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Kavitha KV
P.G. Scholar, Dept of P.G Studies in Agadatantra, SDM College of Ayurveda, Udupi, Karnataka, India

Sudheendra V Honwad
Professor and Head,Dept of P.G Studies in Agadatantra, SDM College of Ayurveda, Udupi, Karnataka, India

Correspondence
Kavitha KV
P.G. Scholar, Dept of P.G Studies in Agadatantra, SDM College of Ayurveda, Udupi, Karnataka, India

An experimental study on the antidote effect of maricha (*Piper nigrum* Linn) kashaya in vatsanabha (*Aconitum ferox*) induced toxicity

Kavitha KV and Sudheendra V Honwad

Abstract
Mahavishas are a group of highly potential drugs and *Vatsanabha* is one among them. which is a cardiac poison by modern concept and main toxic principles are aconitine, pseudoaconitine. The substance which are counteracting or neutralizing the effect of poison are considered as antidotes, in Ayurveda prathyoushada concept can be compared with this. In *Kriyakoumudi* text Maricha kashaya mentioned as an antidote for *Vatsanabha* seed poisoning. To evaluate the antidote effect in *Vatsanabha* poisoning TED dose of Maricha kashaya is used. biochemical parameters, haematological parameters, histopathological studies were compared. *Vatsanabha* showed mild to moderate toxicity in liver, which have been reversed by Maricha kashaya in TED dose which is suggestive by results of biochemical parameters, and histopathological studies.

Keywords: *vatsanabha,* maricha *kashaya*, antidote

Introduction
Ayurveda is an Indian traditional and natural system of medicine which has been practiced since 5000 years. The treatment of Ayurveda is divided into 8 branches (*Astanga Ayurveda*) and *Agadatantra/Damshtrachikitsa* is one among those branches. *Visha* has been broadly classified as Sthavara and Jangama visha [1]. Sthavaravishai.e: plant origin poison is again divided into Mahavisha and Upavisha [2]. *Vatsanabha* is one among the category of Mahavisha and considered as potent cardiac poison [3]. The root of Vatsanabha includes toxic alkaloids like aconitine, pseudoaconitine[4].Vatsanabha is used in many Ayurvedic formulations like *Sanjivanivati, Anandabhairava rasa, Hinguleswara rasa* [5].
When *Vatsanabha* is used as one of the ingredient in the preparation of formulation its shodhana is very essential to reduce its toxicity and to modify its pharmacological actions. If it is used without proper purification it will produce toxic symptoms like burning sensation, nausea, vomiting, respiratory distress [6]. Accidental poisoning is common, since it gets confused with edible horse raddish root [7]. In medical practice always there are conditions to get the adverse reactions of *Vatsanabha* toxicity. So it is very essential to develop a very good and effective antidote.

Aims and objectives
1. To collect a detailed literary review on *Vatsanabha* rootalong with its antidote from different ayurvedic and modern text book.
2. To collect all the information available in the literatures about *Maricha*
3. To determine the toxicity profile of *Vatsanabha* root.
4. To evaluate experimentally, the antidotal effect of Maricha kashaya in *Vatsanabha* induced toxic effect.

Materials and Methods
Vatsanabha and Maricha were procured from SDM Pharmacy Udupi. Wistar strain albino rats of either sex were selected from Animal House of SDM Centre for Research in Ayurveda & Allied Sciences, Udupi. Selected rats were randomly placed under 4 groups and in each group 8 rats were included. They were maintained standard housing condition. the institutional animal ethical committee (SDMCAU/ACA-49/AECE27).
In kriyakoumudi text, dose of Maricha kashaya is given as 2g. The human dose of Maricha kashaya was converted to animal dose by using conversion formula as Human dose x 0.001 x

5. Thus 18 mg/kg body weight of Maricha was used as antidote.

Phase I – Acute toxicity test to determine the LD_{50} dose of the *Vatsanabha root powder*

The toxicant *Vatsanabha* dose was selected based on LD_{50} determined by acute oral toxic study in wistar albino rats. Maximum tolerated dose was calculated by employing OECD 425 guide lines with AOT Software. The LD 50 value was found to be 29.57 mg/kg with a confidence limit of 17.5 to 55 mg/kg

Phase II – Antidotal effect assessment

Group I – Vehicle Control (Fed with distilled water by oral route)

Group II – *Vatsanabha* seed, 1/5th of $LD_{50,}$ i.e 5.9mg/kg body weight of albino rats by oral route.

