EXPLORATION OF MECHANISM OF *HYGROPHILA AURICULATA* TO TREAT CARBOPLATIN INDUCED TOXICITIES BUILT ON NETWORK PHARMACOLOGY

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ABSTRACT

Hygrophila auriculata is a traditional herb used for several ailments, with an unclear mechanism of action. The present study aimed to detect its efficacy on nephrotoxicity and hepatotoxicity in Wistar rats followed by network pharmacology analysis to explain its mechanism of action. 24 rats were divided into 4 groups (n=6). After baseline blood investigations, group 1 was treated with normal saline on 13th day, groups 2-4 with carboplatin, groups 3, 4 with different strengths of *H. auriculata* (day 15 to 30). The active components along with targets of *H. auriculata* were screened and overall network was created using Cytoscape software. We made preliminary predictions about the major active components, targets along with signalling pathways of *H. auriculata* to treat carboplatin induced hepatotoxicity and nephrotoxicity, which could pave way for clinical application of *H. auriculata* against carboplatin induced toxicities.

Keywords: Carboplatin, chemotherapy, plant extract, toxicity, network pharmacology, *Hygrophila auriculata*

ABBREVIATIONS: CCSEA- Committee for control and supervision of experimentation on animals guidelines; CID- Compound Identifier, DAVID - Database for annotation, visualization and integrated discovery, IAEC - Institutional animal ethics committee, IMPPAT- Indian medicinal plants, phytochemistry and therapeutics, KEGG- Kyoto encyclopedia of genes and genomes, $Score_{gda}$ - Score - gene disease association, UniProt- Universal protein resource

INTRODUCTION

Carboplatin, a platinum coordination complex, is used as a chemotherapeutic agent for cancers affecting the ovary, lung, head, neck and brain. The narrow therapeutic index of cisplatin steered the development of carboplatin, which is a less toxic analogue and advantageous with regards to individualized dosing and ease of administration. It is employed as a first-line treatment in advanced ovarian cancer patients, and has been used for the therapy of advanced lung cancer, while it demonstrates inferior activity when compared to cisplatin in head and neck cancer, bladder cancer, and germ cell tumor[1].

Carboplatin-based chemotherapy has been reported to cause bone marrow suppression, which is also its dose-limiting toxicity. Compared to other chemotherapeutic agents, carboplatin is more likely to cause anaemia, neutropenia and thrombocytopenia. It also causes peripheral neuropathy, renal toxicity and hepatic dysfunction as delayed toxicity for which symptomatic treatment has been the current mode of management[2].

The severity of anaemia, which is a regular complication of myelosuppressive chemotherapy, is dependent on the progression of the disease and intensity of therapy. Erythropoiesis may be impaired due to repeated cycles of chemotherapy, leading to a reduction in the quality of life (QOL) owing to symptoms of anaemia. Patients commonly complain of fatigue and dyspnoea on exertion, which can adversely affect their ability to perform daily routine activities[3]. Carboplatin may cause dose-related severe bone marrow suppression, which can result in infection (due to neutropenia) or bleeding (due to thrombocytopenia). Carboplatin-induced anaemia may also require a blood transfusion. Dose reduction is required in such patients, and chemotherapy cycles need

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to be delayed until white blood cell (WBC), platelet, and red blood cell (RBC) counts have recovered[3].

Carboplatin-induced nephrotoxicity involves damage to the glomeruli and tubules, which is associated with morbidity and mortality in patients receiving this drug. Carboplatin can also cause an elevation in serum aminotransferase levels[4]. However, the incidence of acute liver injury is rare and the exact mechanism of liver injury due to carboplatin is not known. Presently, there is a lack of specific drugs for the prevention or management of carboplatin-induced anaemia, nephrotoxicity or hepatotoxicity. There is a need to identify therapeutic agents which can be beneficial for the prevention or treatment of these conditions so that the use of carboplatin can be continued uninterruptedly during the cycles of chemotherapy.

Hygrophila auriculata, an herb generally found in India, is utilized as a vegetable in some states like Orissa, West Bengal, and Chhattisgarh. The stem and leaves of the succulent are usually boiled and used to raise haemoglobin levels in rural pregnant women. This herbal treatment is bereft of side effects and is also demonstrated to be effective. Different parts of the herb including the leaves, roots, and seeds are used in various pathophysiological conditions such as hepatic disorders, rheumatism, gonorrhoea, renal stones and for their anti-tumour activity[5]. Few studies have demonstrated the beneficial effect of *H. auriculata* in various disease conditions like jaundice,

