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ORIGINAL RESEARCH ARTICLE

Hypolipidemic and Antioxidant effects of Seabuckthorn leaf based Herbal formulation

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ABSTRACT

Fruit, seed and leaf of seabuckthorn plant (*Hippophae ramnoides* L. Elaeagnaceae) herb naturally grown at altitude deserts has been studied in depth for nutritional and medicinal application. A herbal formulation (HF) developed with seabuckthorn leaf as major ingredient with added herbs and spices was evaluated for its hypolipidemic and antioxidative properties in normal and hypercholestremic rats exposed to Hexachlorocyclohexane (HCH) induced oxidative stress. HF decreased significantly total cholesterol (TC) Triglyceride (TG) low very low density lipoprotein (LDL + VLDL) and thiobarbutyric acid reactive substances (TBARS) and increased High density lipoprotein (HDL). Converse was true in animals fed with HCH decreased the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) and the levels of glutathione (GSH) but increased malonaldehyde (MDA) in liver. These effects were reversed significantly with the incorporation of HF in the diet. Antioxidant vitamin A and E concentration decreased in HCH fed animals where as the levels of vitamin C increased on HCH diet. The overall effect of HF was same in both normal as well as in hypercholestremic animals. The developed HF has shown to have hypolipidemic antioxidative and heptao protective properties.

Key Words: *Hippophae rhamnoids* L; Hepato-protective; Lipids lowering; antioxidant vitamins

INTRODUCTION

Medicinal properties have been attributed to many plants for thousands of years[1-2]. The plant extracts are widely sold as nutritional supplements or tonic and touted as beneficial for health[3].

Medicinal plants are useful against some kinds of cancers, lipid peroxidation, hypercholesterolemia, immune function, etc, as known in different countries [2]. Herbal formulations are gaining importance for their health benefits due to less of no toxic effects and also could be due to synergistic effects of many bioactive compounds present Seabuckthorn a herb and a medicinal plant native to Asia, naturally grown at altitude deserts the fruit, seed and leaf of which have been utilized for developing many health care products , in the treatment of ultra violet radiation burns, inflammation gastric ulcers, arthritis and metal induced toxicity, in food preservation and cyto protective properties[4-9]. It was used as a medicinal plant in Tibetian and Mangolian traditional medicine[10]. In addition seabuckthorn leaf is found to be rich in phytochemicals, polyphenols, flavonoids and minerals [11].

Hyperlepidemia measured as increased levels of cholesterol, low density lipoprotein and triglycerides with a decrease in high density lipoprotein coupled with elevated oxidative stress measured as increased levels of lipid peroxidation may have negative effect on cardiac function[12-14].

On the other hand Hexaclorocycclohexane (HCH) a chlorinated pesticide used in agriculture sector is known to enhance oxidative stress, hormonal and cell mediated immunity and is metobolised in endoplasmic reticulum cytochromo P-450[15-16]. Produces changes in liver redox system and enhances lipid peroxidation [17-18]. This may be more pronounced in person's employed in the Industry who may be exposed to continuously for longer period. The possibility cannot be ruled out that the pesticide may enter the living system through direct contact polluted air, water, soil and food as residues thus enhancing the oxidative stress and toxicity.

In view of this a herbal formulation (HF) was developed with seabuckthorn leaf as major ingredient with added herbs and spices and was evaluated for its hypolipidemic, hepatoprotective

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and antioxidative properties in normal and hypercholestremic rats exposed to oxidative stress by feeding HCH in the diet for a period of 7 weeks.

MATERIALS AND METHODS :

64 male wistar rats of body weight 70-80g were selected from stock colony of this institute. 32 rats were made hypercholestremic with 1% cholesterol with 0.25% bile salts in the diet ® and remained hypercholestremic through out the study.

Remaining 32 rats were fed with normal synthetic diet. (Table 1) The rats were than divided into four groups of eight rats in each hypercholestremic and normal animals and were housed individually in stain less steel cages maintained at $27^{o} \pm 2^{o}C$ with 12 hours dark and light cycle with free asses to drinking water and diet The details of the experimental design was as follows.

Control (n=8)					Hypercholestremic (n=8)			
Control	HF[1] 2%	HCH[2]	HCH+HF		Control	2% HF	HCH	HF+HCH

Preparation and feeding HCH

2g HCH (M/s Merk India was dissolved in 100 ml of hot ($50^{o}C$) hot peanut oil so as the contain 20mg /ml. 1ml was thoroughly mixed in the diet which was made homogenized by mixing with 5ml of hot water.