Group III – *Vatsanabha* seed, 5.9mg/kg body weight of albino rats with 18mg/kg body weight of albino rats Maricha kashaya in TED dose by oral route.

Assessment criteria

The assessment criteria of antidote effect of Maricha kashaya was made on the basis of

1. Gross behaviour
2. Ponderal Changes
3. Haematology–– haematocrit, haemoglobin concentration, erythrocyte count, PCV, MCV,MCHC, RDWC, RDWSD, Serum sugar
4. Biochemical parameters- Urea, Creatinine, SGOT, SGPT,ALP, Total and direct bilirubin,
5. Histopathological studies of liver, jejunum, heart, kidney, brain

Statistical analysis

The data generated was mentioned as Mean± SEM. Difference among the groups was assessed by employing one way ANOVA with Dunnet'smultiple't' test.for determining the level of significance of the observed effects, as post- HOC test if p value of less than 0.05 was considered as statistically significant

Observation and results

Table 1 showing effect of Vatsanabha and antidote effect of Maricha kashaya on biochemical parameters and table 2 showing effect of Vatsanabha and antidote effect of Maricha kashaya on haematological parameters. Table 3 showing effect of Vatsanabha seed and antidote effect of Maricha kashaya on brain, kidney, liver, jejunum, heart weight changes. Table 4 showing the effect of Vatsanabha and antidote effect of Maricha kashaya on Body weight changes.

Table 1: weight of organs on administration of *Vatsanabha* Group and *Vatsanabha* with Maricha kashaya *(*TED) group

Organ	Normal control	*Vatsanabha* group	*Vatsanabha* +Maricha *kashaya*
jejunum	1.43±0.12	1.33±0.04	1.34±0.07
Heart	0.68±0.04	0.81±0.04	0.75±0.05
Liver	8.08±0.86	11±0.65**	7.54±0.31*
Brain	1.29±0.13	1.53±0.04	1.36±0.08
Kidney	1.43±0.09	1.27±0.08	1.39±0.12

Data: MEAN±SEM, *P<0.05, **P<0.01

Table 2: Activity profile of test drug preparations on biochemical changes in albino rats

Parameters	Normal control	*Vatsanabha*	*Vatsanabha* +Maricha Kashaya TED
Sgot	149.50±11.52	305±58.34**	131±6.15*
Sgpt	81.83±10.05	129.00±20.61*	70±6.15*
Directbilirubin	0.177±0.020	0.082±0.010*	0.072±0.010*
Total Bilirubin	0.080±0.015	0.042±0.003*	0.021±0.005*
Alp	330.60±69.98	509.32±56.19*	310.83±31.29*
Tp	6.4±0.18	5.7±0.23	6.3±0.20
Albumin	4.3±0.07	3.6±0.19	3.5±0.28
Globulin	3.22±0.076	2.80±0.151	3.51±0.252
Urea	42.5±2.8	32.5±2.8	37.0±1.9
Creatinine	0.68±0.21	0.70±0.01	0.56±0.13
Tryglycerides	90.7±13.9	87.1±14.4	90.3±7.4
Sugar	138.5±0.61	137.1±0.70	138.6±1.47

Data: MEAN±SEM, *P<0.05, **P<0.01

Table 3: Activity profile of test drug preparation on Haematological changes in albino rats

Parameters	Normal control	*Vatsanabha* (Compared with Control)	*Vatsanabha* with (TED) Maricha *kashaya* (Compared with *Vatsanabha*)
HB%	14.9±0.439	14.7±0.401	14.9±0.359
RBC	7.6±0.427	7.2±0.206	7.6±0.345
PCV	37±0.274	37±1.36	37±0.91
MCV	53.61±1.048	53.14±1.94	54±1.21
MCH	20.6±1.74	20.25±0.31	19.01±0.51
MCHC	35.3±0.70	36±0.63	35.6±1.1
RDW-CV	25.6 ±0.391	26.15±1.53	24.36±0.24
RDW	27.6±0.74	29.81±3.38	25.11±0.29
PLATELETS	6.96±0.37	6.79±0.52	8.10±0.81
SUGAR	130.07±2.92	130.02±7.61	131±2.21

