Original Research Article

An *in Vivo*Evaluation of the Hepatoprotective Potential of Triphala in CCl4 induced Hepatic Injured Rodent Model.

Abstract:

Liver injury, also known as hepatotoxicity, is a serious health concern since it can irreversibly harm this significant organ's function, leading to life-threatening liver cirrhosis or liver failure. Natural medicinal plants have long been known to play essential roles in up-to-date medication discovery and they are considered as enormous sources of treatments now. Regarding the critical situation of hepatotoxic patients around the globe, among numerous plants with numerous potentials, "Triphala" and it is a popular and highly effective, strong Ayurvedic and Indian traditional medication with exclusive hepatoprotective and antioxidant properties. Triphala is a Sanskrit word that means three fruits and so as the name it is a mixture of the equal amount of the dried fruits of three plants: *Emblica officinalis* (Amalaki), *Terminalia chebula*(Haritaki) and *Terminalia bellerica*(Bibhitaki). In this experiment, CCl4 was used to induce hepatotoxicity and then the hepatoprotective activity of Triphala was assessed through determining the results of different parameters. Afterwards, observing the results it was found that the plant can effectively reverse the disturbed pathological state towards healtyh status in different extent in a does dependent manner. Moreover, any safety concern or possibility of side effects was not aroused throughout the investigation. Therefore, Triphala is considered to have a major aptitude towards hepatic protection and can be counted as an alternative to the marketed synthetic drug, silymarin with more safety and quite equal efficacy. To conclude, more vigorous future study is needed so that this promising plant can be incorporated into hepatotoxic treatments arena.

Keyword: Liver injury, Triphala, CCl4, silymarin, antioxident

Introduction:

The liver is a vital solid organ exclusively found in animals with central controls of several significant biological activities including metabolization, detoxification, vitamin and minerals storage, blood pressure regulation, immunological function, protein synthesis and producing digestive enzymes. Therefore, it can be main target of numerous toxicants and negative impact on this special organ can be provoked by xenobiotics like medicines, dietary supplements, alcohol, chlorinated solvents, peroxidized fats, fungal toxins, radioactive atoms, environmental toxins as well as certain herbs and spices (Thompson et al., 2017). As of now, nearly 71 million individuals are projected to be affected by hepatotoxicity worldwide. This

leads to nearly 1.4 million fatalities each year. As a result, this state of people has become highly alarming, in fact, a global challenge indeed (Jefferies et al., 2018).

Silymarin, Bicyclol, Metadoxine, Curcumin and Glutathione are some of the most appealing and recently identified compounds in the realm of medicine to treat hepatic diseases. These drugs are mainly free radical scavengers with antioxidant effect to reduce oxidative stress of liver. But they are under experimental observation mostly (Li et al., 2021; Muriel & Rivera-Espinoza, 2008). In addition, these available synthetic drugs for liver treatment exhibit several drawbacks including side effects like nephrotoxicity, jaundice, poor absorption, stability, and selectivity (S. A. Ali et al., 2019). Since these treatments have questionable efficiency, therefore, great interest is emerging about alternative and complementary remedies for the management of liver disease. Consequently, medicinal plants have an incredible chance to replace them via genetic alteration incorporating several biotechnological methods (S. A. Ali et al., 2019).

There is a long history of utilizing medicinal herbs to treat hepatic conditions and almost 80 percent of the global population rely on the application of herbal remedies (Ekor, 2014). It has been found that about 160 phytoconstituents of 101 plants have liver-protective properties. Recently, Flavonoids, terpenoids, sterols and other natural compounds derived from medicinal plants, have attracted lots of interest due to their wide range of pharmacological effects, especially antioxidant and hepatoprotective properties (Fitoterapia& 1986, n.d.; M. Gupta et al., 2004).

