

Haemoprotective potential of *Picrorhiza kurroa* against radiation and mercury induced changes in mice

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Abstract- Ionizing radiation (IR) has a sufficient amount of energy to induce physical symptomatology within minutes of exposure, appearing as the acute radiation syndrome (ARS). The prodromal phase of ARS includes nausea, vomiting, and fatigue. The quality of radiation, dose, and dose-rate are all contributing factors to the differential symptoms of ARS. These prodromal symptoms can be followed by dramatic decrease in peripheral blood cell counts, as hematopoietic cells represent a renewal system consisting of cells with fast division rates that are known to be sensitive to IR. Once in the bloodstream mercury go through catalase and peroxidase-mediated oxidation in the red blood cell and tissue and convert into inorganic mercuric mercury and mercurous mercury, a procedure restriction mercury absorption. High level showing to mercury make changes in the central nervous system, possible outcome in irritability, fatigue, behavioural change, tremors, headache, hearing and cognitive loss, dysarthria, in coordination, hallucination, death, hypertension and change in endothelial function. *Picrorhiza kurroa* widely used in traditional systems of medicine and have rendered significant radioprotection in both *in vitro* and *in vivo* model systems. Plants and their constituents with pharmacological activities that may be relevant to amelioration of radiation-mediated damage, including antiemetic, anti-inflammatory, antioxidant, cell proliferative, wound healing and haemopoietic stimulators are also discussed.

In view of above, present study was planned to evaluate the radiation and mercury induced changes in mice and their protection by *Picrorhiza kurroa*. The animals were exposed to 2.5 Gy of gamma rays with or without mercuric chloride treatment. The more severe changes in the combined treatment group showing synergistic effects. The kutki treated groups showed less prominent changes and early recovery may be due to the protection provided by the drug.

INTRODUCTION:

High amount of radiation can damage tissue by changing cell structure and damaging DNA. Damage to blood cells exposed to different dose of radiation. Radiation produces free radical which specially damage DNA. Radiation destroys both mature blood cell and hematopoietic progenitor stem cell in the bone marrow. Decrease of blood cell due to radiation therapy, decrease of hematopoietic progenitor cells and primitive hematopoietic stem cell death of blood cell due to prolonged myelosuppression. Radiation does have some dose- dependent and time-dependent adverse effect on the RBC.

Picrorhiza kurroa (family -Scrofulariaceae, common name: *Picrorhiza kurroa*, katuka, kutaki) is a small perennial herb growing in the mountainous parts of the north- western Himalayan region from Kashmir to Sikkim, Garhwal region in India and Nepal. It has also been observed in Nepal, west China, Pakistan, South-east Tibet and North Burma.

It's growing in wild form in alpine region on rock crevices and also in organic soil. In Nepal, kutki is found in abundance in alpine Himalaya region between altitude of 3500m to 4800m and also in western region of Nepal, where it grows on the rocks crevices on the north facing slopes, cliffs and the turf of glacial flats. *Picrorhiza kurroa* is broadly used in conventional system of medicine and have important radioprotection in both *in vivo* and *in vitro* model system.

Picrorhiza kurroa is considered a bitter drug that is rich in iridoid glycosides with many biological activities such as antioxidant, anticholestatic, anti-inflammatory, anti-periodic, antipyretic, laxative, cholagogue, anti- cancerous, carminative, cardio-tonic, immunomodulatory and hepatoprotective activities. It is also observed as blood purifier, blood pressure reducer and expectorant. Chemical constituents found in this plant are berberine, kurrine, picrorhizetin, kutkisterol, sesquiterpine, apocynine, cathartic acid and kutkin. Its constituents picroliv is also described to possess choleric effect and prevent hepatic injury caused by ethanol, chemicals and microorganism.

Picrorhiza kurroa are the abundant provenance of secondary metabolite such as flavonoids, saponins, alkaloids, triterpenes etc. kutki are also experimentally opposed to several microorganism including gram positive and gram-negative bacteria and fungi. Estimation of insulin level, histological examination of pancreas were accomplished to notice the outcome on pancreatic islets of beta-cell. Furthermore, GLUT-4 content of soleus muscles of rats have been investigated using western blot in order to range over the molecular target of PKC executive of antidiabetic activities. Kutki is protective effect against cyclophosphamide induced immunosuppression. Due to capability of the plant to inhibit the creation of oxygen anions and scavenge free radical, that hepatoprotective activities occur.

