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Role of *Chitraka-Haritaki Rasayana* treatment in reducing the systemic inflammation in individuals exposed to Diesel/Petroleum fumes – A Pilot study

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Abstract:

Objective: To investigate whether the *Chitraka-Haritaki rasayana* treatment is effective in reducing the systemic inflammation in healthy individuals exposed to petroleum fumes in petrol bunks.

Design: Study Type - Interventional, Pilot study; Allocation - Before and after; Endpoint Classification - Efficacy Study; Masking - Open label; Primary Purpose - Preventive treatment.

Setting: Ten healthy volunteers working in petroleum bunk of Udupi municipal area from minimum of six months and having duty of six hours and more per day during the period April 2018 to September 2019.

Participants: 10 healthy volunteers were treated with oral administration of *Chitraka Haritaki Rasayana* (n=10).

Main outcome measures: TNF-α, SGOT, SGPT, ALP, Total protein, Albumin, Urea and Creatinine.

Results: The initial value of TNF-α in exposed group at base line was 13.47 (±SE 1.051) and which reduced to 11.96 (±SE 0.769) after the 48 days of *chitraka haritaki rasayana* treatment. This reduction was statistically significant with p=0.0169.

Conclusion: *Chitraka Haritaki rasayana* treatment is effective in reducing the systemic inflammation there by helpful in preventing the development of chronic health complications.

Key words: *Chitraka Haritaki Rasayana*, Systemic inflammation, Petroleum fumes, DNA damage

Introduction:

Rasayana chikitsa is one among the *Astanga Ayurveda* which deals with preventive, curative and promotes health in all age groups. It is one of the remedy to achieve longevity, immunity, rejuvenation of health and is known to be affective for preventing the effects of ageing, improving the quality of life of healthy as well as diseased individuals. *Rasayana* is a treatment which enhances the *vyadhi kshamatva* of an individual.[1] Aim of the *rasayana* is to reduce the occurrence of any disease, maintenance and improvement of functioning capacity of *dosha* and *dhatu.* As per the disease in literature particular *rasayana* are elaborated termed as *vyadhihara rasayana.*[2] These vyadhihara rasayanas are important for the reconditioning the body against different variety of diseases.[3] These *rasayana* will work on the initial pathology of a disease ie *Khavaigunya* and *srotodusti*. *Rasayana* has its maximum effect if administered after the *kosta shuddi*.[4]

Chitraka-haritaki Rasayana is described in the *Chakradatta*, and can be used as *vata aatapika* and *naimittika*. *Kosta shuddhi* procedure has to be followed before *rasayana* administration (Sush. Chi. 27/3). *Koshta shuddhi* involves cleansing the gastrointestinal tract of the subject by inducing *vamana* (emesis) or *virechana* (purgation), after subject has undergone satisfactory *snehana* (internal and external oleation) and *swedana* (sudation). *Snehana-swedana-shuddhi* is a mandatory procedure before administering any *rasayana* to get the maximum benefits.

Petrol pump workers are constantly exposed to petrol and diesel fumes in addition to hazardous vehicle exhausts and other environmental pollutants at their working places. Petrol is a mixture of volatile hydrocarbons; while diesel is distillate of petroleum which contains paraffin, alkenes and aromatics.[5] Petrol and diesel undergo combustion derived nano particles (CDNPs). These particles are highly respirable and can be remain airborne for longer time periods and can be deposited in greater numbers and deeper into the lungs than the large sized particles.[6] Benzene is the major component of petroleum and activation of benzene leads to continuous production of reactive

oxygen species (ROS) which results into lipid peroxidation, causing damages to DNA, RNA and changes the functions of important enzymes [7]. TNF-α is a cell signalling protein (cytokine) involved in systemic inflammation and is critically involved in the induction of systemic DNA damage.[8] TNF-α induces the enhancement of reactive oxygen intermediates (ROI) leakage from the mitochondrial respiratory chain and that this directly or indirectly leads to DNA damage and various events subsequent to DNA damage, such as impairment of DNA repair, may also be involved in the development of TNF-α cytotoxicity.[9] Many intracellular processes, such as DNA fragmentation, adenosine diphosphate (ADP) ribosylation, phospholipase activation, oxidative stress, an increase in cytosolic Ca^{2+}, and induction of endogenous nucleases are possibly involved in TNF-α induced cell lysis.[10] The literature surveys clearly suggest that elevated level TNF-α, in plasma has role in the systemic inflammation and in the DNA damage.