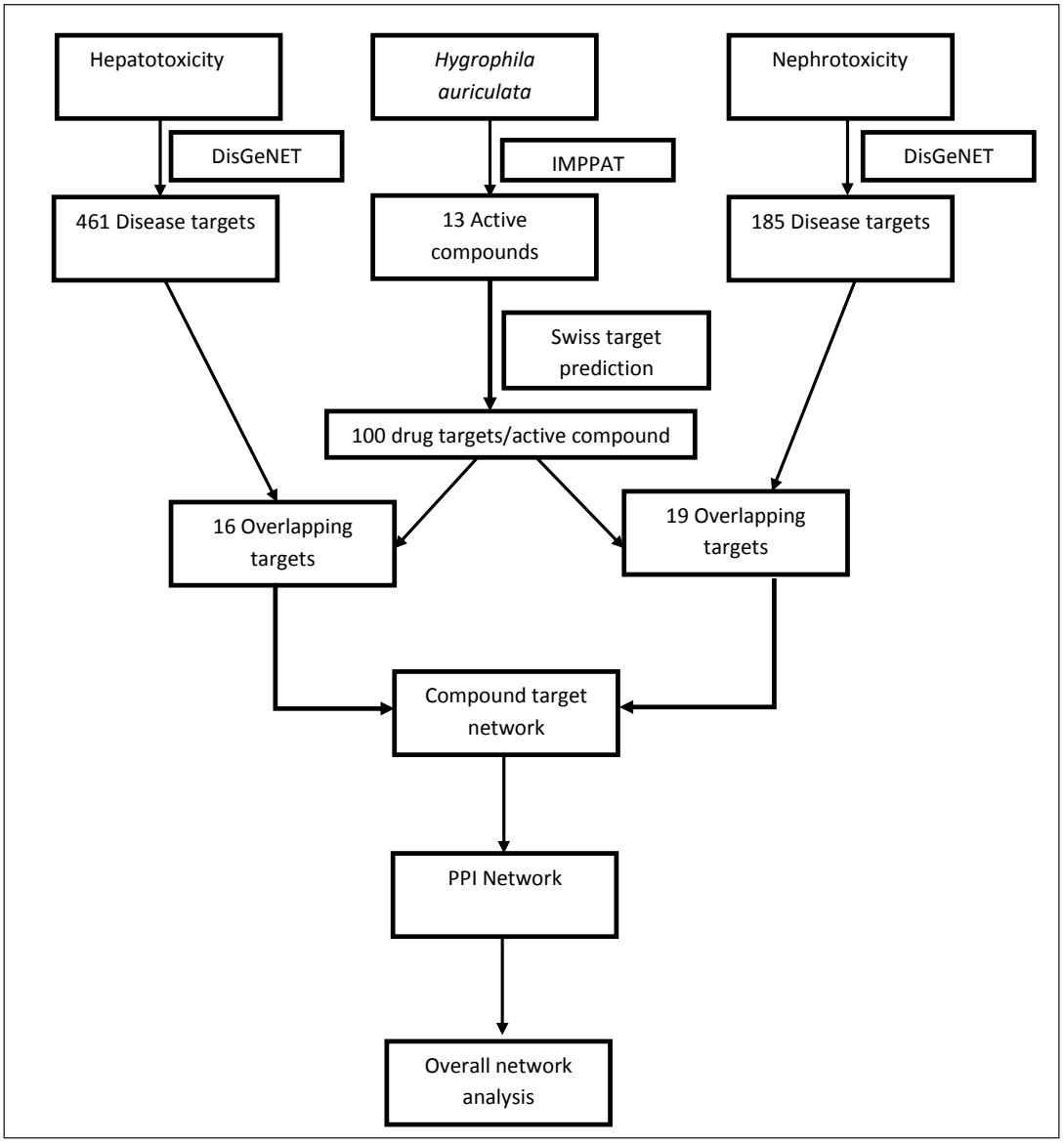

Fig. 1: A flow diagram of network pharmacology for this study

oedema, urinary calculi, anaemia etc[6]. Among these, a study done by Vijayakumar M et al. demonstrated the antidiabetic and antioxidant effect of *H. auriculata* extract in diabetic rats[7]. Another study conducted by Singh A et al. reported the hepatoprotective effect of methanolic extract of *H. auriculata* seeds when administered to rats with induced liver damage[8]. Another study done by Preethi et al. tested the diuretic action of alcoholic extract of the seeds of *H. auriculata,* which demonstrated significant, electrolyte excretion and diuretic properties[9].

Network pharmacology can predict mechanism of action of various ayurvedic medicines by integrating disease and drugs into the biomolecular network[10]. In the current era of evidence-based medicine, network pharmacology is fast emerging as the bridge between Ayurveda and modern medicine to overcome each other's shortcomings. In the present study, the blood and serum parameters were investigated to assess anaemia, hepatotoxicity and nephrotoxicity induced by carboplatin in experimental rodents, and treated with the extract of *H. auriculata* during its flowering stage. Also, the likely mechanism of action of *H. auriculata,* as a hematinic, nephroprotective, and hepatoprotective, in carboplatin-induced anaemia, nephrotoxicity and hepatotoxicity was explored using network pharmacology, and Fig. 1 depicts the workflow of our study.

In order to understand the effect of traditionally used medicinal plants, well-documented research on *H. auriculata* will be helpful to corroborate the potential values of this natural gift, which is lagging behind due to scarcity of research.

MATERIALS AND METHODS

Animals

This study was conducted after approval by Institutional Animal Ethics Committee (IAEC/KMC/115/2019). Animals from the Institutional Central Animal Facility were used, in agreement with Committee for Control and Supervision of Experimentation on Animals guidelines (CCSEA). 24 female albino Wistar rats with weights ranging from 150 to 250 g and aged 8-10 weeks were utilized for this study. Animals were housed under standard conditions which included exposure to 12:12 light-dark cycle, 28 °C temperature, 50 % humidity, water *ad libitum* and were fed with standard food granules.

Drugs, chemicals, and plant product

Carboplatin, manufactured by Fresenius Kabi Oncology Ltd., Gurgaon, Haryana, was purchased from the hospital pharmacy. All the other reagents used were of analytical grade and procured from Coral Clinical Systems, Verna, Goa. *H. auriculata* leaves were obtained from Bangaluru, Karnataka, India, and authenticated by a botany scholar. Glycerol extract of the herb was prepared in 75% glycerol and 25% water. A jar was filled with 60 g of the dried herb. 125 mL of boiling water was poured to dampen the herb. Then 375 mL of vegetable glycerine was poured, and the mixture was stirred with a clean spoon. The lid was put, and the jar was kept in this condition for 6 weeks in dark, and was stirred once a day with a spoon. After 6 weeks, the herbs were strained out and stored in a cool, dry place[11].

Experimental design

In our experiment, 24 rats were arbitrarily distributed into 4 groups (n=6). They were treated for a period of 30 days. Blood was drawn via retro-orbital route with the help of capillary tubes from which haemoglobin (Hb), packed cell volume (PCV), RBC count, kidney and liver function tests were assessed on 0, 15th and 30th days. (On day 13, normal saline was administered to Group 1 (control) via intraperitoneal route (I.P.), and the remaining groups were administered with carboplatin 60 mg kg^{-1} I.P.[12]. From day 15 to 30, Group 1 and Group 2 (carboplatin) were given normal saline I.P. Group 3 - carboplatin + low dose *H. auriculata* (LDHA) was orally treated with 250 mg kg^{-1} of glycerol extract of *H. auriculata* and Group 4 - carboplatin + high dose *H. auriculata* (HDHA) was administered 500 mg kg^{-1} of glycerol extract of *H. auriculata* orally[13].