Both radiation and heavy metals are dangerous for all living being. When person gives to 100 rems radiation, the blood cell number will be decreased that person an easy target to infection. Kutki reduces the deleterious effects of radiation, so that kutki uses as a radio protector. For that reason, in that respect require for compounds, which are efficient in radioprotection, non-toxic, low-price

and easily accessible. Therefore, present study was taken into consideration to evaluate haemo- protective potential of *Picrorhiza kurroa* against radiation and mercury induced changes in mice.

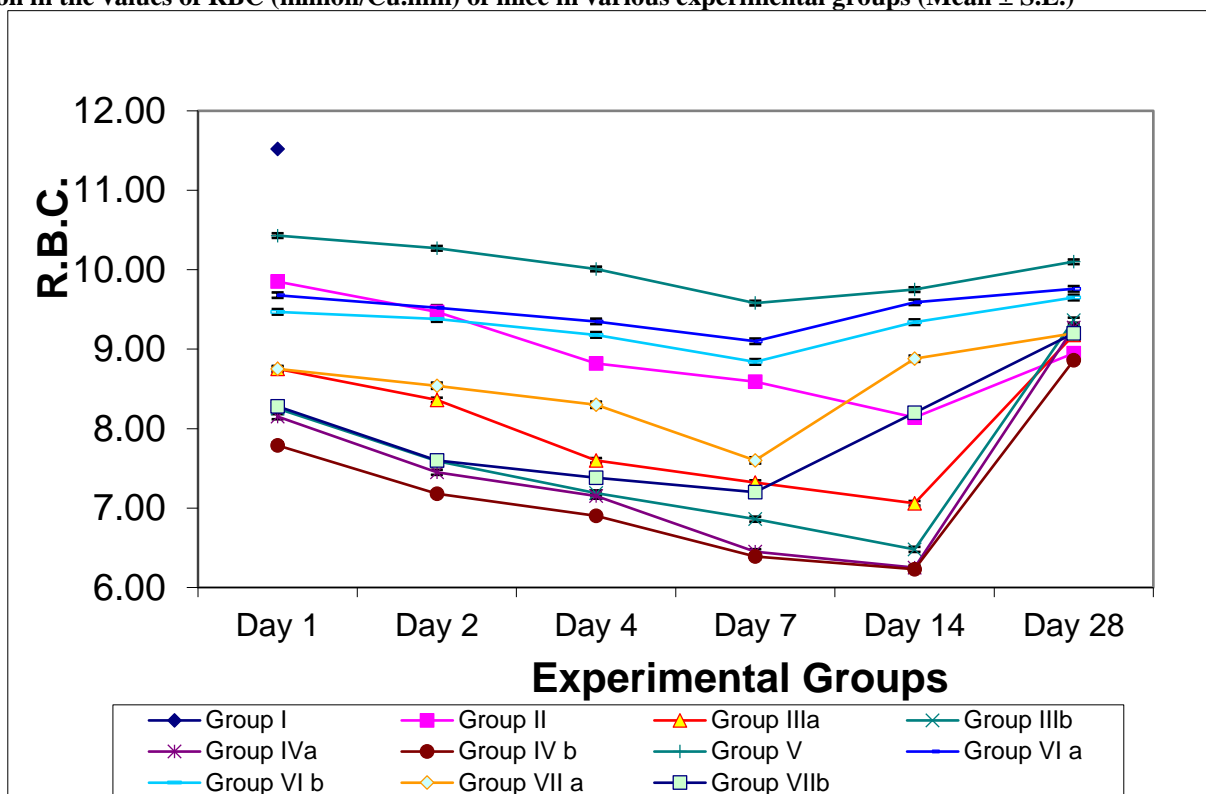
Materials and Methods

For the purpose of study six- to eight-week-old mice were procured from an inbred colony maintained in Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar (India). The adult healthy Swiss albino mice were maintained at animal house of Govt. Dungar college Bikaner (registration no.1066/go/re/s/07/CPSEA) and provided balanced mice feed and water *ad libitum*. As to investigate protective effect of *Picrorhiza kurroa* in mice blood against gamma radiation (2.5 Gy) and mercuric chloride (0.5 ppm) alone and in combination also, the animals were divided into seven groups, (control and experimental). Group I To IV were treated with doses of radiation and mercuric chloride and group V to VII were treated with *Picrorhiza kurroa* extract besides the doses of radiation and mercury. The experimental animals were provided oral doses of *Picrorhiza* was given orally to the drug treated animals from seven days prior to irradiation. A minimum of five animals from each group were sacrificed by cervical dislocation and autopsied at each post treatment intervals of 1,2,4,7,14,28 days. Following parameters were taken into consideration RBC, WBC, Hemoglobin and Packed cell volume,

