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HEPATOPROTECTIVE ACTIVITY OF VASAGUDUCHYADI KWATHA- A COMPOUND HERBAL FROMULATION AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RATS

Kalpu N Kotecha*[1], BK Ashok[2], VJ Shukla[3], PK Prajapati[4], B.Ravishankar[5]

[1] Head, Pharmacology Department, Indian Institute of Ayurvedic Pharmaceutical Sciences, Gujarat Ayurved University, Jamnagar, Gujarat, India.
[2] Drug Discovery Team, Research and Development, Himalaya Drug Company, Makali, Bangalore, India.
[3] Head, Pharmaceutical laboratory,
[4] Director, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India.
[5] Director, Research and development, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

ABSTRACT

Paracetamol (PCM) is widely used as analgesic and antipyretic drug, but at high doses it leads to undesirable side effects, such as hepatotoxicity. The present study evaluates the hepatoprotective activity of *Vasaguduchyadi Kwatha* (an herbal formulation content of 08 drugs) against paracetamol induced toxicity. Honey used as vehicle and Silymarin as a reference standard. Paracetamol induced hepatotoxicity was evaluated by noting the effect of test formulation on increase in serum SGPT, SGOT, ALP activity and bilirubin level. Paracetamol hepatotoxicity was manifested by an increase lipid peroxidation, depletion of reduced glutathione (GSH) and catalase activity in liver tissue. Biochemical parameter observations were supplemented by histopathological examinations. Co-administration of *Vasaguduchyadi Kwatha* (decoction) protects against the paracetamol induced alteration in lipid peroxidation, restored altered serum marker enzymes and antioxidant level towards near normal. Histopathology of liver showed that test formulation attenuated the hepatocelluar necrosis and led to reduction in inflammatory cells infiltration. The results indicate hepatoprotective and antioxidant activity of *Vasaguduchyadi Kwatha* (decoction) against paracetamol induced toxicity.

KEYWORDS: *Vasaguduchyadi Kwatha,* hepatotoxicity, paracetamol, hepatoprotective.

INTRODUCTION

Liver is considered the key organ in the metabolism, detoxification and secretary functions in the body and its disorders are numerous with no effective remedies. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.[1] The Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders[1]. In this modern age it is very important to provide scientific proof to justify the various medicinal uses of herbs

and a satisfactory remedy for serious liver diseases and search for effective and safe drugs for liver disorders continues to be an area of interest[2].

Vasaguduchyadi kwatha (decoction), a compound Ayurvedic formulation is explained in Astangahridaya [3] for the treatment of liver diseases especially for *Kamala* (Jaundice) and *Panduroga* (Anemia).Hence the present work includes the hepatoprotective activity evaluation of *Vasaguduchyadi Kwatha* (decoction) against paracetamol induced hepatotoxicity in rats.

Paracetamol (acetaminophen) is widely used as a hepatotoxic agent for the screening of the antihepatotoxic activity of wide variety of traditional medicinal plants and its formulations. It has been generally reported and accepted that one of the possible mechanisms that may be involved in paracetamol -induced hepatotoxicity has to do with the generation of oxidized reactive intermediates[4]. On extensive review of related literature, no documented evidence was found to show that the *Vasaguduchyadi Kwatha* (decoction) can protect animal livers against toxin-induced hepatotoxicity. This study was therefore undertaken to investigate the hepatoprotective potential of *Vasaguduchyadi Kwatha* (decoction) against paracetamol-induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Test formulation:

The plant materials [Table 1] of the test formulation were collected from pharmacy department of Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, and adjacent area of Jamnagar city after careful botanical identifications by referring to various botanical floras and with the help of pharmacognosist of Institute. These samples were converted to coarse powder (sieve no.44) form and from the powder samples; *kwatha* (decoction) was prepared freshly by referring the classical method[5] just prior to administration to the animals. In brief, 16 parts deionized water and one part drug which were boiled on low flame till 1/4th part was remained. This was filtered and allowed to cool before administration. This prepared *Kwatha* (decoction) contains 25 g of solid material in 100 ml.