Biochemical parameters

RBC count was measured using haemocytometer, Hb estimation with Sahli's hemoglobinometer, PCV, or hematocrit using Wintrobe's tube. The blood indices including Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were also calculated. Biochemical parameters assessed were blood urea using glutamate dehydrogenase (GLDH) kinetic method, creatinine measured by modified Jaffe's kinetic method, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) estimated using modified International Federation of Clinical Chemistry (IFCC) method. All the kits were procured from Agape diagnostics[14-16].

Network pharmacology

IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database was used to search for the active compounds[17]. Canonical SMILES (Simplified Molecular Input Line Entry System) for each active

Table I: RBC count and Hb count of different groups on days 0, 15 and 30

	Day 0		Day 15		Day 30	
Parameter	**RBC count (millions mm^{-3}) Mean± SEM**	**Hb (g dl^{-1}) Mean±SEM**	**RBC count (millions mm^{-3}) Mean ± SEM**	**Hb (g dl^{-1}) Mean ± SEM**	**RBC count (millions mm^{-3}) Mean ± SEM**	**Hb (g dl^{-1}) Mean ± SEM**
Control	5.8067 ± 0.709	16.600 ± 0.186	5.7017 ± 0.682	16.467 ± 0.168	5.8933 ± 0.717	16.433 ± 0.222
Carboplatin	5.8550 ± 0.414	16.400 ± 0.577	4.1367 ± 0.160	10.467 ± 0.440 *	3.4000 ± 0.054[a] *	9.850 ± 0.150 *[a]
Carboplatin + LDHA	5.0483 ± 0.362	15.500 ± 0.486	3.7383 ± 0.325*	10.400± 0.588 *	4.6233 ± 0.243[#]	13.433 ± 1.689 #[b]
Carboplatin + HDHA	5.3300 ± 0.233	15.600 ± 0.225	4.2000 ± 0.232	12.667 ± 0.398 *	4.6783 ± 0.213	14.200 ± 0.737 #[b]

*Repeated measures of ANOVA, Bonferroni's post hoc test *p<0.05 vs day 0; #p<0.05 vs day 15*

Oneway ANOVA on Day 30, followed by Tukey's post hoc test [a] p<0.05 vs control, [b] p<0.05 vs carboplatin

Table II: MCV and MCHC in different groups on days 0, 15 and 30

	Day 0		Day 15		Day 30	
Group	**MCV (Mean ± SEM)**	**MCHC (Mean ± SEM)**	**MCV (Mean ± SEM)**	**MCHC (Mean ± SEM)**	**MCV (Mean ± SEM)**	**MCHC (Mean ± SEM)**
Control	110.9417 ± 13.320	27.9100 ± 0.848	109.965 ± 12.104	27.9567 ± 0.647	109.6250 ± 12.171	27.5267 ± 0.480*
Carboplatin	106.0067 ± 7.697	27.0417 ± 0.927	106.1067 ± 4.611	24.0650 ± 1.026*[a]	147.5700± 0.796*[a #]	19.6367 ± 0.161*[a#]
Carboplatin +LDHA	115.0600 ± 9.703	27.6000 ± 1.329	134.6167 ± 7.715	23.8883 ± 0.923[a]	123.2417 ± 4.885	23.5717 ± 0.567[a]
Carboplatin+ HDHA	117.3983 ± 8.014	25.4350 ± 1.031	122.5250 ± 5.637	24.9050 ± 0.602	126.1600 ± 3.819	24.2100 ± 0.224[a]

*Repeated measures of ANOVA followed by Bonferroni's test post hoc test *p<0.05 vs day 0; #p<0.05 vs day 15*

Oneway ANOVA followed by post hoc Tukey's post hoc test on day 30 [a] p<0.05 vs control, [b] p<0.05 vs carboplatin

Table III: AST and ALT values of different treatment groups on days 0, 15 and 30

	Day 0		Day 15		Day 30	
Parameter	**AST (IU L^{-1}) Mean ± SEM**	**ALT (IU L^{-1}) Mean ± SEM**	**AST (IU L^{-1}) Mean ± SEM**	**ALT (IU L^{-1}) Mean ± SEM**	**AST (IU L^{-1}) Mean ± SEM**	**ALT (IU L^{-1}) Mean ± SEM**
Control	211.04± 7.26	127.45± 11.88	211.81± 7.91	149.66± 14.28	211.31± 7.71	147.82± 13.19
Carboplatin	185.78± 15.86	130.65± 13.88	263.26± 6.76*	187.22± 13.85*	234.55± 31.90*[a]	196.16± 8.69* [a]
Carboplatin + LDHA	189.49± 14.41	125.61± 15.26	261.61± 10.82	212.28± 16.94	170.81± 9.99[#]	142.33± 15.03[#]
Carboplatin + HDHA	193.87± 20.82	139.24± 19.81	249.20± 13.20	208.99± 25.20	190.59± 20.46[#]	124.26± 17.78[# b]

*Repeated measures of ANOVA followed by Bonferroni's post hoc test *p<0.05 vs day 0; #p<0.05 vs day 15*

Oneway ANOVA followed by post hoc Tukey test on day 30 [a] p<0.05 vs control, [b] p<0.05 vs carboplatin

Table IV: Serum urea and creatinine of different treatment groups on days 0, 15 and 30

	Day 0		Day 15		Day 30	
Parameter	**Urea (mg dl^{-1}) Mean ± SEM**	**Creatinine (mg dl^{-1}) Mean ± SEM**	**Urea (mg dl^{-1}) Mean ± SEM**	**Creatinine (mg dl^{-1}) Mean ± SEM**	**Urea (mg dl^{-1}) Mean ± SEM**	**Creatinine (mg dl^{-1}) Mean ± SEM**
Control	36.76±1.18	0.68±0.04	36.49±1.12	0.64±0.03	36.19±1.15	0.68±0.01
Carboplatin	26.74±2.05	0.58±0.04	38.95±4.21	0.94±0.13	43.03±3.34*	0.90±0.11*
Carboplatin+ LDHA	21.45±1.77	0.67±0.02	41.94±9.07	0.94±0.07	20.02±2.85#b	0.57±0.06#b
Carboplatin+ HDHA	18.25±1.02	0.71±0.06	27.04±4.09	1.19±0.10*	26.40±2.91 b	0.54±0.03#b

*Repeated measures of ANOVA followed by Bonferroni's post hoc test *p<0.05 vs day 0; #p<0.05 vs day 15.*

Oneway ANOVA followed by Tukey's post hoc test on Day 30 [a] p<0.05 vs control, [b] p<0.05 vs carboplatin.

