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EFFECT OF BAUHINIA ACUMINATA-L STEM BARK EXTRACT ON DETOXIFICATION OF ARSENIC IN RATS

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Abstract: A study was undertaken to evaluate the efficacy of Bauhinia accuminata (BA) stem bark extract against sodium arsenite (NaAsO2) induced toxicity in adult albino rats. Forty eight adult albino rats having body weight 150-200 gm of either sex were randomly divided into four groups, viz., A0, A1, A2 and A3 each contains twelve rats.Sodium arsenite was administered at 4mg/kg daily in drinking water for 90 days to groups A1, A2 and A3. Groups A2 and A3 were orally treated with BA stem bark extract at 300 mg/kg (1/10th of LD50) and at 150mg/kg (1/20th of LD50) daily respectively from day 91 to 120 day, while only water was given to group A0 (control). Tissue samples, hair, faeces were collected at different days for analysis of total arsenic concentration. Speciation was performed on faeces, liver and hair. The results expresses that the stem bark extract of Bauhinia acuminata (BA) may be effective against induced arsenicosis in rats.

Index Terms **- Arsenic, Rats, Bauhinia acuminata, speciation, total arsenic concentration.**

I. INTRODUCTION

The vascular effects of arsenic in drinking water are health concern contributing to human disease in world wide. Arsenic is a naturally occurring element that has been recognized as a human poison since ancient times. It is ubiquitously distributed in the environment in a number of organic and inorganic forms and thus exposure to this metalloid has become inevitable for both man and animals(1). Because of the abundence of arsenic, human experience duly exposures via ingestion as major route through drinking water and inhalation and skin absorption as a minor route (2). Arsenic occurs both in organic and inorganic form in nature but inorganic species AS(III) and AS (V) are more predominant and posses a threat to human and animal health (3). Groundwater, used for drinking and agricultural purposes, is the main route of arsenic exposure, continuous exposure of arsenic through drinking water has been reported in many countries of the world where the level of arsenic exceeds the safe permissible limit set by WHO(4). Arsenic contained in water, soil or food, ingested arsenic may quickly enter the human body when are containing arsenic dusts is breathed in, the majority of the dust particles settle on to the linins of the lungs (5). Very little internal exposure to arsenic occurs via the material passing through the skin in to the body and so there is little risk at arsenic poisoning posed by this round. Acute arsenic poisoning is uncommon It is usually the result of accidental or suicidal insertion of insecticides or pesticides .Toxicity is manifested by the stomach and intestine damage (diarrhea, vomiting, hemorrhage, electrolyte disturbances), cardiac arrhythmia and face edema. Arsenic acts as a local irritant causing local skin inflammation , ulceration and perforation of the nasal septum. Symptoms of sub acute and chronic arsenic intoxication concern generally the respiratory, alimentary, cardiovascular and nervous systems or bone marrow,(6, 7, 8, 9)the most important organ involved by arsenic is the liver. The toxic effect as not restricted only to the disorder of hepatocytes function and the increased level of enzymes it is often observed that the liver fibrosis leads to chronic hepatic failure. Due to ground water contamination a large number of populations in West Bengal and Bangladesh are suffering from

malanosis, leucao melanosis, Keratosis, Hyperkeratosis, dorsum, non petting oedema, gangrene, Skin cancer and skin lesions in sole and palm (10).

Bauhinia acuminata L belonging to the family, Leguminosae, caesal pinioideae, an evergreen large shrub, grows in many parts of India, several chemical compounds including palmitic acid, three phthalic acid esters, phthalic acid, Gallic acid, Ursolic acid were identified from the leaves of B. acuminata. (11). Bauhinia acuminata have anti inflammatory, anti oxidant and antimicrobial activity (12). However, beneficial effect of Bauhinia acuminata , if any on heavy metal toxicity in general and arsenic toxicity in particular has not been examined. The present work has under taken to examine beneficial effect of *B. acuminata* in arsenicosis,if any.

II. MATERIALS AND METHODs

2.1Chemicals

All chemicals and kits under this study were obtained from Bangalore Geni (India), Congent (India), Merk (Germany), Rankem (India) and Sigma Chemicals (USA).

2.2 Experimental animals

Forty eight adult albino rats (either sexes) having body weight 150-200 gm were procured from registered animal breeder. They were caged in polypropylene cages and were acelimatized in experimental animal room for seven days before starting the experiment. The animals were maintained with standard pellet feed and provided drinking water ad libitum. The Institute of Animal Ethical Committee approved the technical programme.

