

Studies of As₂O₃ Poisoning on Protein, RNA and Glycogen of Albino Rats



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Abstract : The toxic effects of arsenic compounds are known since ancient times and they have been associated with criminal poisoning for many centuries.

The present study deals with the estimation of protein, RNA and glycogen contents in liver, kidney and brain of albino rat after the As₂O₃ administration. Albino rats were given arsenic trioxide (0.2mg/100 gm body weight/day) orally. The animals were sacrificed after 7, 14, 21, 30, 60, 90, 120, 150 and 180 days of poison administration, their liver, kidney and brain were removed, and processed for the estimation of soluble protein, RNA and glycogen contents. The results obtained were compared with control sample.

The results of the study show a significant depletion of protein, RNA and glycogen contents in brain, liver and kidney during the early phase of poison administration, which became prominent during the later period. It is probably due to the inhibitory action of As₂O₃ on protein, RNA and carbohydrate synthesis or stimulation of catabolism through some enzyme reaction.

Key words : Arsenic trioxide, Albino rat, protein, RNA and glycogen.

Introduction

Arsenic is a transition element or metalloid. Its compounds are used as pesticides, fungicides and rodenticides, or used in glass, electroplating, dyestuff, paint and cosmetic industries, which can cause poisoning (NAS, 1977 and WHO, 1981). It is also reported as an environmental pollutant that causes an environmental tragedy in some area of the world in which a large population in drinking arsenic contaminated ground water (Mazumder *et al.*, 1998 and Rehman *et al.*, 2002).

Several arsenic compounds are used as poison since ancient time. Amongst them, arsenic trioxide is the most commonly used

poison. Arsenic trioxide (As₂O₃) is usually used as homicidal, suicidal and accidental poisoning. Moreover, its use as a chronic poison is very common because the chronic As₂O₃ poisoning resemble with the symptoms of death caused by some wasting disease. Hence, it is frequently used as chronic poison in most of the cases of homicidal death.

The adverse health effects of arsenic may be observed on the respiratory, gastrointestinal, cardio-vascular, nervous and haematopoietic systems (Goodman and Gilman, 1985). Arsenicals are absorbed through mucous membranes. The absorption of arsenic compounds in liver, kidney and intestine and other body tissues of rat has been reported by Ducoff *et al.* (1948) and Dutkiewicz (1977).

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The present study is an effort to highlight the effects in protein, glycogen and RNA contents in liver, brain and kidney of albino rats after continuous As₂O₃ administration.

Material and Methods

Substance

Arsenic trioxide (As₂O₃) is selected for the present investigation because it is frequently used in homicidal, suicidal and other cases.

Experimental Animals

Healthy adult albino rats (both male and female) of 4-6 months age and about 120±10 gm weight were selected for the present investigation. The rats were reared in wooden cages and fed regularly with balanced diet and water (Farris, 1954) to avoid the stress of starvation and thirst. The rats were acclimatized to laboratory conditions for 15 days prior to the experimentation.

Dose

The dose of As₂O₃ was chosen on the basis of guidelines suggested by different workers. Hayes (1975) mentioned a sub-lethal dose (LD₅₀) of arsenic trioxide as 72-mg/kg for rats and 70-180 mg for human being. Taking this dose into consideration 0.2 mg/100gm (1/36th of LD₅₀) of Arsenic trioxide (As₂O₃) was administered to rats for sub acute and chronic studies.

Mode of Poison Administration

Arsenic trioxide or Sankhia (As₂O₃) was added in polyethylene glycol (PEG)-400 to form a suspension. The suspension was administered orally to each experimental animal with the help of cannula fixed on a syringe. It was given regularly to the selected groups of experimental animals as the oral dose of 0.2 mg/100gm/ day for the period of 7, 14, 21, 30, 60, 90, 120, 150, and 180 days respectively.