Data: MEAN±SEM, *P<0.05

Table 4: Activity profile of test drug preparation on Body weight changes in different groups

Groups	Body weight changes in 7th day	Body weight changes in 14th day	Body weight changes in 21st day	Body weight changes in 28th day
Normal control	8.01±1.22	14.26±1.79	17.86±2.021	24.288±3.11
*Vatsanabha*group(1)	2.87±3.65	3.59±7.40	3.72±6.07	2.70±4.38**
Vatsanabha+Maricha kashaya *(TED)* group (2)	6.63±1.57	10.35±0.770	11.98±4.620	26.19±5.80**

Data: MEAN±SEM, *P<0.05, **P<0.01

Reports of the histopathological examination

Photomicrograph of albino rats of Normal control group (1×100 magnifications)

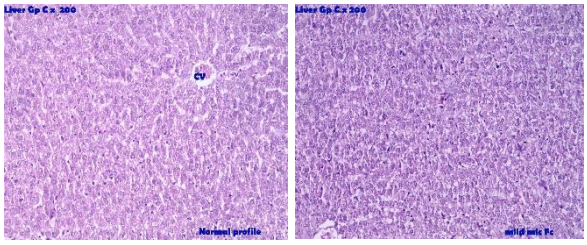

Fig 1a **Fig 1b**

Fig 1a, 1b: sections of liver of normal control group

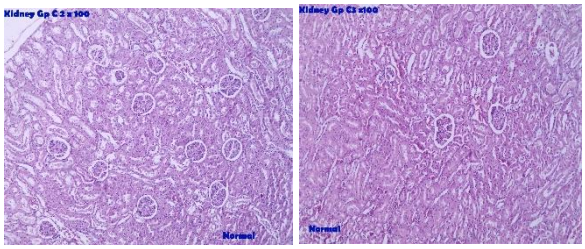

Fig 1c **Fig 1d**

Fig 1c, 1d: sections of kidney of normal control group

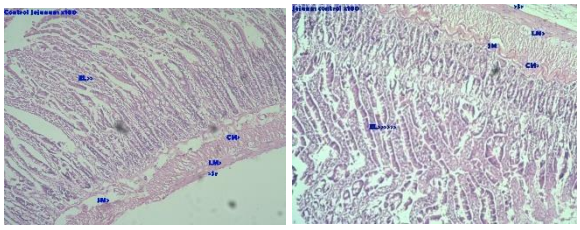

Fig 1e **Fig 1f**

Fig 1e, 1f: sections of jejunum of group normal control group

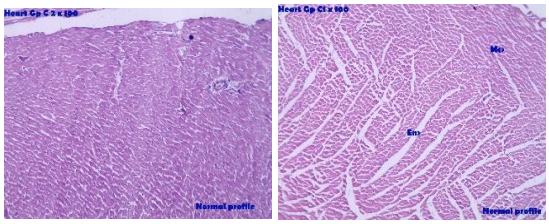

Fig 1g **Fig 1h**

Fig 1g, 1h: sections of heart of normal control group

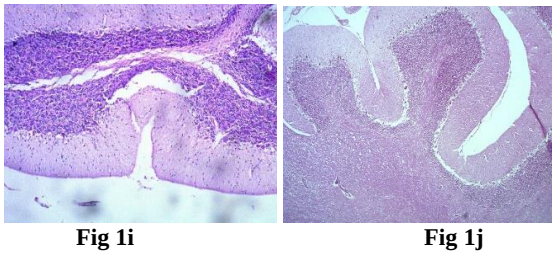

Fig 1i **Fig 1j**

Fig 1i, 1j: sections of brain-cerebellum of normal control group

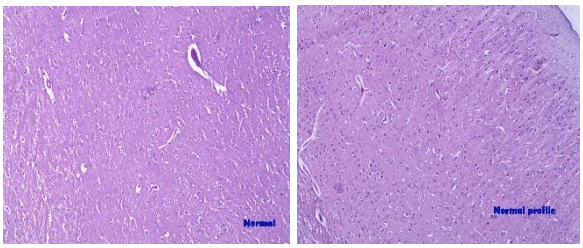

Fig 1k **Fig 1l**

Fig 1k, 1l: sections of brain-cerebrum of normal control group

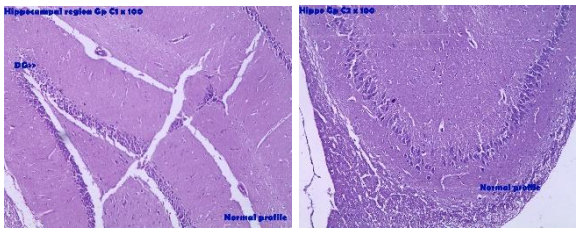

Fig 1m **Fig 1n**

Fig 1m, 1n: sections of brain-Hippocampus and mid-brain of normal control group

All the organs are shown normal cyto-architecture

Photomicrograph of albino rats of Vatsanabha treated group (1×100 magnifications)

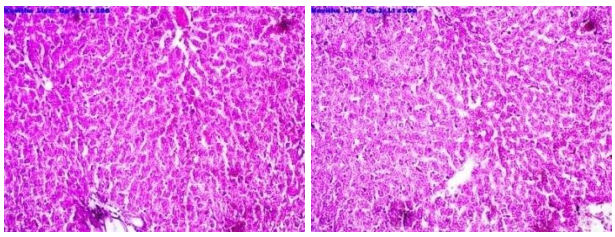

Fig 2a **Fig 2b**

Fig 2a, 2b: sections of liver of group 2 shows moderate cell depletion, fatty degenerative changes, sinusoids obliterated,mild to moderate degenerative changes

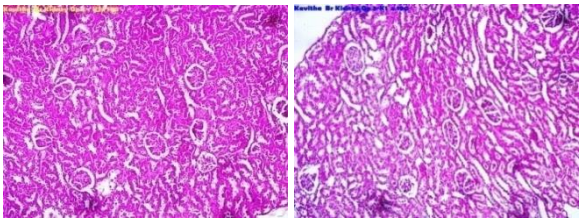

Fig 2c **Fig 2d**

Fig 2c, 2d: sections of kidney of group 2 shows mild hyaline changes, to moderate, mild fatty degenerative changes

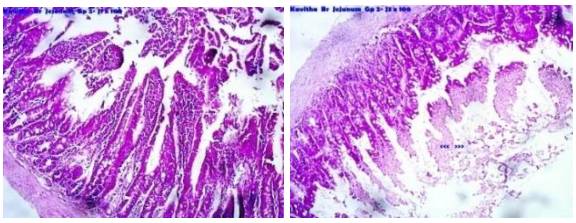

Fig 2e Fig 2f

Fig 2e, 2f: sections of jejunum of group 2 shows Mild to moderate degeneration, shortening of epithelial layer