Table V: The main active constituents of *H. auriculata*

Active compound	Pubchem CID	Molecular formula	Molecular weight
3beta-Hydroxy-20(29)-lupene	CID:16219576	$C_{30}H_{50}O$	426.7g mol^{-1}
Isoflavone glycoside	CID:121596018	$C_{21}H_{20}O_{10}$	432.4g mol^{-1}
Phytosterols	CID:12303662	$C_{29}H_{50}O$	414.7g mol^{-1}
Xylan, Birch	CID:50909243	$C_5H_{10}O_6$	166.13g mol^{-1}
3-Methylnonacosane	CID:85691	$C_{30}H_{62}$	422.8g mol^{-1}
Apigenin 7-glucuronide	CID: 5319484	$C_{21}H_{18}O_{11}$	446.4g mol^{-1}
Beta-D-Xylopyranose	CID:125409	$C_5H_{10}O_5$	150.13g mol^{-1}
Betulin	CID:72326	$C_{30}H_{50}O_2$	442.7g mol^{-1}
Ethanol	CID:702	C_2H_6O	46.07g mol^{-1}
L-ascorbic acid	CID:54670067	$C_6H_8O_6$	176.12g mol^{-1}
L-histidine	CID:6274	$C_6H_9N_3O_2$	155.15g mol^{-1}
Lipase	CID:1369	$C_{16}H_{13}NO_3S$	299.3g mol^{-1}
Nicotinate	CID:937	$C_6H_4NO_2$	122.1g mol^{-1}
Octadeca-9,12-dienoic acid	CID:3931	$C_{18}H_32O_2$	280.4g mol^{-1}
Octadecanoate	CID:3033836	$C_{18}H_{35}O_2$	283.5g mol^{-1}
Oleic acid	CID:445639	$C_{18}H_{34}O_2$	282.5g mol^{-1}
Palmitic acid	CID:985	$C_{16}H_{32}O_2$	256.42g mol^{-1}
Phenylalanine	CID:5460957	$C_9H_{11}NO_2$	165.19g mol^{-1}
Potassium	CID:5462222	K	39.098g mol^{-1}
S-2,6-diaminohexanoic acid	CID:5460926	$C_6H_{15}N_2O_2$	147.2g mol^{-1}
Sterol	CID:1107	$C_{17}H_{28}O$	248.4g mol^{-1}
Triacontane	CID:12535	$C_{30}H_{62}$	422.8g mol^{-1}

Table VI: Potential targets and UniProt information (Chemical and drug induced liver injury)

Sl. No.	Gene name	Protein name	UniProt ID
1	TNF	Tumor necrosis factor	P01375
2	CYP2E1	Cytochrome P450 family 2 subfamily E member 1	PO5181
3	GPT	Glutamic-pyruvic transaminase	P24298
4	IL1-B	Interleukin 1 beta	P01584
5	GSR	Glutathione-disulfide reductase	P00390
6	CAT	Catalase	P04040
7	NR1H4	Nuclear receptor subfamily 1 group H member 4	Q96RI1
8	GSTM1	Glutathione S-transferase mu 1	P09488
9	POLG	DNA polymerase gamma, catalytic subunit	P54098
10	HMOX1	Heme oxygenase 1	P09601
11	ABCB1	ATP binding cassette subfamily B member 1	P08183
12	GCLC	Glutamate-cysteine ligase catalytic subunit	P48506
13	ACTB	Actin beta	P60709
14	CA3	Carbonic anhydrase 3	P07451
15	VWF	von Willebrand factor	P04275
16	APOH	Apolipoprotein H	P02749

Table VII: Potential targets and UniProt information (Acute kidney injury)

Sl. No.	Gene name	Protein name	UniProt ID
1	HAVCR1	Hepatitis A virus cellular receptor 1	Q96D42
2	CLU	Clusterin	P10909
3	LCN2	Lipocalin 2	P80188
4	NPPA	Natriuretic peptide A	P01160
5	PPARG	Peroxisome proliferator activated receptor gamma	P37231
6	B2M	Beta-2-microglobulin	P61769
7	EGFR	Epidermal growth factor receptor	P00533
8	NOS3	Nitric oxide synthase 3	P29474
9	MPO	Myeloperoxidase	P05164
10	NFE2L2	Nuclear factor, erythroid 2 like 2	Q16236
11	BAX	BCL2 associated X, apoptosis regulator	Q07812
12	HMOX1	Heme oxygenase 1	P09601
13	NQO1	NAD(P)H quinone dehydrogenase 1	P15559
14	HBEGF	Heparin binding EGF like growth factor	Q99075
15	HP	Haptoglobin	P00738
16	TLR4	Toll like receptor 4	O00206
17	GFER	Growth factor, augmenter of liver regeneration	P55789
18	ATP5F1B	ATP synthase F1 subunit beta	P06576
19	SIRT1	Sirtuin 1	Q96EB6

compound (excluding those less than five) was obtained from the Pubchem database[18]. The targets for each active compound were predicted by the Swiss Target Prediction database[19]. Using "anemia", "chemical and drug induced liver injury", and "acute kidney injury" as the keywords respectively, anemia, hepatotoxicity and nephrotoxicity related disease targets were collected from the DisGeNET database[20]. Data thus obtained from these databases were merged to obtain a common target (drug and disease), and gene list, and entered in the Microsoft Excel sheet. The potential target and gene list thus obtained were imported into the Cytoscape[21] to construct an active compound and target network. Hepatotoxicity and nephrotoxicity related targets were entered into String database[22] to get "PPI (Protein-Protein Interaction)" network. Universal Protein Resource (UniProt) ID was entered into the Database for Annotation, Visualization and Integrated Discovery (David) database[23] to run the functional annotation clustering and obtain the "KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway". Finally, active compounds of *H. auriculata*, the common targets, and the corresponding KEGG pathways were used to design the complete network encompassing drug compound, target and pathway, and disease through Cytoscape.

