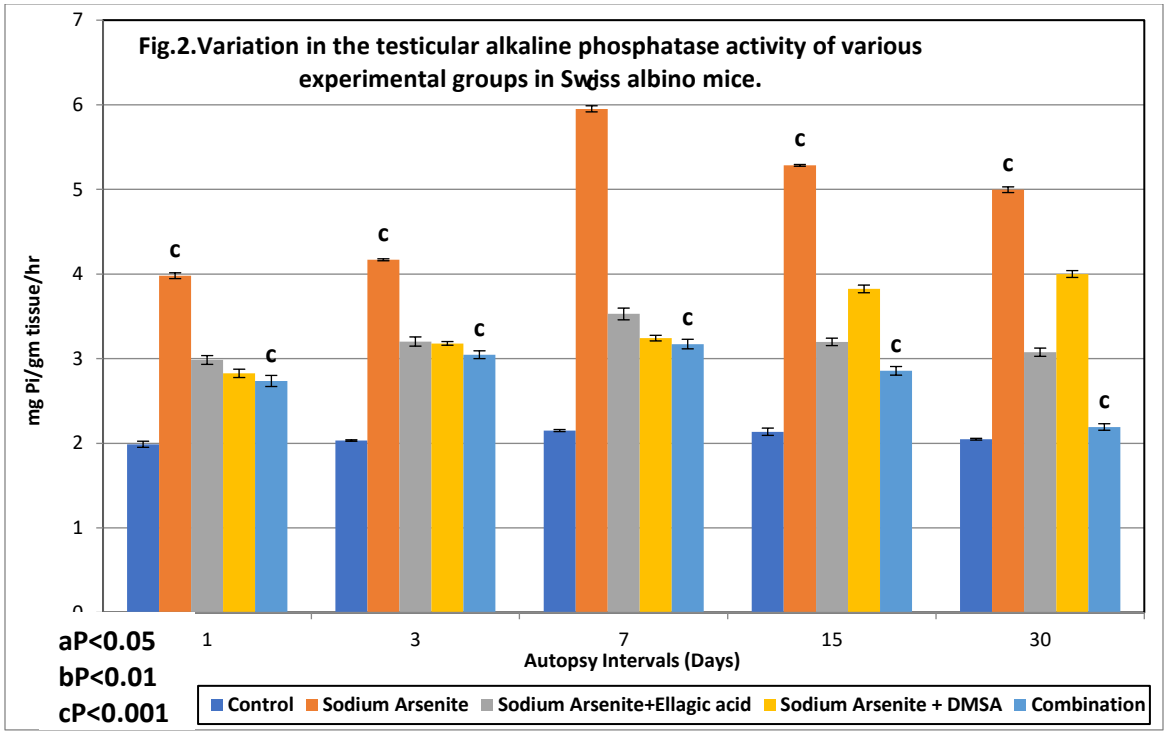


Fig.3.Variation in the testicular lactate dehydrogenase activity of various experimental groups in Swiss albino mice.