TABLE - 1: FORMULATION COMPOSITION OF *VASAGUDUCHYADI KWATHA* (DECOCTION) PER LITRE

Drugs	Quantity used	Latin name	Part used
Vasa	31.25g	*Adhatoda vasica* Nees.	Root
Guduchi	31.25g	*Tinospora cordifolia* (Willd.)Miers.	Stem
Amalaki	31.25g	*Emblica officinalis* Gaertn.	Pericarp
Haritaki	31.25g	*Terminalia chebula* Retz.	Pericarp
Bibhitaka	31.25g	***Terminalia bellerica* Roxb.**	Pericarp
Chirayita	31.25g	*Swertia chirayita* (Roxb.) Karsten.	Whole plant
Kutuki	31.25g	*Picrochiza kurroa* Royle.	Rhizome
Nimba	31.25g	*Azadirachta indica* A.Juss.	Stem bark
16 times (4litre) of water was added and reduced to 1/4th (1litre) of initial volume			

Animals:

Wistar strain albino rats of either sex in the body weight range from 170 - 310g were obtained from animal house attached to Pharmacology laboratory. Six animals in each group were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The animals were acclimatized for seven days before commencement of the experiment in standard laboratory conditions, 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. For their drinking purpose, tap water *ad libitum* was used. Protocol used in this study for the use of animals was approved by the institutional animal ethics committee- Approval number; (IAEC – 04/08-10/PhD/02).

Chemicals and drugs:

Commercially available paracetamol manufactured by Micro Labs. Limited Baddi, Nalagarh, Solon was used to induce liver damage. The reference standard drug used for hepatoprotective evaluation is Silymarin produced by Micro Labs. Limited Baddi, Nalagarh, Solon. Commercially available honey was used as vehicle for *Kwatha* (decoction).All other chemicals and reagents used were of analytical grade.

Statistical analysis:

The results were presented as Mean ± SEM for six rats in each group. Statistical comparisons were performed by unpaired student's t test.

Experimental protocol:

Animals were divided into five groups of six rats each and treated orally as below for 7days. The dose of test formulation , honey and toxicants were calculated by extrapolating the therapeutic dose to rat dose on the basis surface area ratio by referring to the table of Paget and Barnes (1969)[6].

Group 1- Normal control: The animals received water for 7 days.

Group 2- Toxicant control: *The animals* received Paracetamol 3g/kg[7] body wt. in distilled water on 3rd and 5th day.

Group 3 - Vehicle control: The animals received Honey 0.63ml / kg[8] body wt. daily for 7 days + Paracetamol 3g/kg body wt. in distilled water on 3rd and 5th day 2 h after vehicle administration.

Group 4 - Test formulation group: The animals received *Vasaguduchyadi Kwatha (decoction)* 5.04 ml / kg [9]body wt. daily for 7 days + Paracetamol 3g/kg body wt. in distilled water on 3rd and 5th day 2 h after *kwatha* (decoction) administration.

Group 5 - Reference standard group*:* The animals received reference standard Silymarin 100 mg / kg [10]daily for 7 days + Paracetamol 3g/kg body wt. in distilled water on 3rd and 5th day 2 h after reference standard administration.

On 7th day (after 48 h of the 2nd dose of Paracetamol) all the animals were sacrificed by stunning and severing the neck vessels. The blood was collected in the tubes and sent to biochemistry laboratory for biochemical investigations- serum glutamic pyruvic transaminase (SGPT) [11], serum glutamic oxaloacetic transaminase (SGOT) [12], Serum Alkaline Phosphatase(ALP)[13]. Liver was dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain a piece of liver was preserved in 10% formalin for histo-pathological processing. A known amount of liver tissue was homogenized to estimate different biochemical parameters – lipid peroxidation[14], total glutathione[15] and catalase activity[16].

RESULTS

Effect of *Vasaguduchyadi Kwatha* (decoction) and other experiment groups on paracetamol induced liver injury in rats with reference to biochemical changes in serum and tissue homogenate are given in Table 2 and 3.