2.3 Preparation of *Bauhinia acuminata* (BA) stem bark extract

The plant identification was made from BSI (Botanical Survey of India), Howrah, Kolkata having specimen no. WBUAFS/KOL/ 2.

After collection of *Bauhinia acuminata*, stem barks were collected washed with water shade dried and powdered coarsely. A 300 gm of bark powder was placed in soxhlet apparatus and 200 ml of ethanol (99.9%) was added and heated up at 60ºC - 70ºC continuously upto 8 to 12 hrs. After completion of extraction, whole extract was condensed through rotary vacuumevaporator. Condensed extract was kept in room temperature for 2 to 3 days. This semi-solid solution was dissolved in water (Triple distilled) and was analysed for one of the active principle of the extract (Baicalein)before administration to rats.

2.4 Analysis of Baicalein (one of the active principle) in Bauhinia acuminata by HPLC

Analysis of Baicalein in BA extract was done by HPLC as per the method described by De *et al* 2016 (13).

2.5 Determination of LD_{50} of BA stem bark

LD50 of BA stem bark extract in rats was determined as per the method described by Ghosh (14) and it was found to be 3000 mg/kg.

2.6 Estimation of total arsenic

Total arsenic was estimated as per standard method (15) using Atomic Absorption Spectrometer equipped with vapour generation accessories.

2.7 Instrument

A Varian AA240 model AAS equipped with vapour generation accessories (model no.VGA77) was used for total arsenic estimation. Reducing agent (aqueous solution of 0.6% sodium borohydride in 0.5% sodium hydroxide) and acid (40% hydrochloric acid) were prepared freshly before use.

2.7 .1 Instrumental condition

Arsenic hollow cathode lamp; wave length, 193.7nm; slit width, 0.5nm; lamp current, 10.0mA; vapor type, air/acetylene; air flow, 10 l/min; inert gas for hydride generation, argon.

2.8 Arsenic Speciation study

Arsenic speciation in faeces , liver, hair was made according to the method of Datta et al (15) described by De *et al* (13).

2.9 Statistical analysis

The data were analysed statistically by using univariant General Linear Model with two way ANOVA in SPSS 10 version of software.