Some indigenous plants having anti-hepatotoxic potential such as *Silybum marianum, Glycyrrhiza glabra, Phyllanthus amarus, Salvia miltiorrhiza* ,Triphala*(Emblica officinalis, Terminalia belerica, Terminalia chebula),Astragalus membranaceus , Amole tuber, Cochlospermumvitifolium, Heterothecainuloides, Hibiscus sabdariffa, Leucophyllum frutescens, Prostecheamichuacana, Psidium Guajava, Rosmarinus officinalis, Verbena Carolin, Centaurea americana, Juglans mollis, Krameria ramossisima , Turneradiffusa, Capparis spinosa , Cichorium intybus , Solanum nigrum , SapindusmukorossiGaertn., Ginkgo biloba , Woodfordiafruticosa, Vitex trifolia, Schisandra chinensis, Cuscuta chinensis, Lyciumbarbarum, Angelica sinensis ,Diels, and Litseacoreana, Hosta plantaginea, Ligusticum chuanxiong, Daniella oliveri, Garcinia mangostana, Solanum melongena, Vaccinium myrtillus, Picrorhizakurroa ,Citrus medica ,Glycyrrhiza glabra* etc have been used frequently and successfully for the treatment of hepatic disorders based on credible scientific evidence gleaned through the medicinal plants study(M. Ali et al., 2018; Asadi-Samani et al., 2015; Thilagavathi et al., 2023; Torres-González et al., 2014) .

Among these, Triphala is a renowned and admired AyurvedicherbalRasayana formula made up of exactly equal amounts (33.33%) of three dried super-fruits of different plant species, namely "Amlaki" (*Emblica officinalis*) from the Family Euphorbiaceae, "Baheda" (*Terminalia bellerica*) and "Haritaki" (*Terminalia chebula*) both from the Family Combretaceae(R. Gupta et al., 2015; Peterson et al., 2017).It is abundantly propagated in the tropical and subtropical zones of the globe, spreading to the Indian subcontinent, China, Thailand, Sri Lanka, and Middle Eastern countries like Egypt and Iran (Neelam Rawat et al., 2023).

According to Pharmacological tests, it has been revealed that the formula's primary constituents include tannins, gallic acid, ellagic acid, and chebulinic acid, all of which are highly effective antioxidants and may be responsible for several immunomodulatory actions. Other beneficial compounds associated with Triphala include flavonoids including quercetin and luteolin, saponins, anthraquinones, amino acids, fatty acids as well as several types of carbohydrates (Belapurkar et al., n.d.; Lee et al., 2005; Lu et al., 2012).Besides, the hydrolysed form of tannins in it is reported to offer hepatoprotection regarded as the key activators of various biological processes. In addition, no prominent adverse effects can be seen from the water-based derivative of Triphala(Olennikov et al., n.d.; Pawar et al., n.d.; Phetkate et al., 2020; RUSSELL et al., n.d.).

Triphala's three ingredients are employed in the production of several hepatoprotective medications. However, due to the intricacy of the three rasayanas or reviving herbs, in the formulation, Triphala can be characterized as a versatile medication for a variety of uses. Triphala has also been shown to have free radical scavenging, antioxidant, anti-inflammatory, immunomodulating, appetite stimulation, gastric hyperacidity reduction, antipyretic, analgesic, antibacterial, antimutagenic, wound healing, anti-cariogenic, antistress, hypoglycaemic, anticancer, hepatoprotective, chemoprotective, radioprotective effects (Baliga et al., 2012; R. Gupta et al., 2015).

The aqueous and methanol-based extract of Triphala exhibits significant resistance against oxidative damage via its ability to enhance the oxidative stress enzyme system by showing free radical scavenging activities, thereby boosting the antioxidant defence mechanism and assures hepatic protection (R. Gupta et al., 2015; Pundareekaksha Rao P., 2017). Therefore, the present work intends to examine the hepatoprotective activities of Triphala against CCl4-induced hepatotoxicity and histopathological assessments and statistically estimate the ADMET attributes of its phytoconstituents. The outcomes of this study will promote future medicinal uses of Triphala specially in hepatoprotective arena.

Method and materials

Drugs, chemicals and equipment:

Carbon tetrachloride (CCl4) was purchased from the Sigma Aldrich Company, USA. A standard hepatoprotective drug named silymarin (Brand name: silybin) was offered as a gift from Square Pharmaceuticals Ltd. Again, SGPT, SGOT, ALP, Creatinine, Total Cholesterol, HDL, LDL, Triglyceride were assessed by using blood serum analyzing kits, which were supplied from Orbit Trade Ltd, and Gentech Ltd, Bangladesh. These parameters were assessed by Humalyzer 3000 from Germany(Khan et al., 2012).

Collection and extraction of Triphala :

The fruits of Triphala (Amlaki, Baheda, and Haritaki) were gathered at the Dhaka Botanical Garden. Following that, the specimen was validated in accordance with their norms and regulations by the Department of Botany at the University of Dhaka.