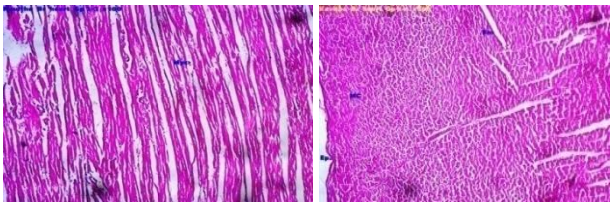

Fig 2g Fig 2h

Fig 2g, 2h: sections of heart of group 2 shows Mild myocarditis and oedema

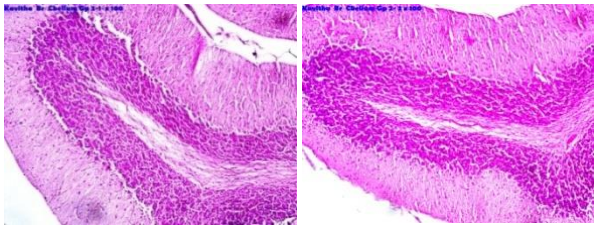

Fig 2i Fig 2j

Fig 2i, 2j: sections of brain-cerebellum of group 2 shows Normal cytoarchitecure

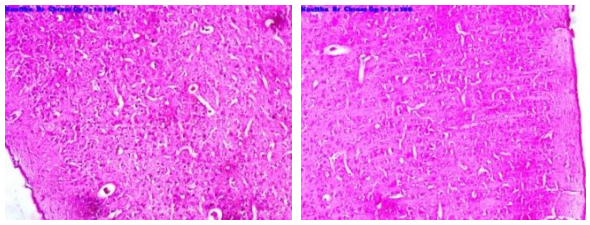

Fig 2k Fig 2l

Fig 2k, 2l: sections of brain-cerebrum of group 2 shows Normal cytoarchitecure

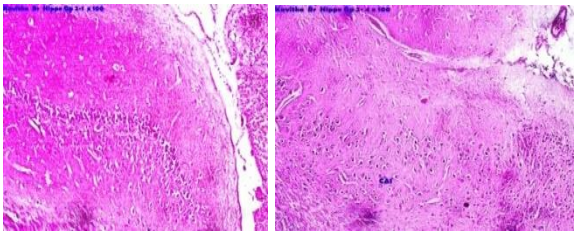

Fig 2m Fig 2n

Fig 2m, 2n. sections of brain-Hippocampus and mid-brain of Vatsanabha group shows normal cytoarchitecture

Photomicrograph of albino rats of Vatsanabha + Maricha kashaya treated group
(1×100 magnifications

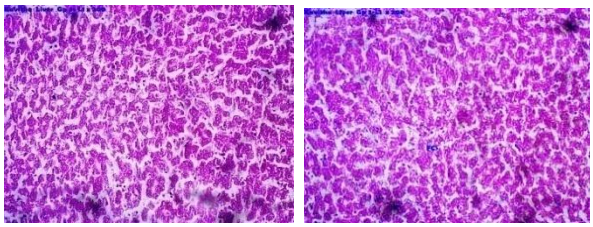

Fig 3a Fig 3b

Fig 3a, 3b: sections of liver of group 3 dose shows mild dialated sinusoids, moderate degenerative changes