IV. RESULTS AND DISCUSSION

4.1 Results

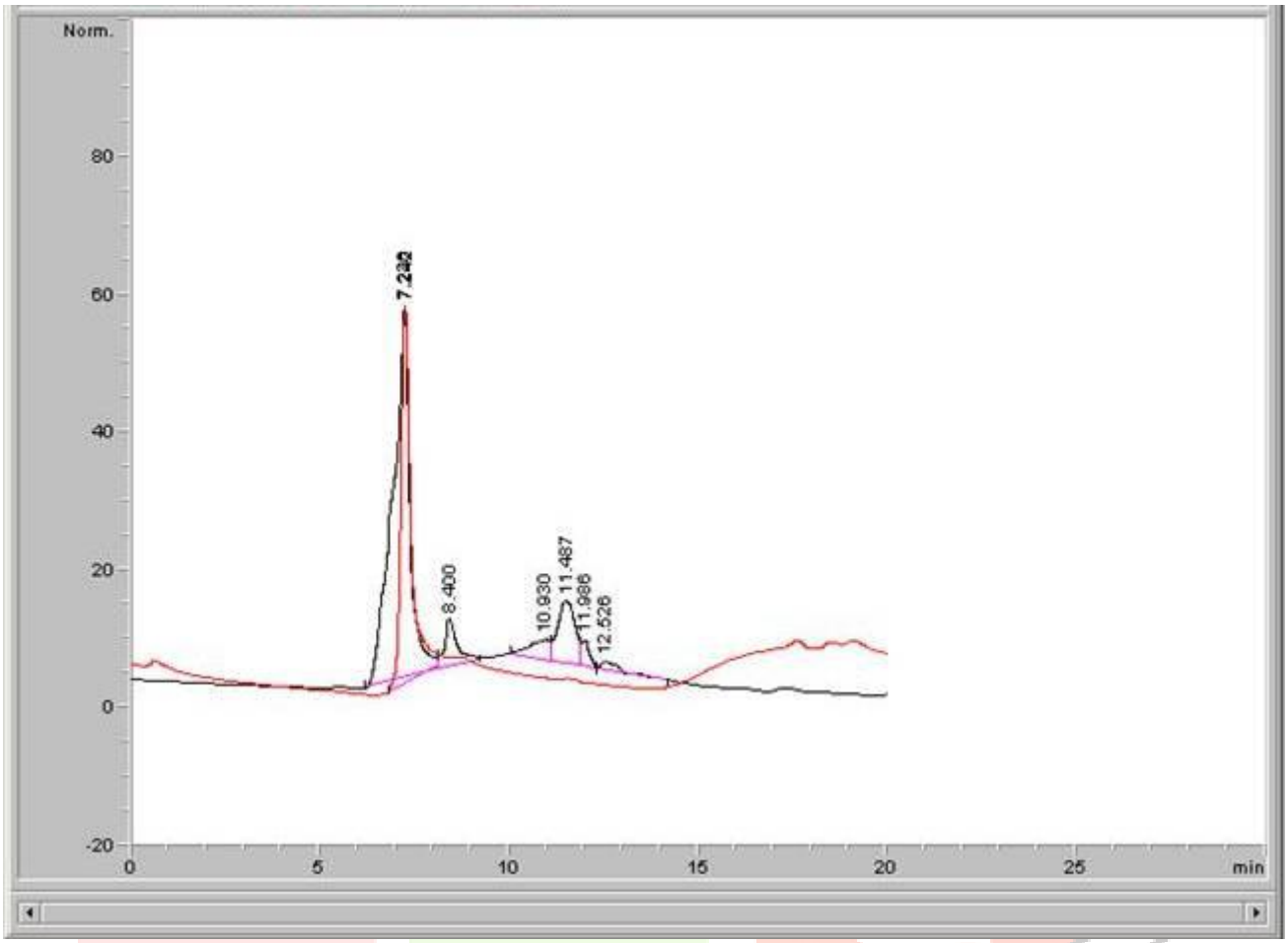
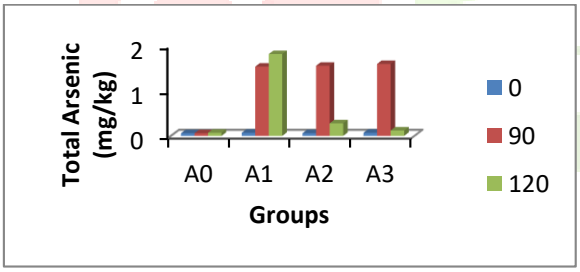
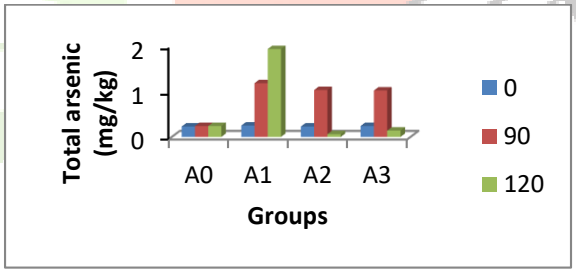


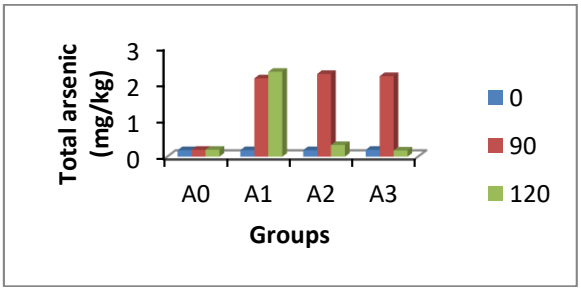
Fig4.1Charomatogram of Baicalein in standard and in plant stem bark extract