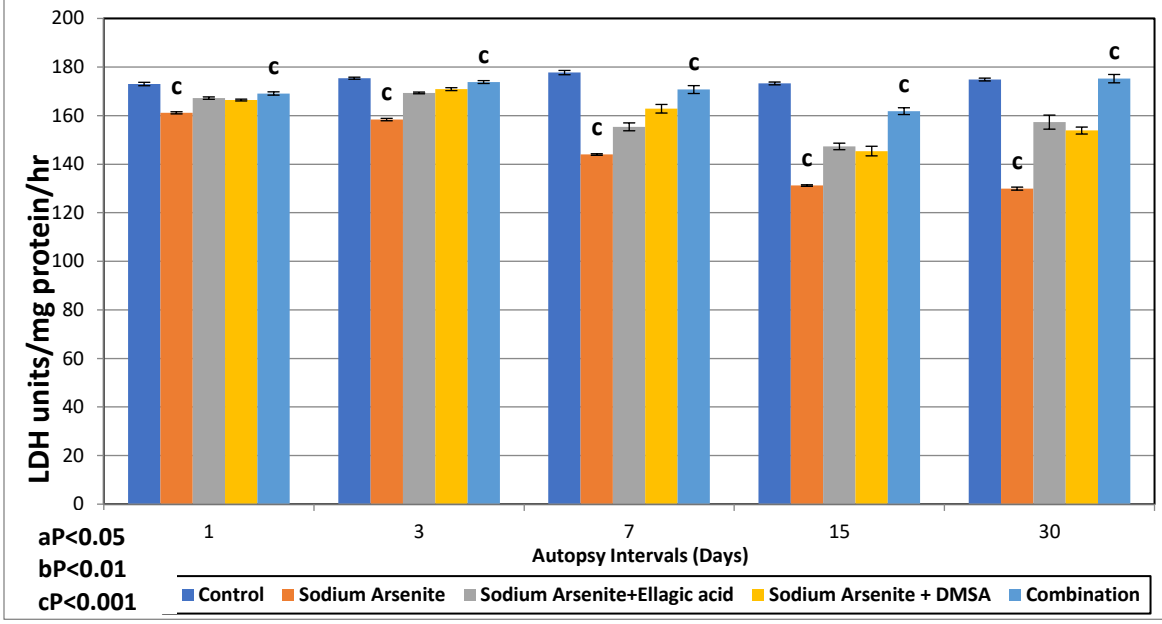


Fig.4.Variation in the testicular lipid peroxidation content of various experimental groups in Swiss albino mice.