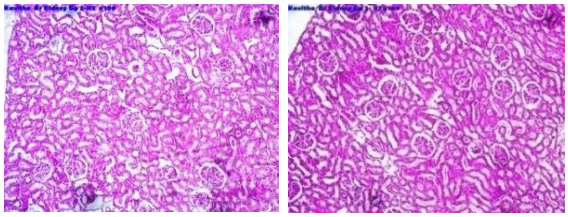

Fig 3c Fig 3d

Fig 3c, 3d: sections of kidney of group 3 shows mild cell infiltration

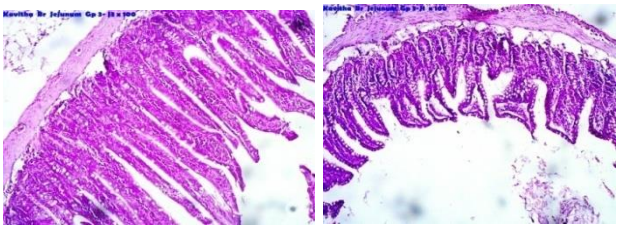

Fig 3e Fig 3f

Fig 3e, 3f: sections of jejunum of group 3 shows shortening of epithelial layer

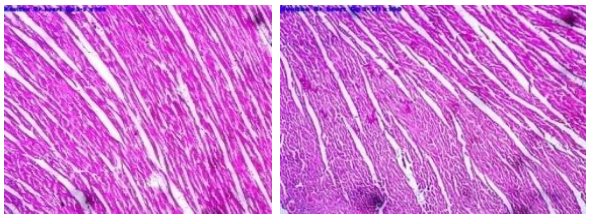

Fig 3g Fig 3h

Fig 3g, 3h: sections of heart of group 3 in shows Normal cytoarchitecture

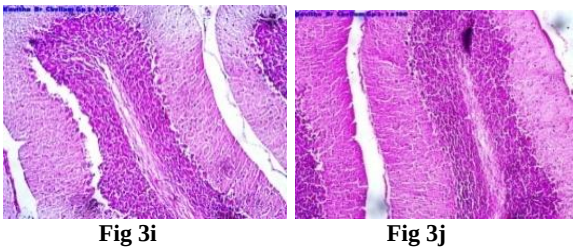

Fig 3i Fig 3j

Fig 3i, 3j: sections of brain-cerebellum of group 3 shows Normal cytoarchitecure

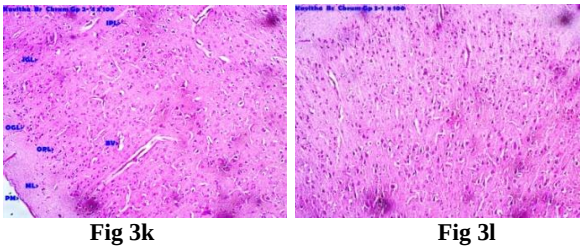

Fig 3k Fig 3l

Fig 3k, 3l: sections of brain-cerebrum of group 3 shows Normal cytoarchitecure

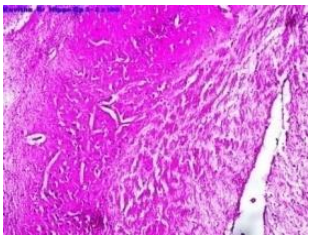
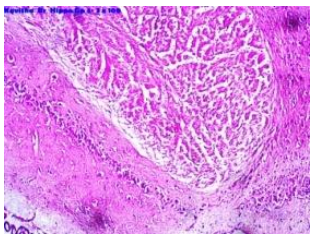

Fig 3m **Fig 3n**

Fig 3m, 3n: sections of brain-Hippocampus and mid-brain of group 3 shows normal cytoarchitecture

Discussion

The experimental study on albino rats was conducted to assess the antidote effect Maricha kashaya *on Vatsanabha* induced toxicity based on the LD_{50} value of *Dattura*.

Ponderal changes

Among the 5 organs studied, in Vatsanabha root group, the weight of the liver significantly increased as compared to normal control group. It may be due to the liver damage caused by toxic principles of Vatsanabha. In Vatsanabha root and Maricha kashaya group significant decrease in liver weight noticed when compared with Vatsanabha root alone group, which suggests the antidote effect of Maricha kashaya in Vatsanabha induced toxicity on liver.

Biochemical Parameters

Among the markers of liver functions - SGOT, SGPT, Alkaline phosphatase, Direct bilirubin, Total bilirubin, TP, Albumin, Globulin- the administration of Vatsanabha roots lead to significant elevation in SGOT, SGPT, Alkaline phosphatase, Direct bilirubin, Total bilirubin and non significant increase in Total protein, non significant decrease in Albumin and Globulin in test group (1). The observed elevation indicates that at the dose level studied, Vatsanabha might have produced liver injury. The transaminases also get elevated when there is inflammation at different sites or more than one organ.

It is observed that among three LFT parameters, SGOT shows an increase in test group (1) when compared to control group which was statistically significant. SGOT usually increases due to some damage process of liver cell. It is remarkable to observe that this elevation was found to be decreased in the anti-dote groups. SGOT shows a decrease when compared to the test group (1) which is statistically significant. This indicates that there might be an antidote effect of Maricha kashaya against Vatsanabha root.