Statistical analysis

In order to make statistical comparisons, the Graph Pad Prism 5.03 Demo Version was used. For intergroup

comparison, one-way analysis of variance (ANOVA) and Tukey's post hoc test were applied. Data comparison within each group at different time periods was done employing repeated measures ANOVA followed by Bonferroni's post hoc test; $p \leq 0.05$ was considered significant.

RESULTS

The data from the Tables I, II, III, and IV convey that 60 mg kg^{-1} of carboplatin caused anaemia, nephrotoxicity, and hepatotoxicity.

Effect on RBC and Hb

Carboplatin significantly decreased the RBC count and Hb on day 30, when compared with day 0 ($p<0.05$). Carboplatin + LDHA (low dose *H. auriculata*) increased the RBC count and Hb on day 30 significantly as compared to day 15 ($p<0.05$) and was comparable to the mean value on day 0. Carboplatin +HDHA (high dose *H. auriculata*) increased the RBC count and Hb on day 30 ($p<0.05$) and was comparable to day 0. Comparison between the groups on day 30 showed that carboplatin reduced the RBC count and Hb significantly ($p<0.05$) compared to control animals. Treatment with LDHA and HDHA increased the mean values of RBC count and Hb in comparison to carboplatin-treated group, but was significant only with HDHA (Table I).

Effect on PCV

Carboplatin significantly decreased the PCV ($p<0.05$) on day 15 and 30 (40.17±0.74), when compared with day 0 (60.67±0.95). Carboplatin + LDHA and carboplatin + HDHA increased the PCV significantly ($p<0.05$) on day 30 (56.67±2.10, 58.67±1.3) when compared to day 15 (43.67±2.37, 50.83±0.70) and was comparable to day 0 (56.5±1.89). Between the groups comparison on day 30 also showed that carboplatin significantly ($p<0.05$) decreased the PCV (40.17±0.74) when compared to control animals. Treatment with LDHA and HDHA increased the mean values of PCV (56.67±2.10, 58.67±1.3) which was significant ($p<0.05$), when compared to carboplatin (40.17±0.74) treated group as shown in Fig. 2.

Effect on MCV and MCHC

Carboplatin significantly increased the MCV on day 30 when compared with day 0 and day 15 ($p<0.05$) and decreased the MCHC on day 30, when compared with day 0 and day 15 ($p<0.05$).

Between the groups,comparison on day 30: carboplatin increased the MCV significantly when compared to the control group ($p<0.05$). Treatment with

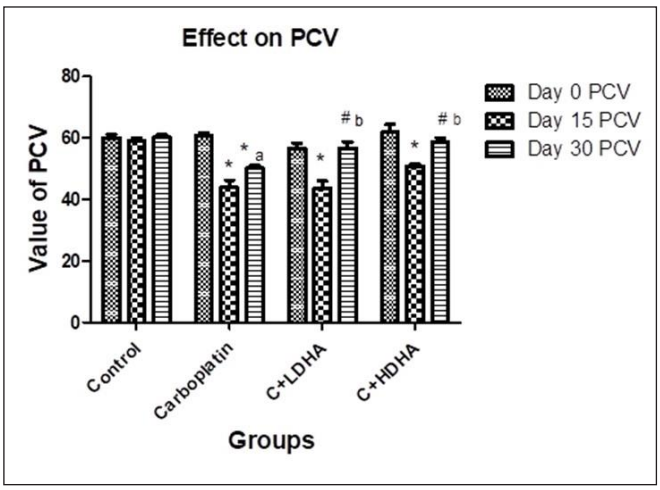

Fig. 2: Effect on packed cell volume

LDHA and HDHA decreased the mean values of MCV when compared to carboplatin-treated group, though not statistically significant. Carboplatin significantly ($p<0.05$) decreased the MCHC as compared to control group. Treatment with LDHA and HDHA significantly increased the mean values of MCHC when compared to carboplatin-treated group ($p<0.05$), as shown in Table II.

Effect on AST and ALT

Carboplatin significantly increased AST and ALT levels ($p<0.05$) on day 15 and day 30, when compared with day 0. Carboplatin + LDHA significantly reduced AST and ALT levels ($p<0.05$) on day 30 when compared to day 15 and was comparable to the mean value on day 0. Also, carboplatin + HDHA significantly reduced AST and ALT levels ($p<0.05$) on day 30 when compared to day 15 and was comparable to day 0. Between the groups comparison on day 30: carboplatin significantly ($p<0.05$) elevated the AST and ALT levels when compared to control animals. Treatment with LDHA and HDHA reduced the mean values of AST and ALT when compared to carboplatin treated group with significance associated with HDHA only (Table III).

Effect on renal function tests

Carboplatin significantly increased the urea and creatinine levels on day 30 when compared with day 0 ($p<0.05$).

Carboplatin + LDHA reduced the urea and creatinine levels on day 30 significantly, when compared to day 15 and was comparable to the mean value on day 0 ($p<0.05$).