Procedure

The albino rats were divided into 10 groups. Each group contained 6 animals. The

first group was kept as control while groups 2 to 10 were given As₂O₃ upto 180 days as mentioned above. The animals were observed for the pharmacological changes during the period of 7, 14 and 21 days for sub-acute study and 30, 60, 90, 120, 150 and 180 days for chronic study. During the study, about 10% mortality was observed in poisoned groups.

The animals were sacrificed, their liver, kidney and brain were removed and processed for the quantitative estimation of protein, glycogen and RNA by using following methods.

Biochemical Estimation

For estimation of protein, glycogen and RNA contents in experimental tissue, Biuret, Anthron and Orcinol methods applied (Jayaraman, 1996 and Plummer, 1985).

Results and Discussion

In the present study, depletion has been observed in protein RNA and glycogen in tissues due to chronic As₂O₃ administration in experimental animals (Tables 1 to 3).

Table-1 shows that there was no significant change in protein concentration of brain, liver and kidney of albino rats up to 14 days. However a significant decrease in protein contents of liver was observed after 14 days. Further in brain and kidney a significantly decrease observed after 21 days of As₂O₃ administration, which become more pronounced after the period of 60 to 180 days.

Probably the hypoproteinaemia in the tissues of experimental animals may be the reason for the decrease in protein concentration of poisoned rats. It is well known that arsenic compounds initially bind to cellular proteins of the tissues and influences protein synthesis at some stage.

Thompson (1948) and Webb (1966) mentioned that inorganic arsenic inhibits enzyme activity and the trivalent inorganic arsenic reacts with the sulfhydryl groups of protein.

Bogdan *et al.* (1994) elucidated that protein contents are inhibited with inorganic arsenic.

The chemical properties of arsenic are similar to those of nitrogen and phosphorus, which are important elements of DNA, RNA and protein. Arsenic increases the normal function of some enzymes through disruption of the formation of ATP from ADP and orthophosphate, which regulate the process of phosphorylation and dephosphorylation. The WHO report (1981) describes that arsenic is known to react strongly with sulfhydryl groups of proteins, by interfering with the normal biochemical function of protein that are regulated by the formation of -S-S- bonds involving the cysteine side chain in the proteins. According to Vahter and Marafante (1989) and Thompson (1993), the detoxification of inorganic As in human body involves several complicated biotransformation processes such as arsenic protein binding in tissues and blood.

Bernstam *et al.* (2002) also found a significant inhibition of DNA and protein synthesis by As (III) exposure.

Simeonova *et al.* (2001) also mentioned that As (III) may alter activating protein -1 (AP-1) and nucleus factor *kappa B* (NF-Kappa B) DNA binding activity.

The findings of the present study are in accordance with the above theoretical assumptions.

The glycogen concentration in liver, brain and kidney of control and As₂O₃ administered rats is shown in Table-2. It reveals that whereas a significantly depletion in glycogen contents was found in liver after 7 days, the remarkable decrease of glycogen quantity was seen after 21 days in brain and kidney. The depletion in glycogen in liver, brain and kidney was aggravated during the later period of As₂O₃ administration.

It is evident from these results that liver has a strong reduction of glycogen content than

spinal cord, brain, and kidney. The liver is an organ, which stores glucose in the form of glycogen. The stored glycogen is converted into glucose, whenever body requires it. As liver gets longer time of interaction with maximum amount of As₂O₃, it in turn inhibits the enzymes in liver, required for glycogenesis and thus causes the reduction of glycogen concentration in liver at an early stage.

These findings are in accordance with the studies of Reichl *et al.* (1990) who reported that glycogen diminution on liver is due to carbohydrate depletion, which is an important characteristic in arsenic toxicity.

Albores *et al.* (1996) also mentioned that As (III) causes hydropic degeneration, total loss of glycogen and necrosis due to decreased adenosine triphosphate synthesis (ATP).

Furthermore, the mechanism of As₂O₃ toxicity in other species as PDH inhibitor with consecutive citric acid cycle and gluconeogenesis inhibitor and excessive carbohydrate depletion had been reported by Reichl *et al.* (1989) and Kawaguchi (1981). These finding also corroborate the results of the present study.