Materials & Methods:

Open labelled pilot study with the approval of Institutional Ethical Committee of Sri Dharmasthala Manjunatheshwara College of Ayurveda Udupi. All volunteers are recruited to the study after taking written consent between April 2018 and September 2019. Healthy volunteers of both sexes between the age group of 20 – 50 years, working in petroleum bunk of Udupi municipal area from minimum of six months and having duty of six hours and more per day were eligible for the study. Volunteers suffering from chronic diseases like diabetes, kidney diseases, cancer and any other chronic illness and on medications were excluded from the study. Volunteers having the habit of cigarette smoking, tobacco chewing, and alcohol consumption of every day were also excluded from the study. Eligible volunteers recruited for the study and treated with *Chitraka Haritaki Rasayana* after kosta shudhi procedure. The 12 gm of *Chitraka Haritaki rasayana* in *leha* form sealed in a packet, and 48 packets were given to each of the volunteers.

Before starting of the rasayana treatment, kosta shudhi procedures were performed for 6 days i.e. on 1st and 2nd day *Chitrakadi vati* was given based on *ama* for *deepana* & *pachana,* on 3rd day *sadhyo snehana* with *gogritha* (100 ml), on 4th day *abhyanga*

& *swedana* followed by *mradu virechana* with *haritaki churna*, and on 5th & 6th day *samsarjana karma*. Volunteers were advised to restrict themselves for bland diet for six days of *kosta shudhi* procedure.

On 7th day or 1st day of *rasayana* treatment participants were asked to consume 12 grams of *Chithraka Haritakai rasayana*, early morning on empty stomach, with warm milk and advised to take breakfast two hours after the consumption of *rasayana*, same is continued for 48 days. Blood samples were collected and analysed for two times, first samples before the *kosta shudhi* procedure and second time after the completion the *rasayana* treatment.

At the same period of time ten apparently healthy volunteers, those are not frequently exposed to petroleum fumes were selected as control group. Volunteers suffering from chronic diseases like diabetes, kidney diseases, cancer and any other chronic illness, on medications, having the habit of cigarette smoking, tobacco chewing, and alcohol consumption of every day were also excluded from the study. Blood samples were collected from control group for one time and no *rasayana* treatment was given.

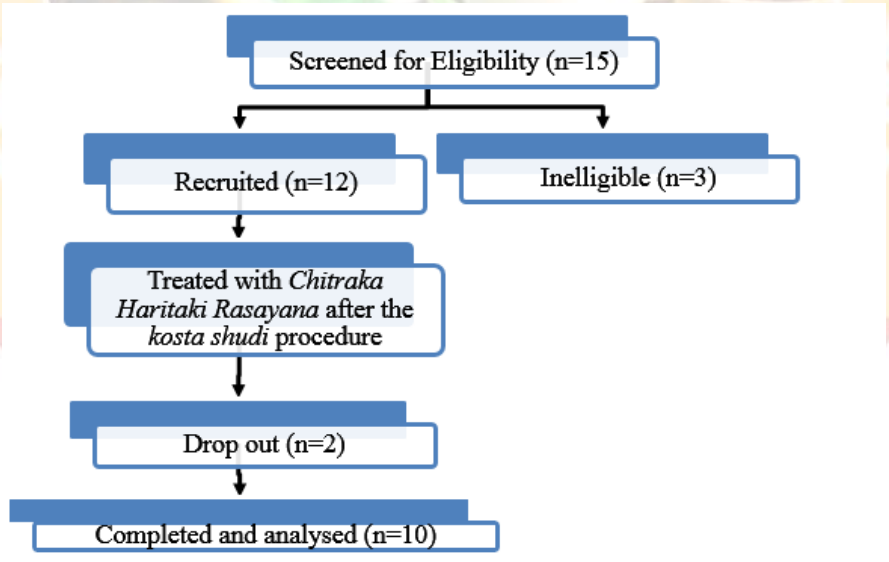

Fig 1: Volunteers flow diagram of treatment group

Blood samples were collected in EDTA and Plain tubes from median cubital vein and analysed for glucose, urea, creatinine, liver function tests, haemogram and TNF α. The data

obtained were analyzed using Graph pad InStat (version 3.05).