Carboplatin +HDHA reduced the urea levels on day 30 and was comparable to day 0. Carboplatin + HDHA

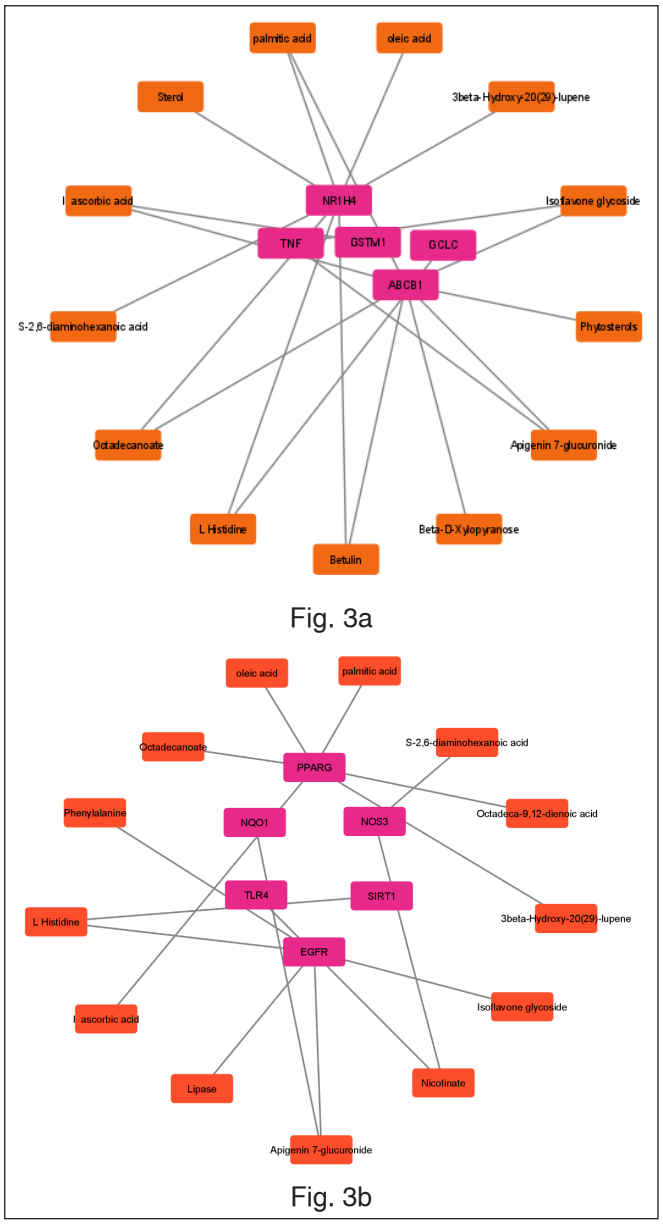

Fig. 3: The active component target network

reduced the creatinine levels significantly on day 30 when compared to day 15 and was comparable to day 0 ($p<0.05$).

On day 30, carboplatin elevated the urea and creatinine values when compared to control group. Treatment with LDHA and HDHA significantly reduced the mean values of urea and creatinine when compared to carboplatin-treated group (Table IV).

Network pharmacology

IMPPAT database was used to obtain 22 active compounds (Table V). As two compounds had canonical SMILES of less than 5, only 20 active compounds were run through Swiss target prediction database, and around 100 targets were obtained for each active compound.

The keywords "anaemia", "chemical and drug induced liver injury" and "acute kidney injury" were searched in DisGeNET which generated a list of 461 and 185 potential targets respectively for "chemical and drug induced liver injury" and "acute kidney injury". Out of these, 16 and 19 potential targets, respectively, were selected based on human gene-disease association score ($Score_{gda}$) of above 0.4 (Table VI, VII). Only 5 potential targets were obtained for anaemia and these were not common with the Swiss target prediction database and hence were not run through network pharmacology analysis.

With the help of Cytoscape, these potential targets (16 for chemical and drug induced liver injury and 19 for acute kidney injury) were analysed to construct the network showing interaction between the active components and targets. There were 18 nodes and 20 edges for liver injury and 19 nodes and 16 edges for kidney injury (Fig. 3a, 3b). PPI network analysis was conducted using string database (Fig. 4a, 4b).