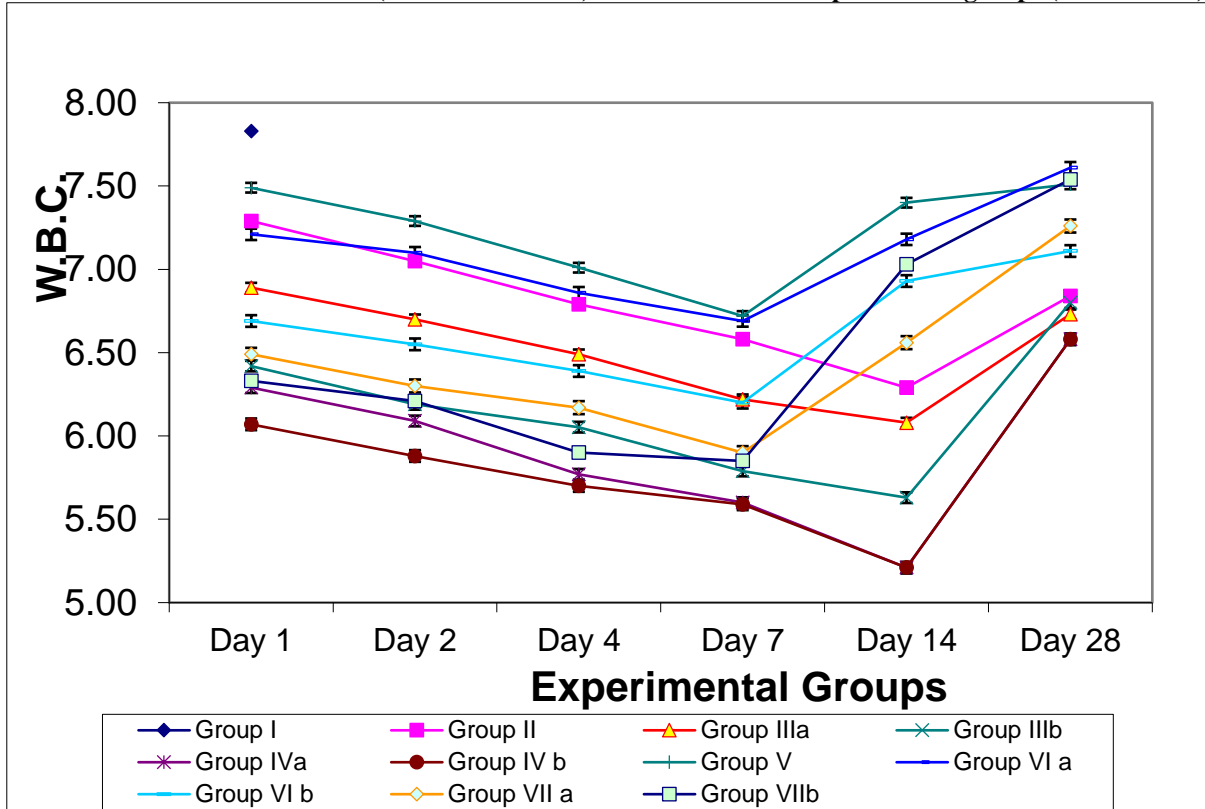
Result

The value of RBC showed a decreasing trend in the non-drug treated groups II, III And IV in the present investigation. The value declined on day 1 and continued to decrease up to day-14. On day-28, the value increased but it was lower than that of the normal value. In the drug treated groups V, VI and VII, the value declined from day-1 to day-7. On day- 14 the value increased and continued so up to day-28.but the decrease in the value was comparatively lesser as compared to non-drug treated groups. The value of WBC also exhibited a trend of decrease in all the groups. In comparison to normal values the Kutki treated groups showed decrease in the value up to day-7 then increases on day -14 and continued up to day-28 in all the groups. The value of hemoglobin