An increase is observed in SGPT in the test group (1) when compared to the control group which was statistically significant. Here increase in the SGPT activity level might be due to liver damage, biliary duct problems. Increased SGPT is also one of the causes involving necrosis of hepatocytes or skeletal muscle cells which goes along with the raise in SGOT. As explained above this elevation was found to be significantly reversed by the anti-dote administration. This is based on the observation that in test group (2) the SGPT shows a decrease when compared to the test group (1) which is statistically significant. It indicates there might be a moderate antidote effect of Maricha kashaya against Vatsanabha root.

There is an increase in the ALP in the test group (1) when compared to the control group which is statistically significant, suggesting a progressing injury to the liver. But in test group (2) the ALP has decreased which is statistically significant, when compared to the test group (1) which indicates that drug was able to minimise the toxic effects in the liver caused by Vatsanabha. An increase is observed in Total Bilirubin and Direct bilirubin in the test group (1) when compared to the control group which is statistically significant. High bilirubin level may indicate liver inflammation, liver scarring or RBC break down more than usual. But in test group (2) Total Bilirubin and Direct bilirubin has decreased which is statistically significant, when compared to the test group (1) which indicates that drug has antidote effect on the toxic effects in the liver caused by vatsanabha.

Among all KFT parameters, a non-significant mild increase was observed in the blood Creatinine level in the test group (1) when compared to the control group. Raised serum Creatinine is usually noticed in conditions like excessive loss of body fluids (dehydration), kidney problems such as kidney damage or failure, muscle problems, such as breakdown of muscle fibers. It may either be due to excessive production or acute kidney injury (AKI) caused by the toxicity of the non-protein haeme pigment that is released from myoglobin.Vatsanabha roots when administered caused diuretic action and when given continuously has lead to dehydration. This might be the reason for the observed mild elevation. Since serum urea is not affected this mild elevation is not likely to indicate kidney malfunctioning. In the test group (2) the Creatinine level has been decreased when compared to the control group which is statistically non significant.