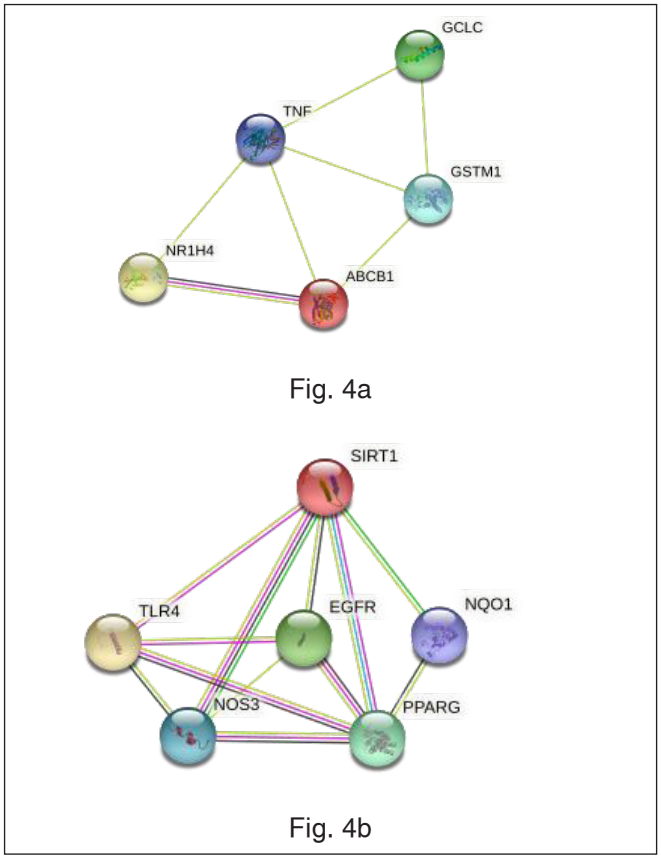

Fig. 4: Protein-protein interaction network analysis

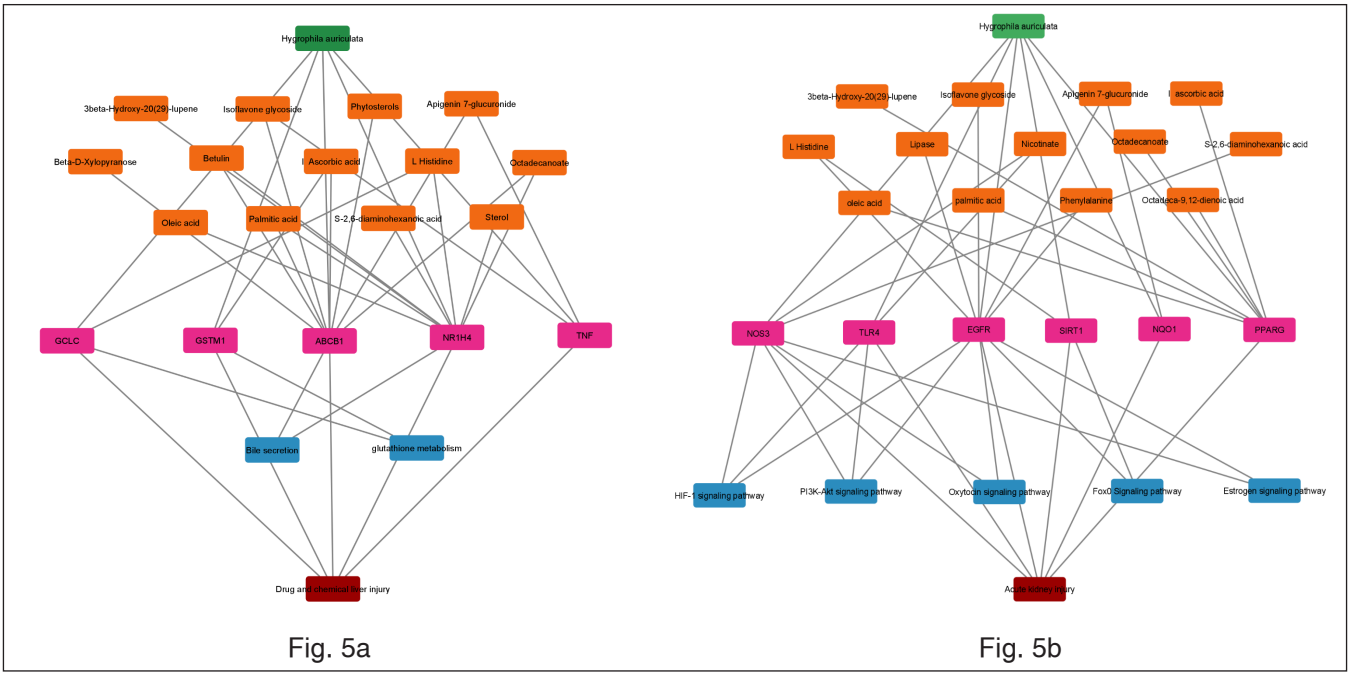

Fig. 5a

Fig. 5b

Fg. 5: Overall network of significant KEGG signalling pathways along with corresponding targets

Green represents H. auriculata, orange denotes active compounds, purple denotes core targets, blue denotes signalling pathways, and dark red denotes drug and chemical liver injury (5a) and acute kidney injury (5b).

The network formed with 18 nodes and 20 edges for liver injury (3a) and 19 nodes and 16 edges for kidney injury (3b). Purple blocks represent the active compounds, orange blocks represent intersecting targets and the edges are denoted as the connection between active component and targets.

Nodes represent genes and edges are interaction between different genes for liver injury (Fig. 4a) and kidney injury (Fig. 4b).

16 and 19 potential targets for liver injury and kidney injury, respectively, were analysed using David (2021 update) database and KEGG signalling pathways were selected. To further understand the molecular mechanism of *H. auriculata* against hepatotoxicity and nephrotoxicity, a network was created based on the important KEGG signalling pathways and their equivalent targets (Fig. 5a, 5b). 22 nodes (1 plant product, 13 compounds, 5 targets, 2 pathways and 1 disease) in the case of liver injury and 26 nodes (1 plant product, 13 compounds, 6 targets, 5 pathways and 1 disease) for kidney injury. Our network analysis suggests that the mechanism of action of *H. auriculata* to treat carboplatin induced hepatotoxicity might be related to nuclear receptor, ATP binding cassette transporter, bile secretion and glutathione metabolism; PPARG receptor, EF receptor, HIF-1 signalling and PI3K-Akt signalling pathway.

DISCUSSION

In the present study, carboplatin induced adverse effects including anaemia, nephrotoxicity and hepatotoxicity and a reversal of parameters has been demonstrated with low and high doses of *H. auriculata.* Carboplatin has reduced RBC count, haemoglobin concentration and MCHC and has increased the MCV, which is suggestive of toxicity due to bone marrow suppression. The reports of this study remain comparable with the previous studies[13].

H. auriculata has been utilized in traditional medicine to treat anaemia, though the exact mechanism is not known. In present study, *H. auriculata,* in both high and low doses, has reversed the anaemia induced by carboplatin by improving RBC count, haemoglobin concentration, MCV and MCHC. One of the previous studies has pointed out that growth factors like cytokines participate in hematopoietic stimulation and may possibly be involved in *H. auriculata* induced hematopoietic stimulation[5].

Carboplatin causes hepatotoxicity by increasing AST and ALT. *H. auriculata* has reduced the elevated AST

and ALT levels back to baseline values in both low and high doses. In one of the previous studies, *H. auriculata* root aqueous extract exhibited hepatoprotective activity in rats with CCl_4-induced liver toxicity and also a significant antioxidant activity *in vitro*. The reversal of drug-induced liver injury was attributed to its free radical scavenging and anti-lipid peroxidative properties[24]. Another study used freshly isolated rat hepatocytes to test the alkaloid fraction of *H. auriculata* leaves (methanol extract) for hepatoprotective efficacy against carbon tetrachloride induced damage. It was observed that low concentrations of 80 to 40 µg mL^{-1} could normalize the altered biochemical parameters, thus exhibiting an antihepatotoxic effect[25].