The fruits were carefully cleaned after which they were left for seven days to dry in the sun. The dried fruits were then stored in a 40°C oven for 7 days. After that, the dried fruits were coarsely powdered. The three distinct powdered fruits were then soaked in three separate bottles of 70% ethanol for around 21 days while occasionally being shaken. Finally, every three days the extract was filtered. The extract was afterwards dried using low temperature and pressure in a rotary evaporator. To extract more accurately, the entire procedure is performed several times.

Then, 45gm of Triphala is made by combining 15gm from each of the three different powder extracts. Finally, utilizing this crude mixture, other pharmacological experiments were carried out.

Animal and housing

140 healthy male Wistar rats were purchased and obtained from Jahangirnagar University in Dhaka, Bangladesh, weighing between 120 and 180 g. Each of them was cautiously nurtured by being kept in the Institute of Nutrition & Food Science at the University of Dhaka for two weeks in an environment that was well -controlled (relative humidity 55±5%, 12±1h light/dark cycle, and temperature 25±3℃). Each group of ten groups was formed with a total of 10 rats, with 3 rats each weighing from 121 -140 grams, 4 rats of 141 - 160 grams, and 3 rats from 161 - 180 grams. All rats received a standard food supplement, clean water and extra room for participating in their typical behaviours. To sum up, all research protocols were followed to the letter by the Institutional Animals Ethics Committee (IEAC).

CCl4 preparation for gastric lavage

In each group, a single oral dosage of CCl4 combined with olive oil as the vehicle was provided on a regular basis at a 1:1 ratio (3 ml/kg of rat body weight). Both the medication and the plant extract had been administered orally. To alleviate liver damage, Triphala extract (500, 750, and 1000 mg/kg) and Silymarin (80, 120, and 150 mg/kg) were administered. A stomach tube was used to give CCl4, Silymarin, and plant extract to animals. To produce hepatotoxicity, carbon tetrachloride (CCl4) was injected intraperitoneally at a dosage of 3 mL/kg.

Animal grouping and treatment procedures are shown in the following Table:

Table 1.Animal grouping and treatment procedures.

Group no.	**Group Status**	**Treatment Specimen**	**The volume of Treatment specimen (mg/kg)**	**Group Abbreviation**
1	Negative control	Physiological saline	10ml/kg	C
2	Disease control	Carbon tetra chloride (CCl_4)	3ml/kg	CCl_4
3	CCl_4 + Marketed	Standardized	3ml/kg+ 140mg/kg	$CCl_4 + S_{140}$

	Silymarin	Silymarin		
4	CCl_4 + Silymarin	Low dose Silymarin	3ml/kg+ 80mg/kg	$CCl_4 + S_{80}$
5	CCl_4 + Silymarin	Medium dose Silymarin	3ml/kg+ 120mg/kg	$CCl_4 + S_{120}$
6	CCl_4 + Silymarin	High dose Silymarin	3ml/kg+ 150mg/kg	$CCl_4 + S_{150}$
7	No disease+ Marketed Silymarin	Standardized Silymarin	140mg/kg	S_{140}
8	No disease + Triphala	Triphala	500mg/kg	T_{500}
9	No disease + Triphala	Triphala	750mg/kg	T_{750}
10	No disease + Triphala	Triphala	1000mg/kg	T_{1000}

Soon after the treatment, all rats were sacrificed ,and blood was obtained via cardiac puncture, Then different parameters such as Serum Glutamate Oxaloacetic Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase(SGPT), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (γ-GT), Creatinine, Total Cholesterol (TC), High-density lipoprotein (HDL), Low density lipoprotein (LDL), Triglyceride (TG), Catalase (CAT), Superoxide dismutase (SOD) and Malondialdehyde (MDA) were determined in all groups to check the impact of CCl4, silymarin and plant extract on rat pathology. Six weeks were the period of the therapy.

Statistical analysis

All the results (raw data) are divided into many groups based on the several study factors that were collected and evaluated on a broadsheet utilizing an MS Excel application. Descriptive statistics were applied to the data, and the results were expressed as mean ± SD. To evaluate statistical significance, the "One Way Anova Test" of "SPSS 16" program was used to analyse inter-group heterogeneity among multiple biological parameters. The occurrences are considered statistically significant and highly significant, with p-values below 0.05 ($p<0.05$) and 0.01 ($p<0.01$), accordingly.