Even though statistically not significant, blood urea level has been increased in thetest group (1), when compared to the control group. Here also in test group (2) the bloodurea level decreased when compared to the test group (1) which is statistically non significant. Blood urea increase may indicative of renal dysfunction, dehydration, gastrointestinal bleeding or catabolic effect of given drug. Moderate but non-significant reversal of this effect was observed antidote group-indicative of the effect of anti-dote on this parameter.

Histopathological Observations

Based on the generated data it can be suggested that administration of Vatsanabha root, in the present dose level, predominantly affects liver function and moderate degenerative changes in liver, heart and jejunum. It has no effect on haematological system and structural integrity of brain and kidney. Most of the toxicant induced changes were significantly and effectively reversed by the administration of the anti-dote Maricha Kashaya at TED dose level. Thus providing strong and un-equivocal evidence for the efficacy of Maricha Kashaya as an anti-dote.

Conclusion

Vatsanabha is one among the drug under the category of Mahavisha. In literary review the reference of *Vatsanabha* was also available since *puranakala* and Acharya's have used in the treatment of many diseases. The concept of *Prathyoushadas* (antidotes) were mentioned in the text book of Keraleeya*vishachikitsa*. These are used when the toxic manifestations of poisonous substances of animal or plant origin happens. These reverses the action of the poison and relieve the person from the toxic manifestations.

Maricha kashaya is mentioned as a good and effective Prathyoushada in the treatment of Vatsanabhavisha by

Kriyakoumudi, Prayoga Samucchayam. When Vatsanabha Administered orally it has been marked mild to moderate toxic effect on liver compared with normal control. The toxic effect was moderately reversed by Maricha kashaya administered at TED dose. Hence it is clear that Maricha kashaya has got antidote effect on Vatsanabha toxicity. Administration of Vatsanabha results in statistically significant increase of SGOT and SGPT which was significantly decreased after administration of Maricha Kashaya in TED dose which suggests moderate recovery from toxicity. From the histopathological study, liver function is affected and moderate degenerative changes in liver, heart and jejunum were noted in Vatsanabha administered group which are reversed after administration of Maricha kashaya.

Vatsanabha has no any effect on haematological parameters and structural integrity of brain and kidney. Most of the toxicant induced changes were significantly and effectively reversed by the administration of the anti-dote i.e. Maricha *Kashaya*at TED dose level. Thus providing strong evidence for the efficacy of Maricha Kashaya as an anti-dote in Vatsanabha toxicity. Hence, from an experimental study on the antidote effect of Maricha kashaya in Vtasanabha poisoning, it is very clear that Maricha kashaya proved to be an effective antidote for Vatsanabha poisoning.

References

1. Acharya Vaghbat, Astanga Hrudayam Uttara Sthana; 35/5; Prof. K.R. Srikantha Murthy; Choukamba Krishnadas Academy; Varanasi. (3):329-586
2. Pranacharya Sri Sadananda Sharmana; Rasatarangini; 24/160; Motilal Banarasidas; Kashi Ayurveda Series;p-642;pp-698
3. Jaising Modi P. A text book of medical Jurisprudence and toxicology Section II; 24th edition; Edited by K. Kannan and Karunakaram Mathiharana published by Lexis Nexis; Hariyana India. 2012; 259-591
4. JLN Sastry. Dravya *Guna vijnana* I. foreword by KC. Chunekar Varanasi. published by choukhambha orientalia.Dec 2003; 11(2):1134
5. Jaising P Modi. Modi A text book of medical Jurisprudence and toxicology Section II 24th edition; Edited by K.Kannan and Karunakaram Mathiharana published by Lexis Nexis; Hariyana India. 2012; 259-591
6. Sekhar Namburi UR. A Text book of Agada Tantra published by chaukhambha publications; Varanasi. 2007; 106:324
7. Sahasrayogam, sujanapriya commentary; edited by K V Krishnan vaidyan and S Gopala pillai. 28th edition; published by Vidyarambham publishers, 2009; 465-544.