content reduced on day-1 This decline was continued up to day -14 in non-drug treated groups against day -7 in the drug treated groups. Thereafter, it increased up to day-28 without reaching to the normal. The packed cell volume showed a decreasing trend in all the groups in the present investigation. The non drug treated groups showed decreasing trend up to day -14 and values recovered on day-28 without reaching to the normal .Kutki treated groups showed increasing trend on day -14 and continued up to day -28.Early onset of recovery in all the parameters while treated with *Picrorhiza* showed the protection provided by the herbal drug against radiation and mercury.

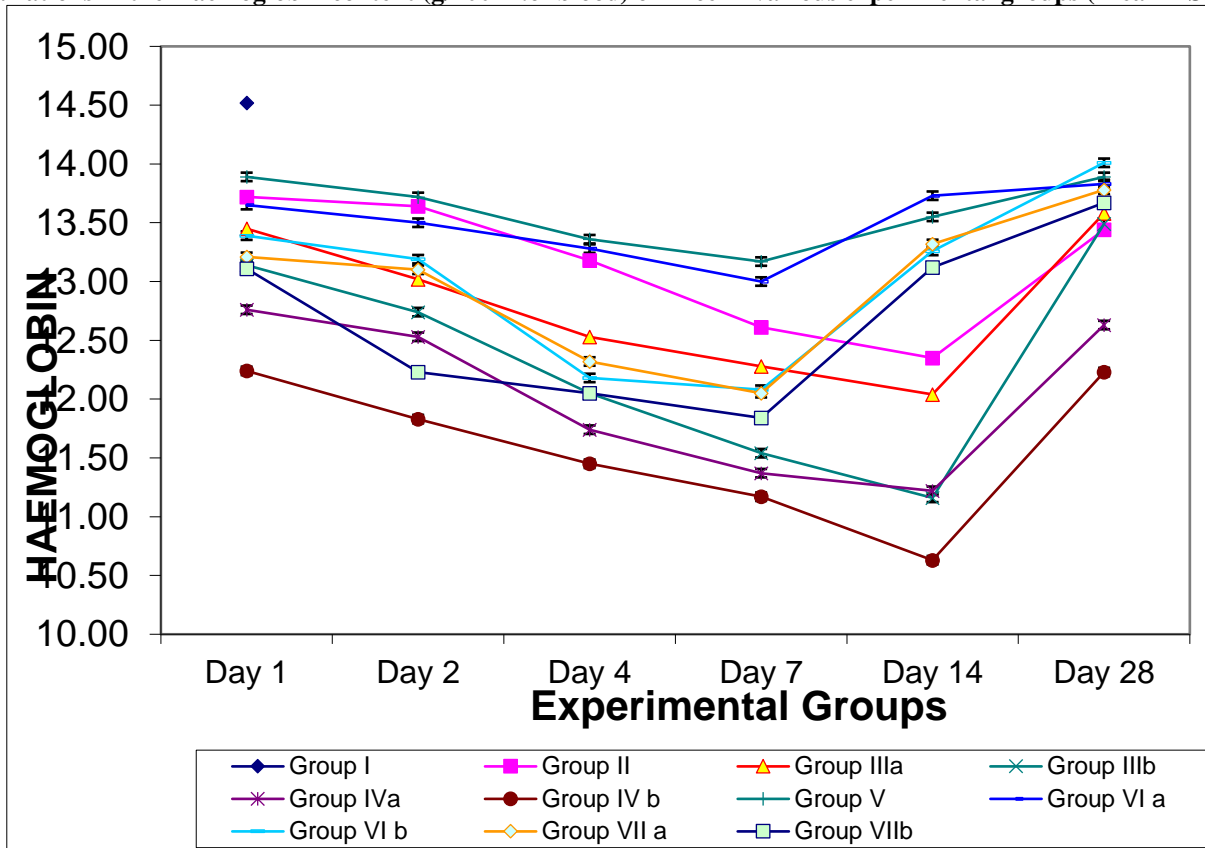
Variation in the values of RBC (million/Cu.mm) of mice in various experimental groups (Mean ± S.E.)