More commonly, patients taking carboplatin have mild and transient elevations in serum aminotransferase levels and very rarely this induces liver failure. But pre-existing liver disease in patients can alter the severity of carboplatin induced hepatotoxicity. Our study has demonstrated the hepatoprotective effect of glycerol extract of *H. auriculata*. To discover the probable hepatoprotective mechanism of *H. auriculata,* thereby generating more evidence for treatment, network pharmacology helped to predict the key active components and targets, with probable signalling pathways of *H. auriculata* to treat carboplatin induced hepatotoxicity.

Based on the network pharmacology analysis, sterol, palmitic acid, oleic acid, octadecanoate *S*-2,6-diaminohexanoic acid, L-histidine, betulin and 3 beta-hydroxy-20(29)-lupene act on NR1H4, which is a bile acid nuclear receptor. NR1H4 exerts hepatoprotective effect by promoting liver regeneration, thereby promoting hepatocyte survival and proliferation. Additionally, vitamin K-independent coagulopathy and neonatal cholestasis with quick progression to end-stage liver disease are both associated with mutations in the NR1H4 gene in humans[26]. ABCB1 is a transporter protein whose overexpression is a risk factor for liver injury. The components of *H. auriculata* such as L-histidine, betulin, octadecanoate, beta-d-xylopyranose and apigenin 7-glucuronide, reduce the expression of ABCB1 and reduce the risk of hepatic injury[27]. In KEGG and complete network analysis, the key targets chiefly belonged to bile secretion and glutathione metabolism pathway, which are highly associated with hepatotoxicity[28], signifying that the mechanism of *H. auriculata* to treat carboplatin induced hepatotoxicity may correlate to bile secretion and glutathione metabolism pathway.

Stearic acid supplementation has been proven to improve liver health after bile duct ligation-induced liver damage in rats. Stearic acid, a component of *H. auriculata,* may reduce liver inflammation by inhibiting inflammatory cell recruitment and/or nuclear factor κB (NF-κB) activity[29,30]. Also, the elevated level of NF-κB after liver damage increases the proinflammatory cytokines, cyclooxygenase-2 and tumor necrosis factor-α [30].

Carboplatin - induced nephrotoxicity has been indicated by the elevated values of blood urea and serum creatinine. Platinum compounds cause nephrotoxicity by causing acute tubular necrosis[31]. The most prominent injury affects the proximal tubules of the outer medulla, especially the straight portion. The affected proximal tubular cells have apical bleeding, brush border loss and disappearance of basal infolding. Though other research papers have shown lesser nephrotoxic effect of carboplatin than cisplatin, however, low plasma protein binding and extensive renal elimination suggest that elevated carboplatin doses have produced undue renal toxicity[32]. Also, there are studies which suggest that reactive oxygen species are involved in the aetiology of nephrotoxicity caused by platinum compounds[33]. *H. auriculata* has reduced blood urea and serum creatinine in both high and low doses. In a previous study, methanol extract of *H. articulata* has prevented the nephrotoxicity produced by cisplatin. It was shown to inhibit the increase in lipid peroxidase (LPO) and superoxide dismutase (SOD) and thus was known to have antioxidant properties[33]. The components of *H. auriculata,* namely oleic acid, palmitic acid, octadecanoate, 3 beta-Hydroxy-20(29)-lupene, octadeca-9,12-dienoic acid and ascorbic acid, target peroxisome proliferator activated receptor-γ (PPARγ**)** according to network pharmacology analysis. The medullary collecting ducts, glomeruli and tubular cells of the kidney tissue have been observed to express PPAR-γ. PPAR-γ inhibits cell proliferation and apoptosis of podocytes[34].

NAD(+)-dependent enzymes with deacetylase/mono-ADP-ribosyl transferase includes sirtuins which are silent information regulator 2 (Sir 2) proteins. These enzymes are in charge of gene transcription, chromosomal stability, and deoxyribose nucleic acid (DNA) repair or recombination. Sirtuins also promote the cell survival in an affected kidney by various mechanisms like response modulation to various stress stimuli, arterial blood pressure regulation, catalase induction leading to protection against renal tubular cellular apoptosis and activates autophagy linked to cellular metabolism and 'red-ox' state. The components of *H. auriculata*, histidine and nicotinate, target Sirtuins for their nephroprotective activity[35]. Nitric oxide (NO) is endogenously synthesized by three different nitric oxide synthase (NOS) isoforms. The activity of these enzymes is

dependent on oxygen and requires L-arginine and several co-factors. The endothelial NOS (eNOS) is expressed in different sites of the renal tissue like the renal vascular endothelium, proximal tubule, ascending loop of Henle, and the collecting tubule. Nicotinate, octadecanoate, 3beta-hydroxy-20(29)-lupene, and octadeca-9,12-dienoic acid target the eNOS or NOS 3 for their nephroprotective action[36]. In KEGG and overall network analysis, the key targets were chiefly involved in HIF 1, PI3K-Akt, estrogen, FoxO and oxytocin signalling pathways, which are highly associated with nephrotoxicity, signifying that the mechanism of action of *H. auriculata* to treat carboplatin induced nephrotoxicity might be related to HIF 1, PI3K-Akt, estrogen, FoxO and oxytocin signalling pathways[37].

Hence, in the present study, *H. auriculata* in both the doses (250 mg kg^{-1} and 500 mg kg^{-1}) has reversed the carboplatin induced anaemia, nephrotoxicity and hepatotoxicity. Though it has been suggested to have antioxidant properties, extensive studies are essential to elucidate its mechanism of action.

CONCLUSION

It is evident from this study that aerial parts of *H. auriculata* produce hematinic, nephroprotective and hepatoprotective effects. The network pharmacology results obtained for therapeutic effect of *H. auriculata* seem to be related to NR1H4, ABCB1 genes in hepatotoxicity and PPARG, SIRT1, NOS3 genes in nephrotoxicity. Molecular docking studies could be conducted to find the binding affinity with the active components of *H. auriculata.* Therefore, it can be concluded that glycerol extract of *H. auriculata* has therapeutic effect against nephrotoxicity and hepatotoxicity caused by carboplatin.

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