Result and Discussion

Table 2. Effect of inter-group heterogeneity among multiple biological parameters

Group No.	SGPT (U/L)	SGOT(U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
1	37.40±3.93	45.45±3.60	0.6±0.04	25.28±3.39	92.40±4.34	69.19±6.19	39.36±5.39	52.39±5.10
2	99.62±8.39	109.34±9.3	2.85±0.97	92.86±9.73	168.34±13.45	45.59±4.18	93.19±11/29	106.4±12.50
3	60.84±6.46	65.32±6.71	1.10±0.82	55.16±7.24	125.16±8.39	58.56±2.32	62.22±6.66	72.20±9.39
4	95.54±7.36	105.22±10.11	2.30±0.76 *	84.14±8.97*	166.31±12.46	47.16±4.54	47.24±4.19*	95.23±11.53*
5	91.22±7.58 *	99.21±8.53 *	1.72±0.08*	77.24±10.42*	158.41±12.38 *	50.20±3.11*	53.30±4.70*	89.46±8.32*
6	84.50±6.36 *	93.57±8.80 *	1.40±0.06*	71.40±9.38*	146.35±9.49*	54.65±5.06*	58.56±3.9*	82.57±3.10*
7	34.21±1.04	48.81±2.34	0.5±0.03	28.80±4.14	95.16±5.51	73.30±6.08	35.26±3.40	55.30±4.10
8	37.35±2.34	42.54±3.10	0.8±0.08	23.15±5.50	90.10±4.23	70.18±5.80	38.40±3.19	52.17±3.19
9	39.16±2.40	42.90±3.34	0.6±0.03	27.90±4.21	94.50±5.26	75.63±4.80	40.43±5.26	56.50±4.18
10	37.54±2.19	44.65±2.81	0.7±0.06	26.24±3.51	97.34±4.10	68.28±5.19	39.16±4.51	50.53±3.21

SGPT:

According to the result, the SGPT level of the CCl_4 treated or disease control group was higher than the value of all the groups. SGPT levels were reduced by the standard drug treated group (Group 3) but in low dose it was reduced non significantly (Group 4). However, altered pathological state was restored in significant ($p<0.05$) and dose dependent manner in the groups treated with high and medium dose drug (Group 5-6). In addition, there is no significant difference of SGPT level of negative control group (Group 1) and plant extract treated groups (Group 8-10). So, it can be said that our plant is not associated with any side effects.

SGOT:

In case of SGOT level, negative control (Group 1) and disease control group (Group 2) displayed two totally contrary circumstances. The drug restored the disturbed pathological state in significant ($p<0.05$) manner (Group 5-6). Moreover, as there is no significant difference of SGOT value among the negative control group (Group 1) and Triphala-treated groups (Group 8-10), no side effects exerting potential were found.

Creatinine:

The disease control group (Group 2) revealed an alarming rise in the creatinine level and it was in striking opposition with the negative control group (Group 1). Silymarin and Triphala treatment presented noteworthy effects by reducing creatinine levels. Besides, there was no notable elevation of this compared to the negative control group (Group 1).

Urea:

In case of urea level, the drug (Group 4-6) lessened the highly elevated urea level of disease control group (Group 2) in dose dependent manner significantly ($p<0.05$). On the other hand, the plant extract treated groups (Group 8-10) displayed that the urea level value was maintained to quite normal value as found in negative control group (Group 1).

HDL:

The largely reduced HDL value of disease control group (Group 2) was proficiently enhanced in the drug treated hepatotoxic groups (Group 5-6) in significant manner ($p<0.05$). Likewise, the plant extract treated groups (Group 8-10) completely kept normal HDL level quite same as the negative control group (Group 1). Hence, no contradiction about the safety of plant extract aroused.

LDL:

For LDL value, the drug treated diseased groups (4-6) displayed significant decrease of CCl_4 mediated highly raised LDL value found in disease control group (Group 2). Besides, the test extract treated groups (Group 8-10) remarkably presented the LDL value with no significant difference with negative control group (Group 1). Hence, side effects exerting potential is absent.

Triglyceride:

The administration of CCl_4 nearly doubled the triglyceride level compared to the negative control group (Group 1). The Hepatic injured rats demonstrated significant decrease ($p<0.05$) of triglyceride level when treated with the drug, Silymarin (Group 5-6). In addition, the plant extract treated groups maintained similar results as the negative control group (Group 1) and ensured safety of plant extract prescribing.

Total Cholesterol:

Notable elevation of total cholesterol level was observed in the hepatic injured group (Group 2). However, the level declined significantly ($p<0.05$) when the injured rodents were treated

with the drug (Group 5-6). Whereas the Triphala extract treated groups (Group 8-10) showed results without any significant difference compared to negative control group (Group 1). Thus, safety of the plant was again ensured.