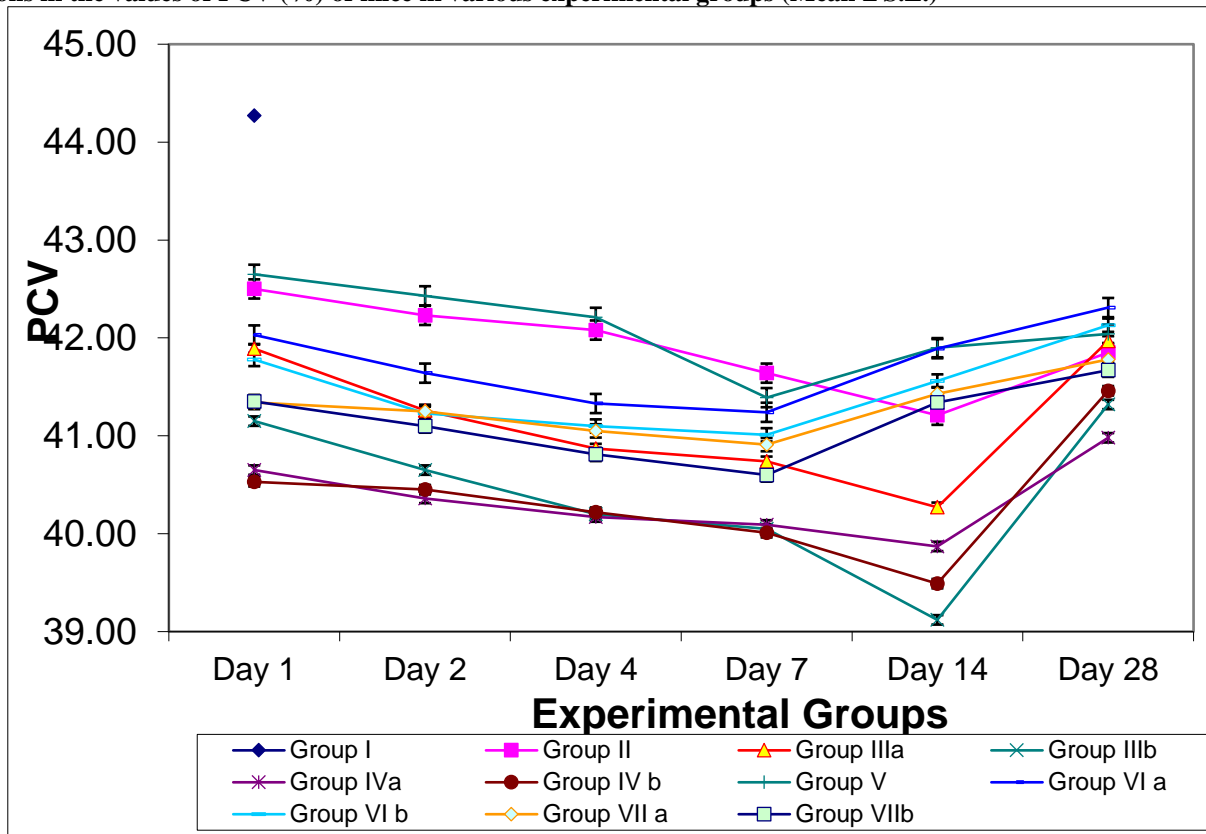
Variation in the values of WBC (thousand/Cu.mm) of mice in various experimental groups (Mean \pm S.E.)



Variations in the Haemoglobin content (g/100ml.of blood) of mice in various experimental groups (Mean \pm S.E.)