According to Table-3 the significant depletion in RNA concentration in liver, brain and kidney was also found during the study period. There was no significant change in RNA concentration of brain and kidney of albino rats upto 30 days. However, a significant decrease in RNA concentration was found in liver and brain, and in kidney after 30 days of As₂O₃ administration, which became stronger during later period of study.

The protein and RNA ratio decreased in rats exposed to As₂O₃ and showed a positive correlation. The loss of RNA and protein contents in tissues indicates the possible interference of As₂O₃ with nucleic acid synthesis.

It is stated that the RNA contents and the RNA/DNA ratio of a tissue are considered

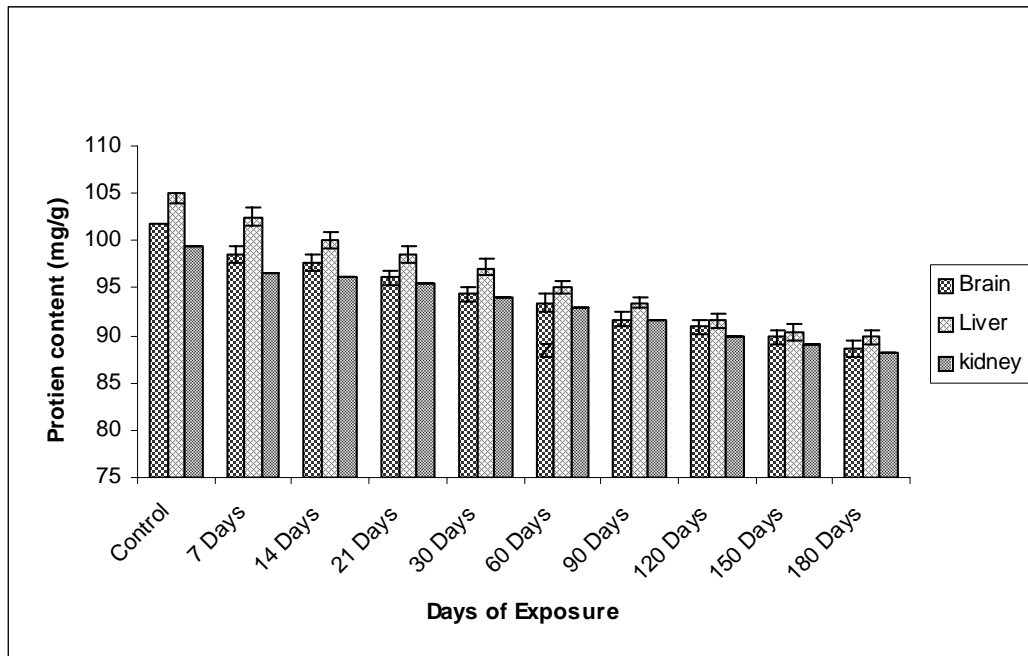


Fig. 1 : Depletion of protein contents in brain, liver and kidney of albino rats after arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