To summarize, our test plant extract showed noticeable positive outcomes in all the countable parameters without any possibility of safety hindrance.

Conclusion:

From the above discussion it is clear that our plant has the potential to impart hepatoprotective activity. Again, the dose dependent approach infers that isolation and purification of desired compounds from the whole extract can exhibit more potentiality and can be incorporated into the hepatotoxicity management system as a medicine.

Reference:

Ali, M., Khan, T., Fatima, K., Ali, Q. ul A., Ovais, M., Khalil, A. T., Ullah, I., Raza, A., Shinwari, Z. K., & Idrees, M. (2018). Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. *Phytotherapy Research*, *32*(2), 199. https://doi.org/10.1002/PTR.5957

Ali, S. A., Sharief, N. H., & Mohamed, Y. S. (2019). Hepatoprotective Activity of Some Medicinal Plants in Sudan. *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/2196315

Asadi-Samani, M., Kafash-Farkhad, N., Azimi, N., Fasihi, A., Alinia-Ahandani, E., &Rafieian-Kopaei, M. (2015). Medicinal plants with hepatoprotective activity in Iranian folk medicine Asian Pacific Journal of Tropical Biomedicine. *Asian Pac J Trop Biomed*, *5*(2), 146–157. https://doi.org/10.1016/S2221-1691(15)30159-3

Baliga, M. S., Meera, S., Mathai, B., Rai, M. P., Pawar, V., &Palatty, P. L. (2012). Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: A review. *Chinese Journal of Integrative Medicine*, *18*(12), 946–954. https://doi.org/10.1007/S11655-012-1299-X

Belapurkar, P., … P. G.-I. journal of, & 2014, undefined. (n.d.). Immunomodulatory effects of triphala and its individual constituents: a review. *Ncbi.Nlm.Nih.GovPBelapurkar, P Goyal, P Tiwari-BaruaIndian Journal of Pharmaceutical Sciences, 2014•ncbi.Nlm.Nih.Gov*. Retrieved September 17, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4293677/

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4 JAN*. https://doi.org/10.3389/FPHAR.2013.00177/FULL

Fitoterapia, S. H.-, & 1986, undefined. (n.d.). Natural products and plants as liver protecting drugs. *Cir.Nii.Ac.Jp*. Retrieved September 16, 2023, from https://cir.nii.ac.jp/crid/1573950400983920256

Gupta, M., Kanti Mazumder, U., Kumar, S., Gomathi, P., & Kumar, R. S. (2004). *Antioxidant and hepatoprotective effects of bauhinia racemosa against paracetamol and carbon tetrachloride induced liver damagein rats*. https://www.sid.ir/EN/VEWSSID/J_pdf/101020040102.pdf

Gupta, R., Gupta, A., Singh, R. L., Ram, L., & Singh, H. (2015). Hepatoprotective Activities of Triphala and Its Constituents. *International Journal of Pharma Research & Review*, *4*(1), 34–55.

Hu, Y.-Y., Liu, C.-H., Wang, R.-P., Liu, C., Liu, P., & Zhu, D.-Y. (2000). Protective actions of salvianolic acid A on hepatocyte injured by peroxidation in vitro. *World Journal of Gastroenterology*, *6*(3), 402–404. https://doi.org/10.3748/wjg.v6.i3.402

Jefferies, M., Rauff, B., Rashid, H., Lam, T., & Rafiq, S. (2018). Update on global epidemiology of viral hepatitis and preventive strategies. *World Journal of Clinical Cases*, *6*(13), 589. https://doi.org/10.12998/WJCC.V6.I13.589

Khan, R. A., Khan, M. R., &Sahreen, S. (2012). CCl4-induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC Complementary and Alternative Medicine*, *12*(1), 1–6. https://doi.org/10.1186/1472-6882-12-178/TABLES/5

Lee, H., Won, N., Kim, K., Lee, H., … W. J.-B. and, & 2005, undefined. (2005). Antioxidant effects of aqueous extract of Terminalia chebula in vivo and in vitro. *Jstage.Jst.Go.JpHS Lee, NH Won, KH Kim, H Lee, W Jun, KW LeeBiological and Pharmaceutical Bulletin, 2005•jstage. Jst.Go.Jp*. https://www.jstage.jst.go.jp/article/bpb/28/9/28_9_1639/_article/-char/ja/