Variations in the values of PCV (%) of mice in various experimental groups (Mean ± S.E.)



Discussion

Natural products from plants are of major pharmaceutical and therapeutic importance, several of which are often obtained from the underground parts of the concerned plants. Deviation from standard rules in modern medicines, where instead of a single isolated fraction, a group of naturally occurring components exerts the desired therapeutic effect, was noted in case of Picroliv or Kutkin of *Picrorhiza kurroa*. Picroliv mainly a glucoside, is one such compound, normally obtained from 3 - 4 years old roots and rhizomes of an endangered medicinal plant - *Picrorhiza kurroa* (kutki) and constitute an important component of many Indian herbal preparations, used mainly for the treatment of a variety of liver ailments. It is an iridoid glycoside mixture containing 60% picroside I and kutkoside in the ratio of 1:1.5. Picroliv has shown efficacy comparable to silymarin in rodent models of galactosamine, paracetamol, thioacetamide and CCl₄ induced hepatic damage. Picroliv has also shown choleric effect in rats and anti-cholestatic effect in rats, guinea pigs and cats treated with paracetamol and ethinyl estradiol. It has also anti-viral and immunostimulant activities and is devoid of any significant CNS and CVS, autonomic and other systemic activity. Because of its apparent ability as a strong hepatoprotective and immune-modulatory compound, it is in high demand in both national and international markets. The review discusses the potential of *Picrorhiza* in various hepatic diseases as well as the chemistry and activity of individual compound of crude drug Picroliv. (H. El-Shanshoury et.al.2016)

Exposure to ionizing radiation is known to have lethal effects in blood cells. It is predicted that an individual may spend days, weeks or even months in a radiation field without becoming alarmed. The study aimed to discuss the evaluation of low dose ionizing radiation (IR) effect on some blood components in animal model. Hematological parameters were determined for 110 animal rats (divided into 8 groups) pre- and post-irradiation. An attempt to explain the blood changes resulting from both irradiation and time is given. There was a significant reduction in WBC counts one day after irradiation at all dose levels compared to the control group and started to be affected at the dose of 0.3 Gy. The significance was increasing with increasing the dose. The degradation rate was 15 times higher than the recovery rate. Although both rates increase with increasing the dose, however, the rate of recovery in the second stage is faster than that in the initial stage. Platelet count shows a slow increase in the rate of recovery with increasing the dose up to 0.4 Gy. After which there is a linear increase up to a dose of 1 Gy with a slope of 21 count/ day/Gy. Additionally, there is an increase in the rate of degradation on the applied dose up to 0.3 Gy with a slope of 61.6 count/day/Gy. The recovery rate of red blood cells count (RBC) increases with the increase in the dose reaching a maximum at about 0.5 Gy. Further increase in dose resulted in a rapid degradation with a minimum count at the dose of 0.75 Gy at the maximum value of 0.5 Gy, the change in count decreases exponentially with the increase in time. The present findings suggest that damage from IR causes a significant reduction in blood cell counts in a dose-dependent manner, which may be considered. (Verma et al ,2009).

While previous studies have focused on the health effects of occupational exposure of radiations on medical radiation workers, few have analysed the dose-response relationship between low radiation doses and changes in blood parameters. Even fewer studies have been conducted on industrial worker populations. Using a prospective cohort study design, this study collected health examination reports and personal dose monitoring data from 705 industrial irradiation workers who underwent regular physical examinations at Dongguan Sixth People’s Hospital. The dose-response effects of low-dose ionizing radiation on blood parameters were assessed using a generalized linear model and restricted cubic spline model. Red blood cell counts decreased then increased,

before decreasing again with increasing ionizing radiation. This was in contrast to the curve of the total platelet count after irradiation. Additionally, a radiation dose of 2.904 mSv was the turning point for the nonlinear curve of hemoglobin count changes. In conclusion, long-term, low-dose ionizing radiation affects blood cell levels in industrial irradiation workers. There is a nonlinear dose-response relationship between red blood cell, platelet, and hemoglobin counts and the cumulative radiation dose. These findings should alert radiation workers to seek preventive medical treatment before the occurrence of any serious hematopoietic disease. (Liu et.al:2022)