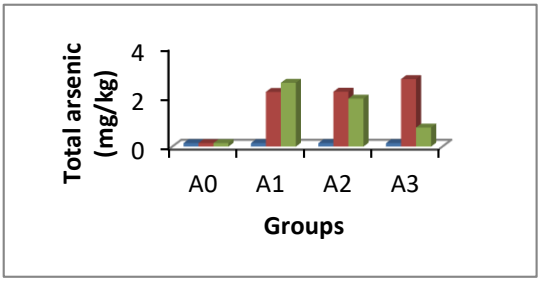
Lung



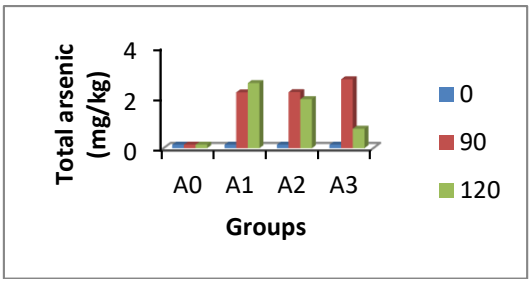
heart



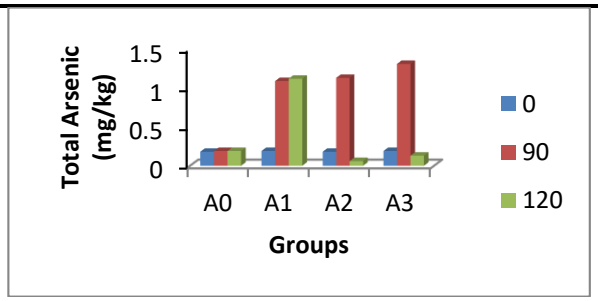
Intestine



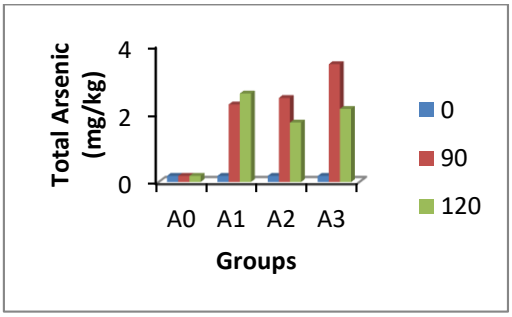
kidney



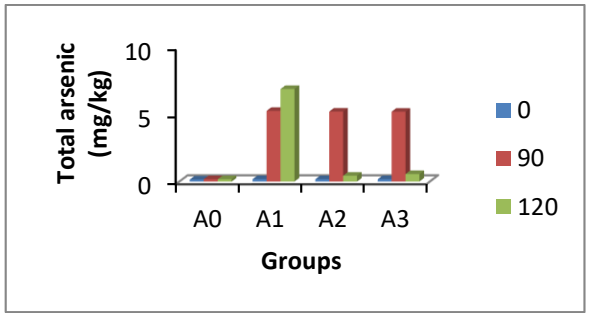
Muscle



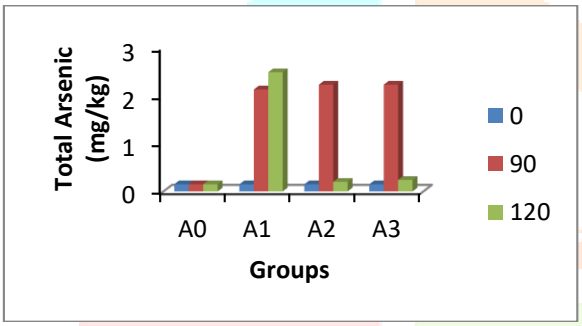
liver



Spleen



hair



faeces

Fig.4.2 : Concentration of total arsenic (mg/kg) in lung , heart , intestine, kidney, muscle, liver, spleen, hair, faeces of rat of different groups and days (n=4).

Table 4.3 : Speciation of arsenic in faeces of rat after daily single oral administration of sodium arsenite 4mg/kg for consecutive 90 days and ameliorative effect by BA stem bark extract at 300 mg/kg and 150mg/kg after 90 days onwards (Mean ±SE, n=4,)

Groups	Arsenite (As^{+3})%			Arsenate (As^{+5})%			Organo arsenic%		
	0	90	120	0	90	120	0	90	120
A_0	26.18 ± 0.59^{y}	28.25 ± 0.61^{xyb}	29.66 ± 0.74^{xb}	9.64 ± 0.22^{y}	10.44 ± 0.13^{xb}	11.00 ± 0.09^{xb}	64.18 ± 0.40^{x}	61.31 ± 0.74^{ya}	59.34 ± 0.70^{ya}
A_1	29.82 ± 1.47^{y}	33.88 ± 0.85^{yab}	47.59 ± 1.16^{xa}	9.69 ± 0.12^{y}	29.81 ± 4.93^{xa}	38.18 ± 1.86^{xa}	60.49 ± 1.58^{x}	36.97 ± 5.71^{yb}	14.23 ± 2.49^{zb}
A_2	26.05 ± 0.88^{y}	37.17 ± 1.90^{xa}	29.55 ± 0.88^{yb}	9.05 ± 0.45^{y}	27.42 ± 1.59^{xa}	12.69 ± 1.23^{yb}	64.90 ± 0.66^{x}	35.42 ± 0.52^{zb}	57.76 ± 2.06^{ya}
A_3	27.00 ± 0.85^{y}	35.12 ± 2.02^{xab}	32.36 ± 1.52^{xyb}	9.89 ± 0.34^{z}	28.30 ± 1.24^{xa}	17.89 ± 2.29^{yb}	63.12 ± 1.02^{x}	36.58 ± 3.26^{zb}	51.18 ± 2.80^{ya}