Li, M., Luo, Q., Tao, Y., Sun, X., & Liu, C. (2021). Pharmacotherapies for Drug-Induced Liver Injury: A Current Literature Review. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/FPHAR.2021.806249

Lu, K., Chakroborty, D., Sarkar, C., Lu, T., Xie, Z., Liu, Z., & Basu, S. (2012). Triphala and its active constituent chebulinic acid are natural inhibitors of vascular endothelial growth factor-A mediated angiogenesis. *PLoS ONE*, *7*(8). https://doi.org/10.1371/JOURNAL.PONE.0043934

Muriel, P., & Rivera-Espinoza, Y. (2008). Beneficial drugs for liver diseases. *Journal of Applied Toxicology : JAT*, *28*(2), 93–103. https://doi.org/10.1002/JAT.1310

Neelam Rawat, Shuchi Mitra, Usha Sharma, & Khem Chand Sharma. (2023). An Overview of TriphalaGuggulu and its Ingredients. *AYUSHDHARA*, 47–59. https://doi.org/10.47070/AYUSHDHARA.V10ISUPPL1.1134

Olennikov, D., Kashchenko, N., Nutrients, N. C.-, & 2015, undefined. (n.d.). In Vitro Bioaccessibility, Human Gut Microbiota Metabolites and Hepatoprotective Potential of Chebulic Ellagitannins: A Case of Padma Hepaten® Formulation. *Mdpi.ComDNOlennikov, NI Kashchenko, NK ChirikovaNutrients, 2015•mdpi.Com*. https://doi.org/10.3390/nu7105406

Pawar, V., Lahorkar, P., of, D. N.-I. J., & 2009, undefined. (n.d.). Development of a RP-HPLC method for analysis of TriphalaCurna and its applicability to test variations in TriphalaCurna preparations. *Ncbi.Nlm.Nih.GovV Pawar, P Lahorkar, DBA*

NarayanaIndianJournal of Pharmaceutical Sciences, 2009•ncbi.Nlm.Nih.Gov. Retrieved September 16, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2865809/

Peterson, C. T., Denniston, K., & Chopra, D. (2017). Therapeutic Uses of Triphala in Ayurvedic Medicine. *Journal of Alternative and Complementary Medicine*, *23*(8), 607. https://doi.org/10.1089/ACM.2017.0083

Phetkate, P., Kummalue, T., Rinthong, P. orn, Kietinun, S., &Sriyakul, K. (2020). Study of the safety of oral Triphala aqueous extract on healthy volunteers. *Journal of Integrative Medicine*, *18*(1), 35–40. https://doi.org/10.1016/J.JOIM.2019.10.002

Pundareekaksha Rao P. (2017). Antioxidant Effect of Triphala - Critical Review. *Journal of Ayurveda and Integrated Medical Sciences*, *2*(01), 213–219. https://doi.org/10.21760/JAIMS.V2I1.7513

RUSSELL, L., Mazzio, E., Badisa, R., … Z. Z.-A., & 2011, undefined. (n.d.). Differential cytotoxicity of triphala and its phenolic constituent gallic acid on human prostate cancer LNCap and normal cells. *Ar.Iiarjournals.OrgLH RUSSELL, E Mazzio, RB Badisa, ZP Zhu, M Agharahimi, DJ Millington, CB GoodmanAnticancer Research, 2011•ar.Iiarjournals.Org*. Retrieved September 16, 2023, from https://ar.iiarjournals.org/content/31/11/3739.short

Thilagavathi, R., Begum, S. S., Varatharaj, S. D., Balasubramaniam, A. kumar, George, J. S., & Selvam, C. (2023). Recent insights into the hepatoprotective potential of medicinal plants and plant-derived compounds. *Phytotherapy Research*, *37*(5), 2102–2118. https://doi.org/10.1002/PTR.7821

Thompson, M., Jaiswal, Y., Wang, I., & Williams, L. (2017). Hepatotoxicity: Treatment causes and applications of medicinal plants as therapeutic agents. *The Journal of Phytopharmacology*, *6*(3), 186–193. www.phytopharmajournal.com

Torres-González, L., Waksman-de Torres, N., Pérez-Meseguer, J., Muñoz-Espinosa, L., Salazar-Aranda, R., & Cordero-Pérez, P. (2014). Review of plants with hepatoprotective activity evaluated in Mexico. *MedicinaUniversitaria*, *16*(63), 78–86. https://www.elsevier.es/en-revista-medicina-universitaria-304-articulo-review-plants-with-hepatoprotective-activity-X1665579614366029