Picrorrhiza species has been explored since 1940s for its chemical composition. Since then, several phytochemicals have been isolated from different parts of this plant species which have shown the presence of glycosides, aromatic esters, bis-iridoid, phenyl propanoids, alcoholic compounds and fatty acids. Iridoid glycosides viz. kutkins, kutkosides and picrosides, cucurbitacins, triterpenes and simple phenols such as apocynin are the most explored phytoactives of *P.kurroa* for their biological activity and potential clinical applicability. The active principles of *P.kurroa* are the iridoid glycosides picrosides I, II and III. Kutkin is a mixture of picrosides I and II. *P.kurroa* also contains Picroside IV and V, verminoside, catapol, veronicoside, specioside 6-feruloylcatapol, pikuroside, aucubin and many more. *P.kurroa* also contains other active constituents such as apocynin, drosin, nine cucurbitacin glycosides, D-mannitol, phenolic acids and phenylethanoids. As has been reported by Sah & Varsheya, 132 chemical constituents belonging to different class of compounds from roots, rhizomes, seeds, stem and leaves of two Picrorrhiza species (*P. kurroa* Royle ex Benth and *Picrorrhiza scrophulariiflora* Pennel) are listed between 1949 and 2013 (Shah and Varshney, 2013). In the last decade or so, 53 more phytoconstituents have been identified. (Prakash et.al,2020).

Conventionally utilize of *Picrorrhiza kurroa* incorporate chronic constipation, skin-related problems, burning sensation, chronic reoccurring fever, jaundice, heart problem, breathing, digestion, allergy, tuberculosis, blood related problems, prediabetes, obesity, laxative, cholagogue and liver stimulatory. Autoimmune disintegration of insulin manufacturing pancreatic beta- cells generated insulin insufficiency and hyperglycaemia in type 1 diabetes mellitus. Several chemical drugs have been utilizing for cancer treatment, but the cause of genotoxic, carcinogenic and teratogenic effect limits their utility. Anti-oxidant, anti-inflammatory activity of picrosides as the central mechanism in decreasing oncogenesis. Work of picrosides on detoxifying enzyme, cell cycle regulation and induction of signal transducers inhibiting apoptosis has been analysed. AFPR has anti-hyperuricemic activity ascribed to the inhibition of uric acid production in the liver and possible to the increase of urate excretion in the kidney, and possess nephroprotective effect in hyperuricemic rats due to its anti-inflammatory and anti-oxidant activity. (Q et.al;2018). The *Picrorrhiza kurroa* extract's main active part for liver protection is kutkin. Due to suppression of xanthine oxidase inhibitors, metal-ion chelators, oxygen anion production, free radical scavenging and anti-lipid peroxidation are mostly hepatoprotective activity of kutkin. The plant extract of kutki can renew the beta cells of pancreas, increase the production of insulin and help decrease the blood glucose level. (Soni 2019)

The challenge of bioavailability of *P.kurroa-Kutki* phytoactives has been explored through the application of nanoencapsulation technique. Alcoholic extract of *P. kurroa-Kutki* was successfully encapsulated into pluronic-F-68-PLA nanoparticles by nanoprecipitation method. Encapsulation efficiency for picrosides I and II was determined $60.17 \pm 2.8\%$ and $67.2 \pm 7.4\%$ respectively. The authors stated that the release dynamics profile suits intestinal absorption and uptake in humans and could be a promising approach for enhancing intestinal absorption, biocompatibility as well as bioavailability, making it a suitable form of administering the alcoholic extract. However, limited studies on oral bioavailability indicate the need for further work along with exploring the potential for encapsulation and controlled release of the preparations, followed by appropriate studies for safety and toxicity of the nano-encapsulates. (Jia et. al, 2015)

S Ma et al., 2020, have discussed in details the therapeutic potential of picroside-II for organic ischemia and reperfusion injury (to organs and tissues such as cerebrum, myocardium, kidney, testes, skeletal muscles and erythrocytes), liver damage, inflammation, cancer metastasis and osteoclastogenesis. Although the effects of picroside-II are multiple and complex, with the intricate involvement of a number of pathways, the mechanisms of picroside-II appear to be mainly acting through anti-oxidant, anti-inflammatory and anti-apoptotic mechanisms