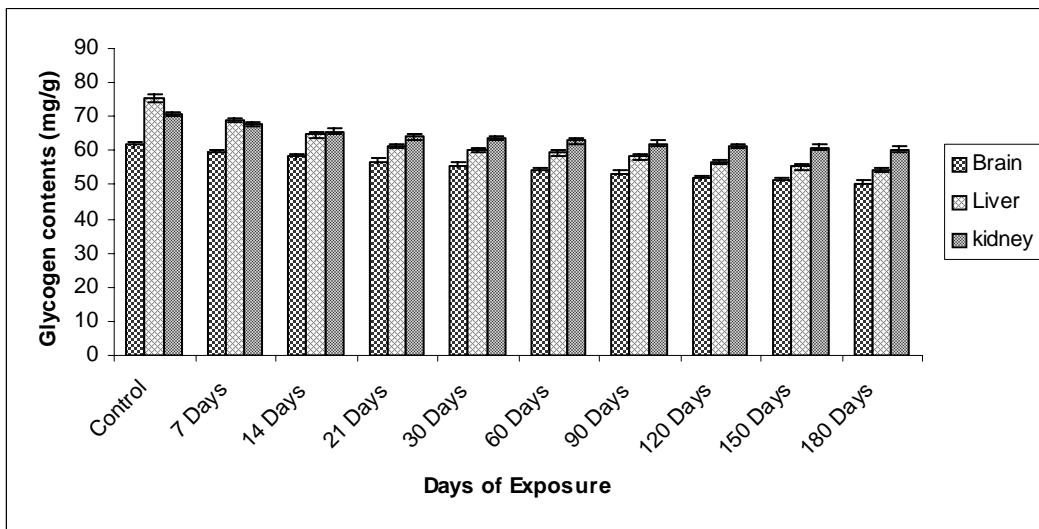


Fig. 2 : Depletion of glycogen contents in brain, liver, kidney of albino rats during chronic arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

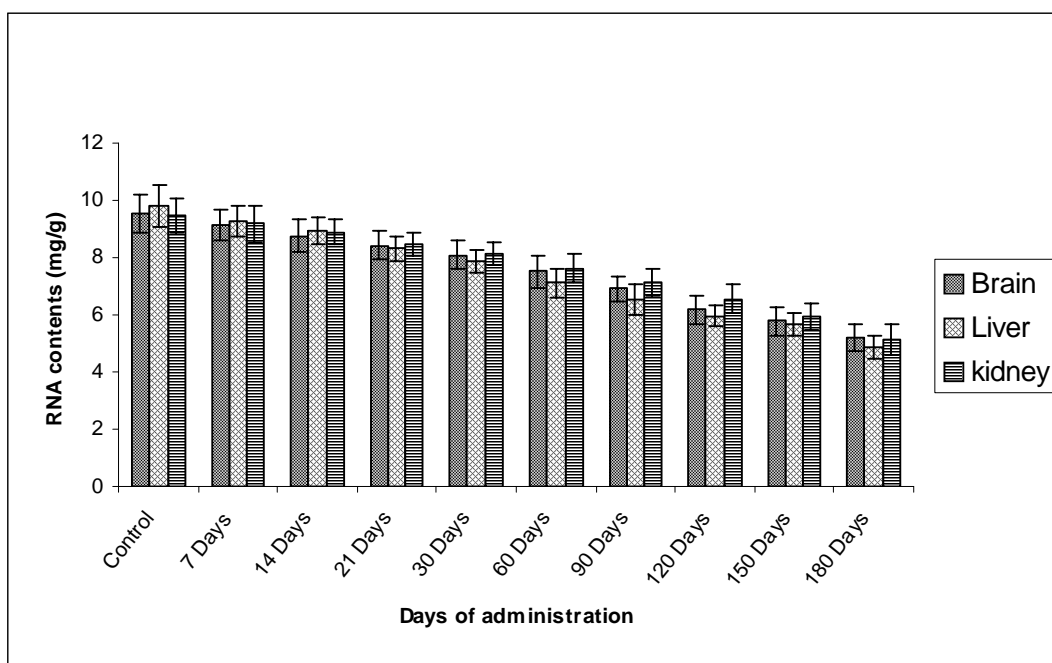


Fig. 3 : Depletion of RNA contents in brain, liver and kidney of albino rats arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