TABLE 2: Effect of *vasaguduchyadi kwatha* (decoction) on serum biochemical parameters against paracetamol induced hepatotoxicity

Groups	**Dose Drug(ml /kg) + toxicant (g/kg)**	**SGPT (IU/L)**	**SGOT (IU/L)**	**Alkaline Phosphatase activity (IU/L)**	**Bilirubin (T) (mg/dl)**
Normal control	Q.S.	39.50 ± 5.29	128.67 ± 16.95	148.17 ± 12.15	0.400 ± 0.07
Paracetamol control	3.0 g	108.67 ± 10.30###	285.33 ± 28.81###	430.67 ± 20.13###	0.550 ± 0.07
Honey control + Paracetamol	0.63ml + 3.0g	118.80 ± 30.10	212.80 ± 13.85	227.40 ± 32.73***	0.560 ± 0.04
Vasaguduchyadi Kwatha (decoction) + Paracetamol	5.04ml + 3.0 g	56.00 ± 5.69**	166.83 ± 18.93**	286.50 ± 27.01**	0.417 ± 0.08
Silymarin+ Paracetamol	100mg + 3.0g	60.20 ± 4.66**	184.40 ± 19.65*	288.40 ± 26.41**	0.600 ± 0.05

Values are Mean ± SEM, n = 6 animals in each group, ###P<0.001 in comparison of paracetamol to normal control (Un paired‘t’ test),*P<0.05, **P<0.01, ***P<0.001 in comparison of honey control, *vasaguduchyadi kwatha* (decoction), Silymarin to paracetamol control (Un paired‘t’ test). SGPT- serum glutamic pyruvic transaminases, SGOT- serum glutamic oxaloacetic transaminase

TABLE 3: Effect of *vasaguduchyadi kwatha* (decoction) on liver tissue homogenate biochemical parameters against paracetamol induced hepatotoxicity

Groups	Dose Drug(ml/kg) + toxicant (g/kg)	Lipid peroxidation (µmole of MDA released/g wet tissue)	Total glutathione (ng/mg wet tissue)	Catalase (µmole H_2O_2 consumed / min / mg protein).
Normal control	Q.S.	10.447 ± 1.07	13.89 ± 0.95	8.406 ± 1.02
Paracetamol control	3.0 g	34.879 ± 1.00###	8.54 ± 0.86##	4.089 ± 1.12#
Honey control + Paracetamol	0.63ml + 3.0g	5.055 ± 2.58***	13.67 ± 2.68	15.39 ± 4.10**
Vasaguduchyadi Kwatha (decoction) + Paracetamol	5.04ml + 3.0 g	15.502 ± 2.81***	13.63 ± 1.85*	8.257 ± 0.67*
Silymarin+ Paracetamol	100mg + 3.0g	5.864 ± 1.55***	11.78 ± 1.25	10.154 ± 3.27

Values are Mean ± SEM, n = 6 animals in each group, # P<0.05, ##P<0.01, ###P<0.001 in comparison of paracetamol to normal control (Un paired‘t’ test). *P<0.05, **P<0.01, ***P<0.001 in comparison of honey control, *vasaguduchyadi kwatha* (decoction), Silymarin to Paracetamol control (Un paired‘t’ test)

The administration of paracetamol resulted in a marked increase of SGPT, SGOT, ALP and serum bilirubin levels compared to the normal control group, reflecting the liver injury caused by paracetamol. Whereas blood samples from the animal treated with *Vasaguduchyadi Kwatha* (decoction) showed significant decrease in the level of serum markers which are comparable to the values in the standard drug treated group of animals, indicating the protection of hepatic cell against paracetamol damage.

In liver tissue homogenate parameters administration of paracetamol leads to marked elevation of lipid peroxidation and decrease in liver glutathione content and catalase activity. The significant decrease in liver peroxidation and significant elevation of glutathione content and catalase activity was observed in *Vasaguduchyadi Kwatha* (decoction) administered group and standard drug treated group.

Histopathological profile of animals is depicted in Figure 1,2,3,4 and 5.