Preparation of *Rasayana*:

Chitraka Haritaki rasayana: Based on Ayurvedic School of Pharmaceutical practices *Chitraka Haritaki rasayana* is prepared follows.

Chitraka, *Guduchi*, *Dashamula* and *Amalaki* are taken in equal quantity and are coarsely powdered and *Chaturthavashesha Kashaya* is prepared separately. *Avalehya Paka* is prepared by mixing all the four *Kashayas* together along with *Guda*. When *Paka Lakshana* are obtained fine powdered *Haritaki Churna* added and mixed well. *Prakshepaka dravya* are also added, stirred thoroughly and cooled. *Madhu* is added to the *avaleha* when the mixture has sufficiently cooled down.

Results:

Ten volunteers exposed to petroleum fumes were completed the *rasayana* treatment during the period from April 2018 to September 2019 from the petrol bunks in the Udupi municipal area having duty hours of six and more per day. These volunteers received the medication as per the protocol.

Mean age of the volunteers in the exposed group was 27.1 years (SD±4.433). Among the 10 volunteers 40% were women. Mean age of the volunteers in the control group was 26.8 years and 50% were women.

Table1. Show comparison of biochemical & haematological parameters in blood in exposed group (base line value) and in control group.

Test	Group (N=10)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
Haemoglobin	Control	12.60	1.160	0.367	14.4	11.0	12.45	2.581	0.0194
	Exposed	13.83	0.963	0.304	15,3	12.6	13.75		
RBC Count	Control	4.62	0.457	0.145	5.43	3.96	4.59	2.186	0.0431
	Exposed	5.09	0.501	0.159	5.77	4.41	5.12		
Platelet Count	Control	2.43	0.478	0.151	3.15	1.82	2.33	0.1024	0.9197
	Exposed	2.41	0.392	0.124	3.20	1.84	2.39		
Haematocrit	Control	38.25	3.515	1.111	43.5	34.0	37.60	2.258	0.0374
	Exposed	41.50	2.892	0.915	46.3	37.0	41.50		
MCV	Control	82.94	2.223	0.703	86.4	80.1	83.25	1.486	0.1581
	Exposed	84.85	3.404	1.076	89.1	80.9	84.60		
MCH	Control	27.32	0.561	0.177	27.9	26.4	27.50	2.539	0.0247
	Exposed	28.27	1.041	0.329	29.8	26.7	28.50		

MCHC	Control	32.94	0.427	0.135	33.7	32.2	32.85	1.816	0.0870
	Exposed	33.32	0.505	0.159	34.1	32.5	33.45		
SGOT	Control	24.20	3.878	1.225	30.2	18.9	23.00	0.9415	0.3605
	Exposed	26.08	4.984	1.576	34.5	18.1	24.95		
SGPT	Control	21.82	3.399	1.075	28.3	16.7	20.85	1.435	0.1693
	Exposed	24.04	3.516	1.112	28.0	17.3	24.80		
ALP	Control	157.6	42.31	13.38	211.0	95.0	165.50	0.2503	0.8054
	Exposed	162.9	51.91	16.41	248.0	110	136.50		
Bilirubin Total	Control	0.829	0.130	0.041	1.00	0.62	0.85	0.0795	0.9376
	Exposed	0.833	0.092	0.029	0.98	0.68	0.84		
Bilirubin Direct	Control	0.156	0.061	0.019	0.24	0.05	0.175	0.6253	0.5406
	Exposed	0.176	0.081	0.025	0.33	0.09	0.145		
Total Protein	Control	7.71	0.578	0.183	8.4	6.6	7.85	1.627	0.1222
	Exposed	7.24	0.707	0.224	8.0	6.2	7.55		
Albumin	Control	4.83	0.362	0.115	5.2	4.0	4.65	2.739	0.0146
	Exposed	4.24	0.268	0.085	4.8	3.9	4.15		
Creatinine	Control	1.03	0.172	0.054	1.3	0.78	1.065	1.235	0.2338
	Exposed	1.13	0.190	0.060	1.4	0.85	1.140		
Urea	Control	32.5	6.621	2.094	42.0	24.0	31.5	1.483	0.1574
	Exposed	36.3	4.668	1.476	44.0	29.0	36.5		
Glucose	Control	100.3	10.16	3.211	113.0	85	100.50	0.1345	0.8946
	Exposed	99.7	9.800	3.099	114.0	83	100.50		

Table2. Show comparison TNF alpha (Marker of systemic inflammation & DNA damage) in exposed group (base line value) and in control group.