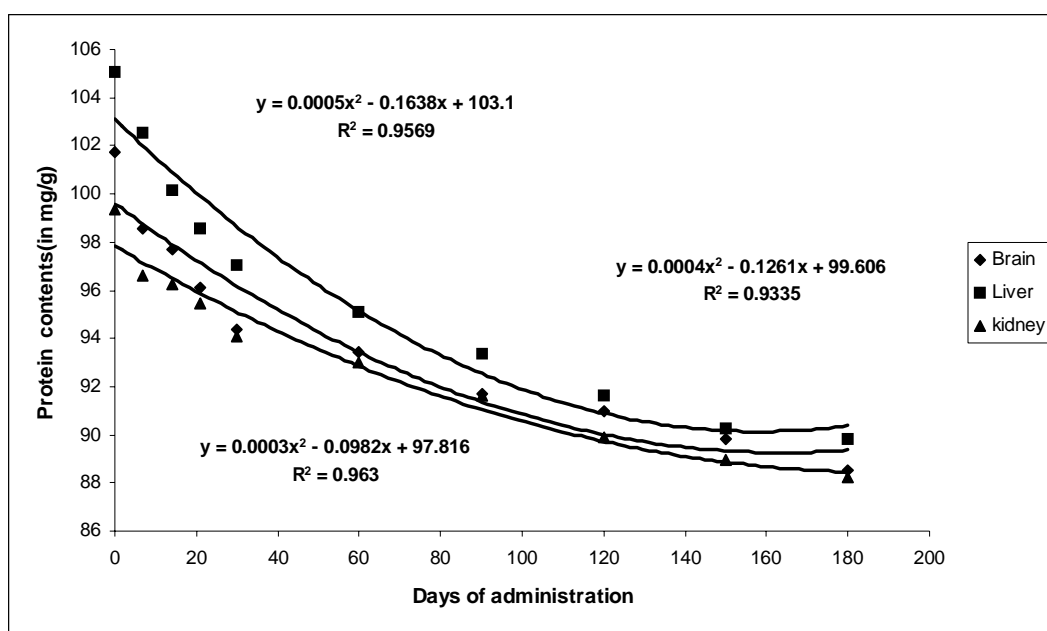


Fig. 4 : Regressed line and equation on protein contents (Y) on day (X) of albino rats chronic arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

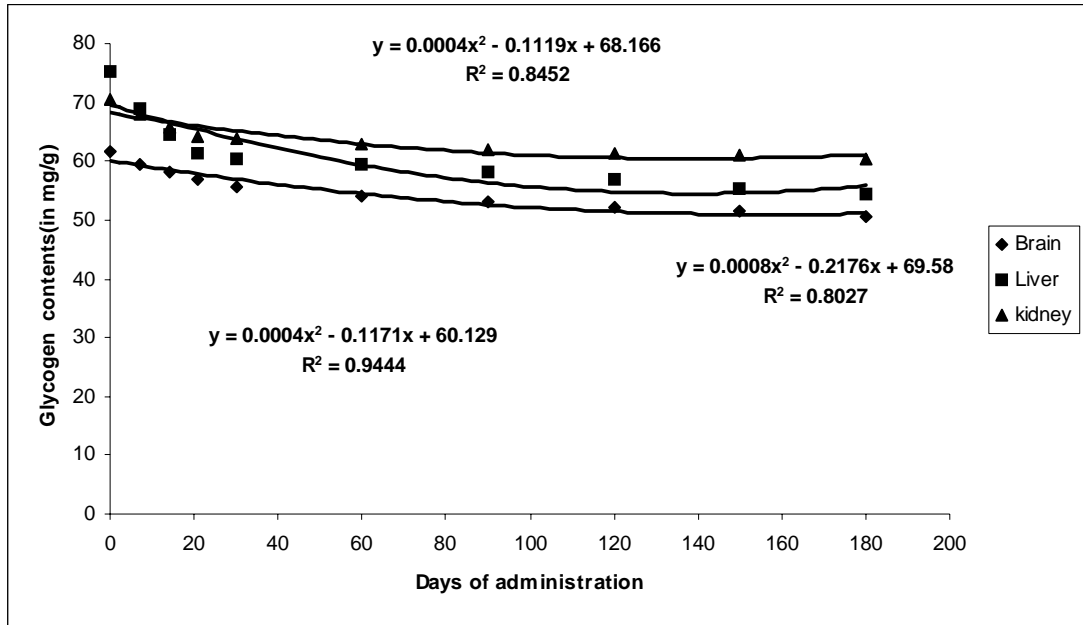


Fig. 5 : Regressed line and equation on glycogen contents (Y) on day (X) of albino rats of chronic arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