Histopathological Profile

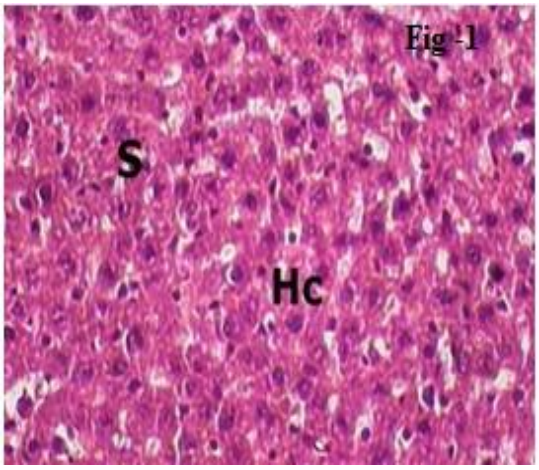

Fig 1- representative sections of liver of albino rats from normal control group showing Hc-Hepatic Cell, S-Sinusoid and normal cyto architecture.

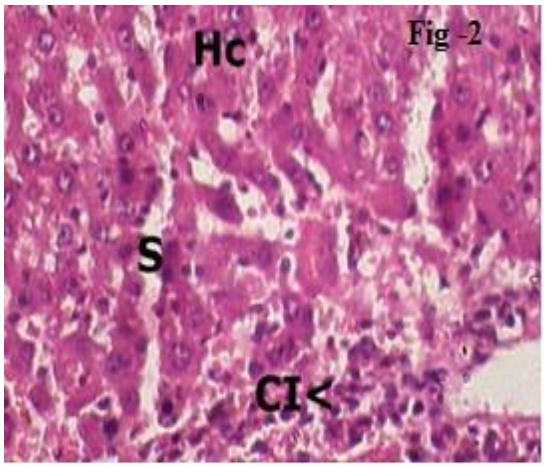

Fig 2- representative sections of liver of albino rats from paracetamol control group showing Hc-Hepatic Cell, S-Sinusoid Periportal, CI-cell infiltration, necrosis and fatty degenerative changes.

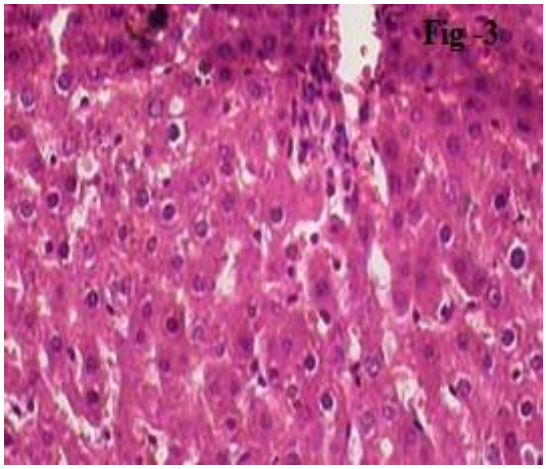

Fig 3- representative sections of liver of albino rats from paracetamol plus honey group showing only mild fatty changes.

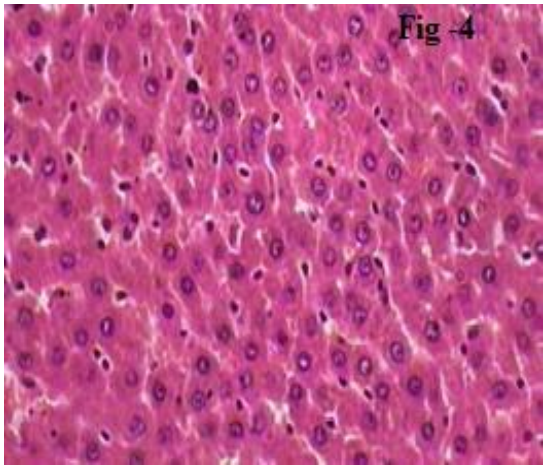

Fig 4 - representative sections of liver of albino rats from paracetamol plus *vasaguduchyadi kwatha* (decoction) group showing almost normal cytoarchitecture.

