



Research Protocol for Assessment of Solitary and Combined effect of *Guduchi* and *Punarnava* on Structural and Functional Changes of Ageing in Liver and Kidney in Wistar Rats

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Authors' contributions:

This work was carried out in collaboration among all authors. Author KT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PDD and PD managed the analyses of the study. Author KT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i30A31630

Editor(s):

(1) Dr. Prem K. Ramasamy, Brandeis University, USA.

Reviewers:

(1) Bandameedi Ramu, Mewar University, India.

(2) Sobha Rani, Karnataka Veterinary, Animal and Fisheries Sciences University, BIDAR, India.

(3) K. R. Chandrashekar, Yenepoya (Deemed to be University), India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68579>

Study Protocol

Received 08 March 2021

Accepted 14 May 2021

Published 02 June 2021

ABSTRACT

Background: The liver and kidney play a central role in metabolic and detoxification processes leads to structural degeneration of organs with aging, which may hamper the functional ability of these organs. *Acharya charaka* suggests to use drugs of *vayasthapana mahakashaya* in different combinations to prevent ageing. *Guduchi* (*Tinospora cardifolia*) and *punarnava* (*Boerhavia diffusa*) are two drugs chosen to evaluate the solitary and combined effect on structural and functional changes in liver and kidney of Wistar rat.

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Materials and Methods: After identification and phytochemical study of test drugs, the 32 healthy, male rats of 18 months will be divided into four different groups, with eight rats in each group. 1st to 4th group will receive distilled water, *guduchi kwath*, *punarnava kwath*, *guduchi and punarnava kwath* respectively for 30 consecutive days at the rate of 8.1ml/kg dose, orally. Before administration of control drug and test drug blood sample assessment will be done for LFT, KFT, and SOD level. The same investigation will be repeated after one month trial period. Then after 60 days, 120 days and 180 days, two animals from each group will be sacrificed, organs such as liver and kidney will be extracted and preserved for histopathological study, and blood samples will be collected for investigation. The remaining animals will be returned to the animal house for their reuse.

Expected Results: *Guduchi* and *punarnava* may provide strength to liver and kidney and thus helpful in delaying degenerative changes of organs.

Keywords: Aging; *guduchi*; *punarnava*; *vayasthapana*; liver; kidney.

1. INTRODUCTION

The process of aging refers to the decay of an organism's structure and function, in which molecular and cellular modification can have various effects at the individual level with time. Damage that occurs in the molecule, cell, and tissues affects the physiological functions and leads to many diseases [1], as cardiovascular diseases, diabetes and neurodegenerative disorder [2].

Aging is influenced by genetic factors, environmental factors, and reactive oxidative species (ROS) [3,4].

Cells are the basic building block of a body. Tissues are groups of cells that have a similar structure, and different kinds of tissues together form an organ. As changes occur in cells and tissues, organs also experience the changes of ageing.

The liver is one of the largest organs in the body and plays a central role in metabolic processes. hepatocyte (liver cell), hepatic stellate cell, Kupffer cell are basic structures involved in liver. Liver carries, blood, nutrients, medication, and toxic substances. These substances are processed, deposited, modified, detoxified, and return to the blood or released into bowels to be eliminated, thus we can observe the changes in the basic structure of the liver with aging. Even with an incredible ability to heal itself, the liver has vulnerabilities with alcohol, infections, toxins, and a general genetic disorder [5]. Kidney act as a filter to remove waste products, maintain healthy balances of water, salt, and minerals [6]. kidney shows changes in nephrons, in number of glomeruli, and cortical volume. As kidney stops functioning toxin begins to build up in the body,

eventually, this leads to liver failure [7]. The continuous function of detoxification and filtration leads to changes in structures of hepatocytes (liver cells), hepatic sinusoidal endothelial cells, Kupffer cells of liver and nephron, glomeruli, cortical volume of the kidney. These changes would consequently have direct impact on the functional capacity of the organ liver and kidney. Thus ageing becomes a major risk factor for a variety of liver and kidney diseases [8,9].

Acharya Charaka indicates to use the *vayasthapana mahakashaya* for *vayasthapana* purpose. Acharya Chakrapani stated that *vayasthapana* means *vayastarunsthapayatiti vayasthapana* [10], which means establishing the young stage of life. This suggests that *vayasthapana mahakashaya* may have a property to keep the body organs healthy for a long time. And healthy organs perform their functions properly and maintain their structural integrity and strength for a long time. The use of *vayasthapana mahakashaya* may prove beneficial in regards to aging changes that occur in body organs.