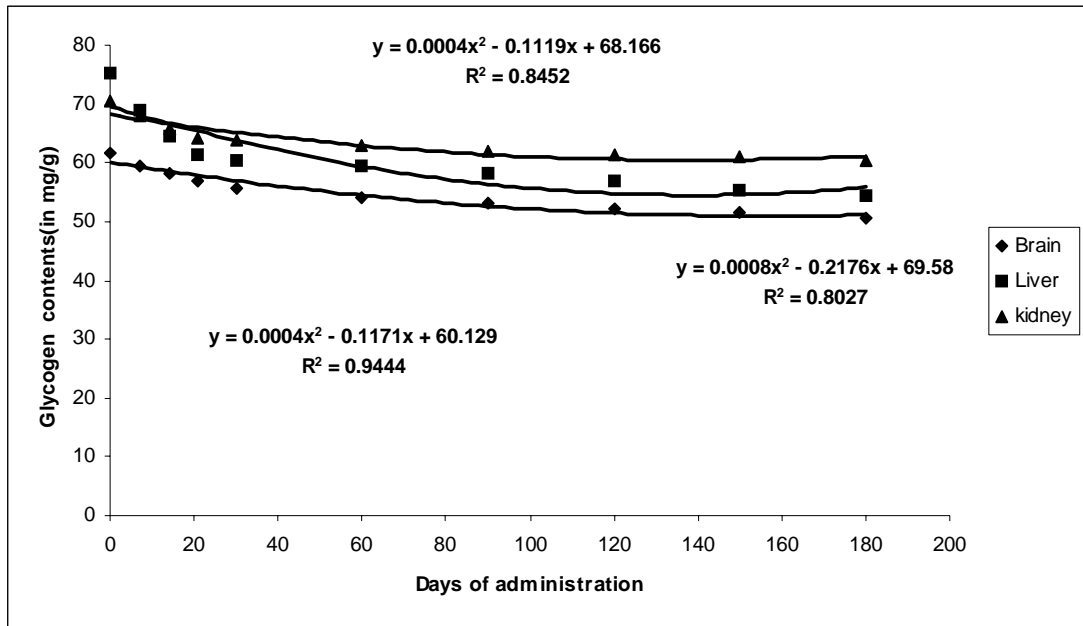


Fig. 6 : Regressed line and equation on RNA contents (Y) on day (X) of albino rats on chronic arsenic trioxide administration. Values are expressed as mean±S.E. (n = 6)

Table 1 : Statistical values of protein contents in brain, liver and kidney of albino rats during chronic sankhia (As₂O₃) administration
n=6 Dose = 0.2 mg/100gm/day oral

S. No.	Tissue	Control	EXPERIMENTAL GROUP (Days of exposure)											
			Sub acute study			chronic study								
			7	14	21	30	60	90	120	150	180			
1	BRAIN	101.73 ± 0.932	98.59 ± 0.77 *	97.69 ± 0.76 *	96.13 ± 0.75 P<.01	94.40 ± 0.87 P<.01	93.47 ± 0.74 P<.001	91.68 ± 0.75 P<.001	90.96 ± 0.71 P<.001	89.86 ± 0.90 P<.001	88.56 ± 0.81 P<.001			
2	LIVER	105.08 ± 1.09	102.51 ± 0.85 *	100.13 ± 0.83 P<.01	98.57 ± 0.94 P<.01	97.08 ± 0.67 P<.001	95.12 ± 0.70 P<.001	93.40 ± 0.54 P<.001	91.64 ± 0.85 P<.001	90.26 ± 0.78 P<.001	89.85 ± 0.78 P<.001			
3	KIDNEY	99.37 ± 0.86	96.59 ± 0.86 *	96.23 ± 0.83 *	95.43 ± 0.77 P<.02	94.08 ± 0.76 P<.01	92.99 ± 0.92 P<.001	91.62 ± 0.82 P<.001	89.93 ± 0.84 P<.001	88.94 ± 0.75 P<.001	88.21 ± 0.80 P<.001			