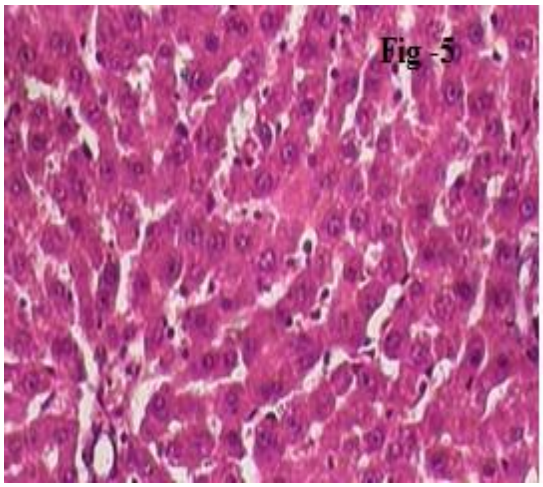

Fig 5 - representative sections of liver of albino rats from paracetamol plus *s*ilymarin group showing almost normal cytoarchitecture.

Histopathological studies showed paracetamol produced extensive vascular degenerative changes and centrilobular necrosis, fatty changes and severe cell infiltration in hepatocytes. Reversal of these degenerative changes was observed with vehicle, *Vasaguduchyadi Kwatha* (decoction) and standard treated group.

DISCUSSION

Paracetamol a widely used antipyretic-analgesic drug produces acute hepatic damage on accidental over dosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite- N–acetyl–p–benzoquinamine (NAPQI) [17] which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction [18]and the development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in paracetamol bioactivation to NAPQI. P450 2E1 have been suggested to be primary enzymes for paracetamol bioactivation in liver microsomes[19]. Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity[20].

SGPT predominantly found in mitochondria of hepatocytes. Hence is more specific to liver, and thus is a better parameter for detecting liver injury. Serum ALP and bilirubin is also associated with liver cell damage. The SGPT, SGOT and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury [21, 22]. Administration of paracetamol caused a significant elevation of enzymes level such as SGPT, SGOT, ALP and

bilirubin level which can be attributed to the damage of structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity [23, 24].

In the present study administration of paracetamol caused a significant elevation of enzyme levels such as SGPT, SGOT, ALP and non significant elevation of total bilirubin when compared to control. There was a significant restoration of these enzyme levels on administration of the test formulation and reference standard. The reversal of increased serum enzymes in paracetamol induced liver damage by the *Vasaguduchyadi Kwatha* (decoction) may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[25]. Effective control of ALP and bilirubin points towards an early improvement in the secretary mechanism of the hepatic cells.

The increase in lipid peroxidation level in liver induced by paracetamol leads to tissue damage and failure of antioxidant defense mechanism and prevents formation of excessive free radicals[26]. Treatment with *Vasaguduchyadi Kwatha* (decoction) has significantly reversed these changes. Hence it is likely that the mechanism of hepatoprotection of *Vasaguduchyadi Kwatha* (decoction*)* may be due to their antioxidant effect.

Glutathione is one of the most abundant tri-peptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols[27]. Decreased level of glutathione is associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of *Vasaguduchyadi Kwatha* (decoction) and Silymarin increased glutathione level which once again highlights one of the mechanisms behind the observed hepatoprotective activity with the adjuvant and the test formulation.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals.[26] Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. In our study catalase activity was found to be increased in *Vasaguduchyadi Kwatha* (decoction) administered group which further indicate they have hepatoprotective activity.

Histopathological study of liver showed paracetamol to produce extensive vascular degenerative changes and centrilobular necrosis, fatty changes and severe cell infiltration in hepatocytes. Reversal of these degenerative changes was observed with vehicle, test formulation and reference standard but it was more remarkable in test formulation. All these results indicate a hepatoprotective potential of *Vasaguduchyadi Kwatha* (decoction).

CONCLUSION

Vasaguduchyadi Kwatha (decoction) shows hepatoprotective potential and possible mechanism for their hepatoprotective activity may be their membrane stabilizing activity, healing of hepatic parenchyma and the regeneration of hepatocytes and reduction in reactive free radical induced oxidative damage to liver- antioxidant activity. In accordance with these results, it may be hypothesized that tannin-gallic acid, alkaloids, coumarin glycosides, flavonoid, phenolic compound and triterpenoids which are present in the preparation of *Vasaguduchyadi Kwatha (decoction)* [28]could be considered responsible for their hepatoprotective activity.

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For Correspondence
Dr. Kalpu Kotecha
Email: k_kalpu@rediffmail.com