Test (pg/ml)	Group	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha	Control (10)	9.32	1.360	0.430	11.4	7.3	9.15	3.654	0.0018
	Exposed (10)	13.45	3.306	1.045	18.8	8.5	13.25		

Table 3 & 4 represents the results of the *Chitraka Haritaki rasayana* treatment. The base line mean value of TNF-α was 13.47 (±SE 1.057) and reduced to 11.96 (±SE 0.769) after the intervention. This improvement was statistically significant with p=0.0169 indicating after the treatment systemic inflammation and DNA damage is considerably reduced.

Table 3: Values of haematological, biochemical parameters in exposed group before and after *rasayana* treatment.

Test	*Rasayana* treatment (N=10)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
Haemoglobin	BT	13.83	0.963	0.304	15.3	12.6	13.75	3.609	0.0057
	AT	13.3	0.867	0.274	14.4	12.0	13.30		
RBC Count	BT	5.09	0.501	0.159	5.77	4.41	5.12	3.290	0.0094
	AT	4.74	0.461	0.146	5.55	4.10	4.72		
Platelet Count	BT	2.41	0.392	0.124	3.20	1.84	2.39	0.8718	0.4059
	AT	2.43	0.350	0.111	3.10	1.91	2.41		
Haematocrit	BT	41.50	2.89	0.915	46.3	37.0	41.5	3.080	0.0131
	AT	40.54	2.89	0.915	45.3	37.0	41.2		
MCV	BT	84.85	3.40	1.976	89.1	80.9	84.6	1.282	0.2320
	AT	85.65	2.84	0.897	90.2	81.5	85.9		
MCH	BT	28.27	1.041	0.329	29.8	26.7	28.5	0.729	0.485
	AT	28.40	0.912	0.288	30.0	27.0	28.6		
MCHC	BT	33.32	0.505	0.160	34.1	32.5	33.5	1.308	0.2234
	AT	33.15	0.375	0.119	33.7	32.4	33.2		
SGOT	BT	26.08	4.98	1.576	34.5	18.1	24.9	2.221	0.0534
	AT	25.01	3.69	1.166	30.7	18.4	24.5		
SGPT	BT	24.04	3.52	1.112	28.0	17.3	24.8	2.613	0.0281
	AT	22.55	2.54	0.804	26.0	17.8	22.5		
ALP	BT	162.9	51.91	16.41	248	110	136.50	2.881	0.0182
	AT	152.3	58.27	18.43	252	97	130.50		
Bilirubin Total	BT	0.833	0.092	0.029	0.98	0.68	0.84	1.500	0.1679
	AT	0.825	0.082	0.026	0.96	0.70	0.83		
Bilirubin Direct	BT	0.184	0.076	0.024	0.33	0.09	0.17	1.980	0.0791
	AT	0.165	0.054	0.017	0.25	0.10	0.16		
Total Protein	BT	7.24	0.707	0.224	8.0	6.2	7.55	3.317	0.0090
	AT	7.46	0.670	0.212	8.2	6.4	7.70		
Albumin	BT	4.24	0.268	0.085	4.8	3.9	4.15	2.648	0.0266
	AT	4.42	0.326	0.103	4.9	4.0	4.40		
Creatinine	BT	1.127	0.189	0.059	1.4	0.85	1.14	0.4621	0.6496
	AT	1.089	0.178	0.056	1.38	0.84	1.07		
Urea	BT	36.3	4.668	1.476	44.0	29.0	36.5	3.822	0.0041
	AT	33.8	4.686	1.482	42.0	28.0	33.0		

Glucose	BT	99.7	9.799	3.099	114.0	83.0	100.5	0.1092	0.9155
	AT	100.1	10.29	3.254	113.0	84.0	99.5		

Table 4: Values of TNF- α in exposed group before and after *rasayana* treatment.