Values are mean with SE.
Rows bearing different superscript (x-z) differ significantly (P<0.05).
Columns bearing different superscript (a-d) differ significantly(P<0.05).
Group A_0 : Received water for 120 days orally.
Group A_1 : Received sodium arsenite at 4mg/kg orally once daily for 90 days.
Group A_2 : Received sodium arsenite at 4mg/kg orally once daily for 90 days and received $^{1}/_{10}^{th}$ of LD_{50} BA (300 mg/kg) from day 91 to 120 day once daily orally.
Group A_3 : Received sodium arsenite of 4mg/kg orally once daily for 90 days and received $^{1}/_{20}^{th}$ of LD_{50} of BA (150 mg/kg) from 91 day to 120 day once daily orally.

Table4.4 : Speciation of arsenic in liver of rat after daily single oral administration of sodium arsenite 4mg/kg for consecutive 90 days and ameliorative effect by BA stem bark extract at 300 mg/kg and 150mg/kg after 90 days onwards (Mean ±SE, n=4)

Groups	Arsenite (As^{+3})%			Arsenate (As^{+5})%			Organo arsenic%		
	0	90	120	0	90	120	0	90	120
A_0	21.00 ± 0.58^{z}	23.97 ± 0.85^{yc}	26.50 ± 0.35^{xb}	2.20 ± 0.15^{y}	2.33 ± 0.09^{yb}	2.80 ± 0.09^{xc}	76.80 ± 0.72^{x}	73.74 ± 0.86^{ya}	70.70 ± 0.42^{za}
A_1	21.10 ± 0.55^{y}	45.33 ± 1.76^{xab}	52.30 ± 1.96^{xa}	3.23 ± 0.15^{z}	10.25 ± 0.38^{ya}	12.08 ± 0.31^{xa}	76.00 ± 0.76^{x}	44.42 ± 2.13^{yb}	35.63 ± 2.24^{zb}
A_2	21.44 ± 0.45^{z}	48.07 ± 1.15^{xab}	25.74 ± 0.65^{yb}	2.52 ± 0.34^{z}	10.92 ± 0.44^{xa}	4.11 ± 0.12^{yb}	76.04 ± 0.78^{x}	41.02 ± 0.51^{zb}	70.15 ± 0.63^{ya}
A_3	22.17 ± 0.94^{z}	49.55 ± 0.87^{xa}	29.69 ± 1.48^{yb}	2.55 ± 0.37^{z}	11.07 ± 0.64^{xa}	4.73 ± 0.52^{yb}	75.28 ± 1.30^{x}	39.38 ± 0.59^{yb}	65.59 ± 1.42^{za}

Table 4.5 : Speciation of arsenic in hair of rat after daily single oral administration of sodium arsenite 4mg/kg for consecutive 90 days and ameliorative effect by BA stem bark extract at 300 mg/kg and 150mg/kg after 90 days onwards (Mean ±SE, n=4)

Groups	Arsenite (As^{+3})			Arsenate (As^{+5})			Organo arsenic		
	0	90	120	0	90	120	0	90	120
A_0	25.85 ± 1.16^{y}	28.20 ± 0.55^{yb}	32.79 ± 1.04^{xb}	8.46 ± 0.27^{y}	9.52 ± 0.23^{yb}	11.98 ± 0.48^{xb}	65.69 ± 1.43^{x}	62.23 ± 0.78^{xa}	55.29 ± 1.35^{ya}
A_1	27.23 ± 0.58^{y}	37.62 ± 0.88^{xa}	42.30 ± 1.82^{xa}	8.73 ± 0.27^{y}	15.80 ± 2.53^{yab}	25.89 ± 2.04^{xa}	64.03 ± 0.31^{x}	46.58 ± 3.40^{yb}	31.82 ± 3.75^{zb}
A_2	26.67 ± 1.71^{y}	36.03 ± 1.86^{xa}	29.54 ± 0.88^{yb}	9.28 ± 0.37^{y}	18.72 ± 0.02^{xa}	14.12 ± 1.62^{xyb}	64.05 ± 1.78^{x}	45.25 ± 3.88^{yb}	56.35 ± 1.09^{xa}
A_3	26.28 ± 1.14^{z}	35.69 ± 1.01^{xa}	31.08 ± 1.00^{yb}	9.77 ± 0.74^{y}	20.04 ± 1.95^{xa}	15.97 ± 1.65^{xyb}	63.95 ± 1.82^{x}	44.27 ± 2.38^{zb}	52.96 ± 1.29^{ya}

4.6Discussion

One of the active compound Baicalein (containing Flavonoids) was estimated in Ethanolic stem bark extract of Bauhinia acuminata (16, 17).