* - Non-significant

Table 2 : Statistical values of glycogen contents in brain, liver and kidney of albino rats during chronic sankhia (As₂O₃) administration
n=6 Dose = 0.2 mg/100gm/day oral

S. No.	Tissue	Control	EXPERIMENTAL GROUP (Days of exposure)											
			Sub acute study			chronic study								
			7	14	21	30	60	90	120	150	180			
1	BRAIN	61.71 ± 0.88	59.59 ± 0.78 *	58.28 ± 0.88 *	56.87 ± 0.79 P<.01	55.57 ± 0.83 P<.01	54.18 ± 0.99 P<.001	53.27 ± 0.80 P<.001	52.05 ± 0.64 P<.001	51.39 ± 0.58 P<.001	50.48 ± 0.77 P<.001			
2	LIVER	75.36 ± 0.96	68.91 ± 0.74 P<.01	64.60 ± 0.86 P<.001	61.38 ± 0.61 P<.001	60.35 ± 0.66 P<.001	59.39 ± 0.87 P<.001	58.17 ± 0.65 P<.001	56.83 ± 0.67 P<.001	55.24 ± 0.79 P<.001	54.23 ± 0.74 P<.001			
3	KIDNEY	70.67 ± 0.75	67.88 ± 0.77 *	65.69 ± 0.78 P<.01	64.18 ± 0.93 P<.01	63.76 ± 0.79 P<.001	62.92 ± 0.90 P<.001	62.11 ± 0.76 P<.001	61.46 ± 0.77 P<.001	60.94 ± 0.82 P<.001	60.38 ± 0.85 P<.001			

* - Non-significant

Table 3 : Statistical values of RNA contents in brain, liver and kidney of albino rats during chronic sankhia (As₂O₃) administration
n = 6
Dose = 0.2 mg/100gm/day oral

S. No	Tissue	Control	EXPERIMENTAL GROUP (Days of exposure)									
			Sub acute study					chronic study				
			7	14	21	30	60	90	120	150	180	
1	BRAIN	9.54 ± 0.68	9.12 ± 0.54 *	8.76 ± 0.56 *	8.42 ± 0.49 P<.05	8.10 ± 0.52 P<.05	7.52 ± 0.58 p<.01	6.927 ± 0.42 p<.01	6.18 ± 0.50 p<.01	5.77 ± 0.53 p<.01	5.21 ± 0.46 p<.001	
2	LIVER	9.80 ± 0.72	9.26 ± 0.52 *	8.92 ± 0.48 *	8.31 ± 0.42 *	7.86 ± 0.38 P<.05	7.12 ± 0.49 p <.01	6.54 ± 0.52 p<.01	5.96 ± 0.36 p<.01	5.67 ± 0.42 p<.01	4.88 ± 0.40 p<.001	
3	KIDNEY	9.48 ± 0.58	9.18 ± 0.62 *	8.88 ± 0.44 *	8.46 ± 0.40 *	8.14 ± 0.42 P<.05	7.62 ± 0.50 p <.01	7.12 ± 0.47 p<.01	6.56 ± 0.52 p<.01	5.93 ± 0.46 p<.01	5.12 ± 0.53 p<.001	

as to indicate the intensity of protein synthesis. These two parameters show a positive correlation with the protein and RNA contents indicates the intensity of protein synthesis in a tissue. In view of the observations on protein and RNA contents in the present investigation, a deficient synthesis of any type of RNA should have its reflection in a corresponding failure to protein synthesis.

Petres *et al.*, (1977) reported that a general explanation for the inhibitory effect of inorganic arsenic on cell metabolism is the known strong affinity of arsenic to enzymes, especially to those containing sulfhydryl groups.

Nakamuro and Sayato (1981) elucidated comparative cytogenic effects on the synthesis of DNA, RNA and protein in cultured mammalian cells due to arsenic.

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