Vayasthapana mahakashaya mentioned in the text is a combination of ten drugs. It's very difficult to evaluate the exact effect of each drug on the structure of the organs.

Charakacharya has mentioned using the drugs in combination or individual as per requirement [11].

The individual effect of *guduchi* (*Tinospora cardifolia*) against carbon tetrachloride-induced hepatic damage, cadmium induce oxidative stress and hepatotoxicity [12] and gentamycin-induced renal toxicity has been studied [13,14]. Similarly, the individual effect of *punarnava* (*Boerhavia diffusa*) on gentamicin-induced nephrotoxicity and thioacetamide intoxicated rats

has been studied [15,16]. Animal experiment carried out to investigate organ toxicity profile of tenofovir and tenofovir nano particle on liver and kidney [17] However, combined effect of *guduchi* and *punarnava* drugs have not been studied by previous researchers in intoxicated rats, also the solitary effect of *guduchi* and *punarnava* on normal age-related structural and functional changes occurs in the liver and kidney in the animal experiment not studied. Thus an attempt will be made to study the solitary effect of *guduchi* and *punarnava* and combination effect of these two on structural and functional age-related changes that occur in liver and kidney of Wistar rats. The antioxidant properties of combined *guduchi* and *punarnava kwath* will be studied.

2. MATERIALS AND METHODS

2.1 Plant Materials

The raw material will be collected from Manas Ayurveda, Nagpur. Identification and authentication of raw material will be done by the subject expert of *Dravyaguna* or Botanist.

2.2 Preparation of Test Drug

The raw materials of *guduchi kwath*, *punarnava kwath*, *guduchi* and *punarnava kwath* will be separately shade dried and disintegrated into fine pieces and powdered by hammer mill pulverizer of mesh size 60 and boiled with water in the ratio of 1:16 and reduced to 1/8 and filtered through fine cloth [18].

2.3 Animal Experiment

Male Wistar rats of age 18 months [19], weighing between 150-280 gm will be selected for the experiment. A total of 32 rats will be divided into four different groups of 8 animals in each group as follows:

Group 1- normal control group received only distilled water

Group2 - treated with *guduchi kwath* (GK) with a dose of 8.1ml/kg.

Group 3 - treated with *punarnava kwath* (PK) with a dose of 8.1ml/kg.

Group 4 - treated with *guduchi* and *punarnava kwath* (GPK) with a dose of 8.1ml/kg.

2.4 Dose Selection and Fixation

A dose of 90 ml of *kashaya* will be fixed to adult rats as per the *Bhavprakash Nighantu* [20]. Dose

fixation will be calculated by extrapolating the human dose to animal's dose based on ratio of body surface area with reference to the table of Paget and Barnes [21].

Conversion formula: = Human Dose × 0.018
(conversion factor for Rat)

Test drug *guduchi kwath*, *punarnava kwath*, *guduchi punarnava kwath* dose.

= Human Dose × 0.018 (conversion factor for Rat)

= 90 × 0.018 = 1.62ml/200g body weight of Rat

= 8.1ml/kg

2.5 Route of Administration

Orally with help of 2 ml syringe.

2.6 Antioxidant Assay DPPH [22]

1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assays are one of the most extensively used authentic activity of the extract (*kwatha*). It is mentioned in terms of radical scavenging ability or hydrogen donating capacity using the stable radical DPPH. The authentication activity will be measured at 515 nm after thirty minutes of incubation. DPPH solution 300 um will be prepared in methanol and 95 ul of DPPH will be added to each well, prepared *kwatha* of drug, 5 ul will be added to the respective well. Ascorbic acid will be used as a positive control.

The scavenging activity percentage is calculated with the help of following formula

Percentage of scavenging = $[AC - AS / AC * 100]$

Where

AC – is absorbance of control

AS – is absorbance of sample (*guduchi+punarnava*)

A huge reduction in the absorbance of the reaction mixture indicates the significant free radical scavenging activity of the drug compound.

2.7 Animal Experimental Procedure

Thirty-two rats will be divided into four different groups of 8 animals each.