It is evident from Fig.2 that total arsenic content in hair, faeces and vital organs did not alter significantly in group A0 (control) at different days compared to its '0' day value. It is also clear from the figures that total arsenic content in organs (lung, liver, kidney, heart, spleen, intestine and muscle), hair and faeces was significantly increased on 90 and 120 day in group A1 (untreated control) compared to respective "0" day.

The total arsenic content in vital organs, hair and faeces was also significantly ($P<0.05$) increased on 90 day in group A2 and A3 compared to respective '0' day but decreased on 120 day compared to 90 day following treatment with BA stem bark extract in rats at both the dose levels. But level of arsenic was not reduced in A1 on 120 day. Arsenic content in the above organs were significantly higher in all arsenicosis of rats. The concentration of arsenic was also higher in lungs, kidney, hair and faeces. Hair is one of the routes of elimination of arsenic. Arsenic accumulated to the greatest extent in the liver, spleen, kidney, lungs and gastrointestinal tract.

But clearance of arsenic from these organs is rapid following administration of BA stem bark extract.

Bertelero et al (18) reported that exposure to either arsenite or arsenate leads to an initial accumulation in the liver, kidney, lungs in most animal species which is similar to the present findings.

It is evident from Tables 1,2 and 3 that arsenite fraction in faeces, liver and hair did not alter significantly in group A0 at different day compared to its '0' day value. On the other hand arsenite fraction was significantly ($P<0.05$) increased on day 90 and 120 in group A1 but arsentie fraction was significantly ($P<0.05$) increased on day 90 but decreased on day 120 following administration of BA extract in groups A2 and A3 animals.

Further it is observed from Tables 1,2, and 3 that arsenate fraction in faeces, liver and hair did not alter significantly in group A0 at different days with respect to its '0' day value. But arsenate fraction was significantly ($P<0.05$) increased on the day 90 and 120 in group A1 whilst arsenate fraction was significantly ($P<0.05$) increased on the day 90 but decreased on day 120 in groups A2 and A3 animals.

On the other hand, organo-arsenic fraction in faeces, liver and hair did not alter significantly in group A0 at different days with respect to its '0' day value. But organo arsenic fraction was significantly decreased ($P<0.05$) on day 90 and 120 in group A1 while organo-arsenic fraction was significantly ($P<0.05$) decreased on day 90 but increased on day 120 for groups A2 and A3 animals.

Generally arsenate fraction in liver, hair and faeces was very low of the arsenic exposed animals compared to arsenite. Benramdane et al (19) also observed a very less accumulation of arsenate in tissues of intoxicated human.

During the carbon metabolism and methylation of arsenic, methylated arsenic metabolite like monomethyl arsenic acid (MMAIII) or even trimethyl arsenic acid may form during different methylation in human and animal (20).

It is not possible to separate all organo-arsenic species in the present study, hence a total orgno arsenic species was determined. It is reported that BA contains certain active principle like ursolic acid (11) the compound having seven methyl groups in its structure which may donate methyl group to inorganic arsenic through methylation resulting more productions of organo-arsenic in those organs of rats (21,22).

In the present study, in liver faeces and hair, organo-arsenic part was significantly lower compared to '0' day value in liver, faeces and hair . But the concentration of arsenite value was higher followed by arsenate value compared to respective '0' day in arsenicosis rats, and this is the fact that inorganic part of arsenic is responsible for toxicity. But an increased level of organo arsenic and decreased level of both arsenite and arsenate level has been observed in animals of groups A2 and A3 treated with stem bark extract of BA at different dose level.

4.7Conclusion :

It may be concluded that Bauhinia acuminata stem bark extract may have some ameliorative effect on reducing arsenicosis through trans methylation pathway of metabolism of arsenic .

III.ACKNOWLEDGMENT

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IV.ETHICAL STATEMENT

The Institutional Animal Ethics Committee approved the technical programme.

V.CONFLICT OF INTEREST

This is my self funding work. So there is no potential conflict of interest.

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