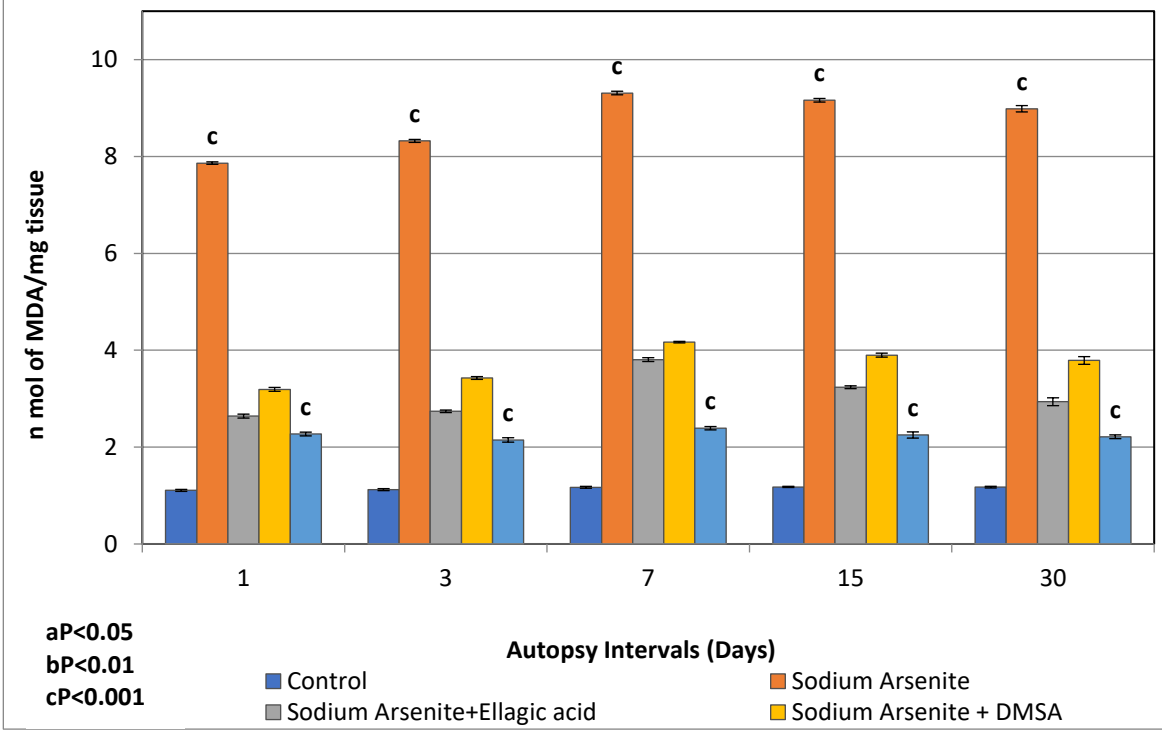
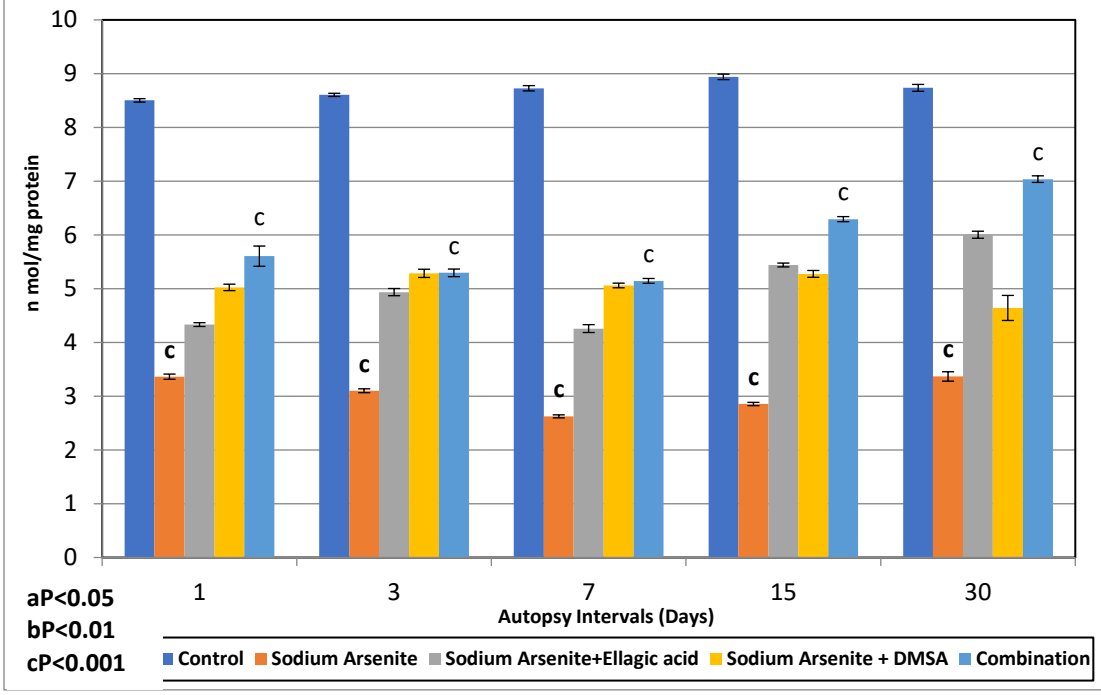


Fig.5.Variation in the testicular reduced GSH Level of various experimental groups in Swiss albino mice.



A balanced level of ALP is required for normal protein phosphorylation, apoptosis, cellular growth, and migration of germ cells, and elevated levels are often associated with disease conditions (Sharma *et al.*, 2014). The oxidative stress on the testes was observed with the elevated LPO levels, which was maximum in the control group-II .This oxidative damage was reduced by the chelation of arsenic in DMSA experimental group -III with metal chelation and excretion. In the Ellagic acid experimental group –IV, the anti-oxidative properties reduced the arsenic-induced oxidative stress, evident with the higher glutathione values. The reduction in arsenic-induced ROS and mitochondrial damage after arsenic exposure of rats has been reported by Ellagic acid treatment in rats (Keshtzar *et al.*, 2016). The highest protection was afforded in the combination group V (DMSA+Ellagic acid), with a highly significant reduction of arsenic-induced oxidative stress in the form of low LPO levels and elevated antioxidant status with higher GSH levels.

Thus, it can be concluded that the combination therapeutic approach of DMSA and ellagic acid with more comprehensive protective coverage is a good option for managing arsenic-induced testicular toxicity.

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