Group 1, normal control (NC) group received only distilled water

Group2 treated with *guduchi kwath* (TGG) with a dose of 8.1ml/kg.
 Group 3 treated with *punarnava kwath* (TGP) with a dose of 8.1ml/kg.
 Group 4 treated with *guduchi* and *punarnava kwath* (TGGP) with a dose of 8.1ml/kg. (Table 1)

All the above groups except group 1, will be orally administered test drug, with dose 8.1ml/kg for 30 days. On the first day before administration of the test drug the blood sample will be collected through orbital puncture of all 32 animals for assessment of LFT, KFT, and SOD level. After 30 days of administration of the last dose of the test drug the animal will be given rest overnight and the blood sample will be collected again through orbital puncture of all 32 animals for assessment of LFT, KFT, and SOD level.

After than at the end of 60 days two animals from each group will be sacrificed by administrating a light dose of ether anesthesia, blood will be collected by jugular vein for assessment of LFT, KFT, and SOD level. The liver and kidney will be separated and preserved in formalin and for histological studies. Similarly, at the end of 120 days two animals, from each group, will be sacrificed and liver and kidney will be separated and preserved in formalin for histological studies. The last two animals, from each group, after 180 days will be sacrificed by the administration of the light dose of ether anesthesia, blood will be collected by jugular vein for assessment of LFT, KFT and SOD level. The liver, and kidney will be separated and preserved in formalin for the histological studies. (Fig. 1).

Table 1. Drug intervention plan

GrNo	Group name	Age	Group code	Sample size	Intervention/ drug	Route of drug administration
1.	Normal control group -	18 months	NC	8	Distilled water	Oral
2.	Trial group <i>guduchi</i>	18 months	TGG	8	<i>Guduchi kwath</i>	Oral
3.	Trial group <i>punarnava</i>	18 months	TGP	8	<i>Punarnava kwath</i>	Oral
4.	Trial group <i>guduchi</i> and <i>punarnava</i>	18 months	TGGP	8	<i>Guduchi and Punarnava kwath</i>	Oral

2.8 Outcome Measures

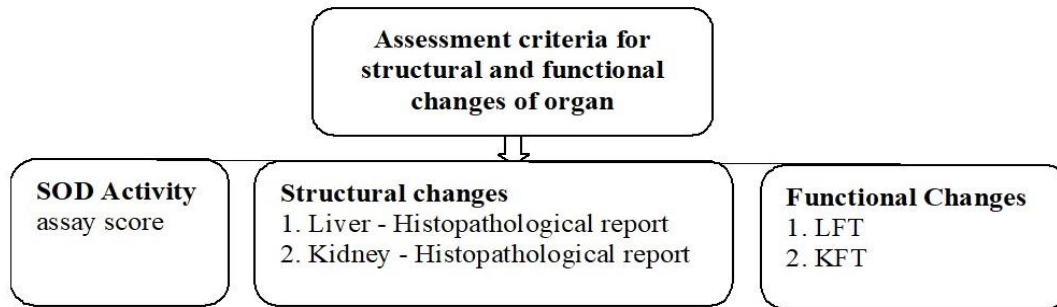


Fig. 1. Outcome measures

2.9 Methods of Data Collection

Data will be collected by laboratory reports and histology reports.

2.10 Statistical Analysis Methods

All data management will be done in an excel sheet. All the values will be expressed as mean, mean± standard deviation. Statistical significance will be analyzed using the student ‘t’ test. repeated measure ANOVA followed by post hoc test will use to assess the statistical significance between the different groups. Results will be considered on a 95% confidence limit. All the statistical analyses will be performed using statistical software SPSS.

2.11 Expected Results

Guduchi and *punarnava* may provide strength to organ liver and kidney and helpful in delaying degenerative changes of Organ.

3. DISCUSSION

Drugs of *Vayasthapana mahakashaya guduchi* and *punarnava* may be the better source of natural antioxidants and it may be helpful to prevent and delay damage to cells and contribute to the development of a noble treatment strategy that can delay the progression of degeneration. It may help in delaying aging process in individuals also. A number of studies on different liver conditions [23-26] and kidney diseases [27-30] were reviewed. Khan et al. reported a study on Evaluation of In Vitro Anti-Cancer Activity of *Kukkutanakhi Guggula* on Liver, Prostrate, Ovary and Renal Cancer [31]. Similar studies on Rat and Mice models were reported by Khatib et al. [32,33].

4. CONCLUSION

Ageing becomes a major risk factor for liver and kidney diseases. Conclusion will be made on the basis of would be result, the effect of *guduchi* and *punarnava* on structural and functional changes on organ liver and kidney may helpful in delaying age and better functioning of the organ.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Protocol is approved by institutional animal ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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