Test (pg/ml)	*Rasayana* Treatment (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha	BT (10)	13.47	3.34	1.057	19.0	8.5	13.25	2.924	0.0169
	AT (10)	11.96	2.43	0.769	17.0	8.4	11.95		

Discussion & Conclusion:

This pilot study was conducted to find out the health benefit of *rasayana* treatment in petroleum bunk workers as they were exposed to petroleum fumes which contain pollutants like carbon monoxide and benzene. This study is important for further research to establish the effect of chronic exposure of petroleum fumes on systemic inflammation and genotoxicity, and further to determine the effectiveness of *rasayana* treatment in reducing the systemic inflammation and genotoxicity. Haematological and biochemical parameters and marker for systemic inflammation TNF alpha were measured in the exposed and control groups. In haematological parameters (Table no.1) we observed significant increase in the baseline values of haemoglobin, RBC count and haematocrit in the exposed group compared to control group. Petroleum bunk workers were exposed to air pollutants like Carbon monoxide (CO) which causes hypoxia and stimulates the haemopoises in the bone marrow. CO having very high affinity to haemoglobin than oxygen firmly binds and forms carboxyhemoglobin and interferes with oxygen transport capacity of blood cells, finally resulting hypoxic hypoxia. This is very strong stimulus for the production of red blood cells by stimulating erythropoietin, which finally results in the increased production of Hb and RBC in the blood circulation.[11] Significant decrease in Hb, RBC counts and haematocrit values (Table 3) was observed after the treatment with *Chitraka haritaki rasayana*, indicating that *rasayana* treatment decreases the effect of hypoxia and increases the oxygen carrying capacity of blood cells, which in turn slows down the stimulatory

effect of CO on erythropoises in the bone marrow.

We have also performed Liver function and kidney function tests to evaluate the hepatotoxic and nephrotoxic potential of petroleum fumes. Hydrocarbons present in the petroleum fumes are converted in to free radicals during oxidation process mainly in liver and kidney cells, and these free radicals causes peroxidation with some cellular components like membrane lipids resulting in change in the membrane integrity.[12] Transaminases i.e. SGOT & SGPT are useful biomarkers to assess the integrity of hepatocytes and increase level of these enzymes in plasma linked to hepatocellular damage.[13] It was observed that SGOT, SGPT, ALP, Bilirubin total & direct were within the reference range of normal individuals in both exposed and control group, but higher mean value was found in these parameters in exposed group and difference is statistically insignificant (Table 1). After the *rasayana* treatment significant decreases was observed in SGPT and ALP level (Table 3), showing improvement in integrity of hepatocytes. Mean value of total protein and albumin were low in exposed group indicating that exposure to petroleum fumes interferes with synthetic activity of the liver, and after the treatment significant increase in the total protein and albumin level observed showing the role *rasayana* treatment in improving the synthetic activity of the liver. Exposure to benzene in petroleum fumes associated with kidney injury. [14] We observed higher level of urea, creatinine levels in exposed group compared to control group, but statistically insignificant (Table 3). After the treatment with *rasayana* it was observed that urea and creatinine levels decreased having statistical significance in urea level.

TNF alpha is one important the biomarkers of systemic inflammation and DNA damage was assessed in exposed and control group, and observed significantly higher level of TNF alpha in exposed group (Table 2). After the treatment with *Chitraka haritaki*, significant decrease in the level of TNF alpha was observed in the exposed group (Table 4). Decrease in the level of TNF alpha after the treatment suggests protective action of *rasayana* against systemic inflammation and DNA damage.

This is a preliminary study carried out to find out the effectiveness of *rasayana* treatment to prevent the systemic inflammation and long term health complications in individuals exposed to petroleum fumes. As this study is with very small sample size so it is very early to generalize the finding of this study in people exposed to petroleum fumes. Further research with large sample size is required to establish the effectiveness of *chitraka haritaki* rasayana treatment in reducing the systemic inflammation and DNA damage in healthy population exposed to petroleum fumes.

Ethical approval:

This study was approved by the Institutional research ethics committee.

Conflict of Interest:

There is no conflict of interest.

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