Conventionally, for most of the clinical indications *P. kurroa-Kutki* rhizome powder is used in a dose range of 300 mg–500 mg, two to three times a day, for an adult. For laxative purpose the dose used is 2 gms to 4 gms as a single dose (Gogate, 1982). Although *P.kurroa-Kutki* is also considered as a detoxicating agent (*Vishapaha*), it has been reported to cause toxicity if not used in an appropriate dose for the correct indication. As per our clinical experience of using *P. kurroa-Kutki* and its traditional products, it is observed to have developed adverse effects such as increased bowel frequency, diarrhoea, abdominal gurgling, abdominal colic etc. (Raut et.al,2023).

REFERENCES:

1. Ashwinikumar Raut, Hiteshi Dhama-Shah, Aashish Phadke, Anand Shindikar, Shobha Udipi, Jayashree Joshi, Rama Vaidya, and Ashok D.B. Vaidya(2023): *Picrorrhiza kurroa*, Royle ex Benth: Traditional uses, phytopharmacology, and translational potential in therapy of fatty liver disease. *J Ayurveda Integr Med.* ; 14(1): 1005582.
2. Deepika Soni, A. G. (2019). "Picrosides" from Picrorrhiza kurroa as potential anti-carcinogenic agents . *Biomedicine and pharmacotherapy*, 1680-1687.
3. Gogate V.M. I ed. 1982. Dravyagunavidnyana, continental prakashan for Maharashtra vidyapeeth granthanirmitti mandal Pune; pp. 261–263. [Google Scholar]
4. JJ, Liu N, Ma Z, Gong ZJ, Liang YL, Cheng Q, Zhong XG, Yao ZJ(2022): Dose Response Effects of Low-Dose Ionizing Radiation on Blood Parameters in Industrial Irradiation Workers. *Dose Response* 5;20(2):15593258221105695. doi: 10.1177/15593258221105695. PMID: 35693871; PMCID: PMC9174562.
5. H. El-Shanshoury , G. El-Shanshoury , A. Abaza (2016): Evaluation of low dose ionizing radiation effect on some blood components in animal model. *Journal of radiation research and applied sciences* Volume 9, Issue 3 Pages 207-362

6. Jia D., Barwal I., Thakur S., Yadav S.C. Methodology to nanoencapsulate hepatoprotective components from *Picrorhiza kurroa* as food supplement. *Food Biosci.* 2015;9(1):28–35. [[Google Scholar](#)]
7. Ma S., Wang X., Lai F., Lou C. (2020): The beneficial pharmacological effects and potential mechanisms of picroside II: evidence of its benefits from in vitro and in vivo. *Biomed Pharmacother.* 2020; 130:110421. [[PubMed](#)] [[Google Scholar](#)]
8. Prakash V., Kumari Anjana, Kaur H., Kumar M., Gupta S., Bala R.(2020) :chemical constituents and biological activities of genus *picrorhiza*: an update. *Indian J Pharmaceut Sci.* :562–577. [[Google Scholar](#)]
9. Praveen C. Verma, Vaishali Basu, Vijayta Gupta, Gauri Saxena and Laiq Ur Rahman (2009): Pharmacology and Chemistry of a Potent Hepatoprotective Compound Picroliv Isolated from the Roots and Rhizomes of *Picrorhiza kurroa* Royle ex Benth. (Kutki). *Current Pharmaceutical Biotechnology* Volume: 10 Issue: 6
10. Sah J.N., Varshney V.K. Chemical constituents of *Picrorhiza* genus (2013): A review. *American Journal of Essential Oils and Natural Products.* ;1(2):22–37. [[Google Scholar](#)]
11. Su, Q., Su, H., Zhihuan, N., Li, D., Wang, L., Chu, S., . . . Chen, N. (2018). Hypouricemic and Nephroprotective Effects of an Active Fraction from *Polyrhachis Vicina* Roger On Potassium Oxonate - Induced Hyperuricemia in Rats. *National Library of Medicine*, 220-233.