Feeding HF

Seabuckthorn leaves were dried, powdered and analysed for various parameters **Table 2** Raw materials were mixed and powdered in a motorized mixer and analysed for proximate composition[19] (table 3 & 4) cloraphil, carotenoids ascorbic acid[20] total phenols[21], flavonoids[21] and antioxidant activity [22] were also determined. HF was mixed thoroughly in the diet at 2% level. Total experimental duration was 7 weeks.

Food intake and body weight gain were monitored once in a week. At the end, the animals were sacrificed under mild anesthesia (neubutal 50 mg / kg body weight). Blood was collected directly from heart into heparinised centrifuged tubes and centrifuged at 1000 rpm for 10 mins. to obtain plasma.Organs namely heart, liver and kidney were weighed. Liver was stored in liquid nitrogen until completion of the analysis.

Chemical Assay

Plasma was analysed for TC, TG, High Density Lipoprotein (HDL), Low and Very Low Density Lipoproteins (LDL+VLDL) by enzymatic methods with biochemical kits manufactured by m/s Coral Clinical Systems, Verna, Goa, India and marketed by Crest Company, Bombay, India.

TBARS in blood was analysed by the method Farelough and Waselimger et al [23].(year) Hepatic MDA assay was carried out by homogenizing 0.5g liver and precipitating with 10% TCA and reacted with 0.35% thiobarbutyric acid reaction mixture consisting of sodium dodecyl sulphate, ferric chloride and BHT in 0.1 M glycine buffer. After boiling and cooling OD was taken at λMax 532 and MDA was calculated using a molar extinction coefficient of $1.56x10^{5}/M$[24].

Liver catalase activity was assayed according to the method of Cohen et al [25]. Glutathione peroxidase (GSHPx) was determined by the method of Weiss et al [26] in the supernatant of liver homogenate prepared in phosphate buffer (0.5 M pH 7.0) using H_2O_2 and NADPH as substrate. Superoxide dismutase (SOD) was determined by inhibition of cytochrome C reduction mediated via superoxide anions generated by xanthine-xanthine oxidase and monitored at 550 nm. One unit of SOD is defined as the amount required for inhibiting the reduction of cytochrome C by 50% [27]. Liver glutathione was determined the method of Ellman [28].

Table 1 Composition of control diet) Hypercholestremic diet ((g/ 100g)

Casein	20	Casein	20
Methionine	0.2	Methionine	0.2
Vitamin mix*	2	Vitamin mix	2
Mineral mix**	4	Mineral mix	4
Peanut oil	19	Peanut oil	19
Cod liver oil	1	Cord liver oil	1
Sucrose	10	Sucrose	10
Corn starch	43.8	Corn starch	42.55
		Cholesterol	1
		Bile salts	0.25

*Vitamin mix was prepared as per Indian standard IS 7481 1975

** Mineral mix was purchased from SISCO Research Lab, Bombay, India

Cod liver oil provides 1500 IU vitamin A and 100 IU vitamin D

0.01g of □ tocopherol was added into coconut oil and then mixed with diet

Table 2 : Composition of Seabucthorn leaf

Sl. No.	Parameters	g/100g (dry)
	Protein	2.78[19]
2	Fat	2.67[19]
3	Ash	6.80[19]
4	Reducing sugar	8.53[20]
5	Non reducing sugar	Traces[20]
6	Chlorophyll	0.28[20]
7	Carotenoids	0.15[20]
8	Total phenols	0.04[20]

Table 3 : Composition of SBT based herbal formulation

Raw materials	G/100g
Tulasi dried	2.5
Mint leaves	2.5
Cloves	2.5
Cardamom	5
Cinnamon	5
Coriander seed	10
Jeera	10
Ginger dried	2.5
Seabuckthorn leaves dried	60

Table 4 : Composition of HF(%g)

Moisture	6.8[19]
Protein	2.84[19]
Fat	2.77[19]
Total ash	6.9[19]
Insoluble ash	4.76[19]
fiber	14.7[19]
Total chlorophyll %	0.028[20]
Carotenoids mg/g	102.75[20]
Ascorbic acid %	0.028[20]
Total sugar	8.53[20]
Total phenols mg/g	1.1[21]
Total flavonoids mg/g	1.16[22]
Antioxidant activity %	55.3[22]

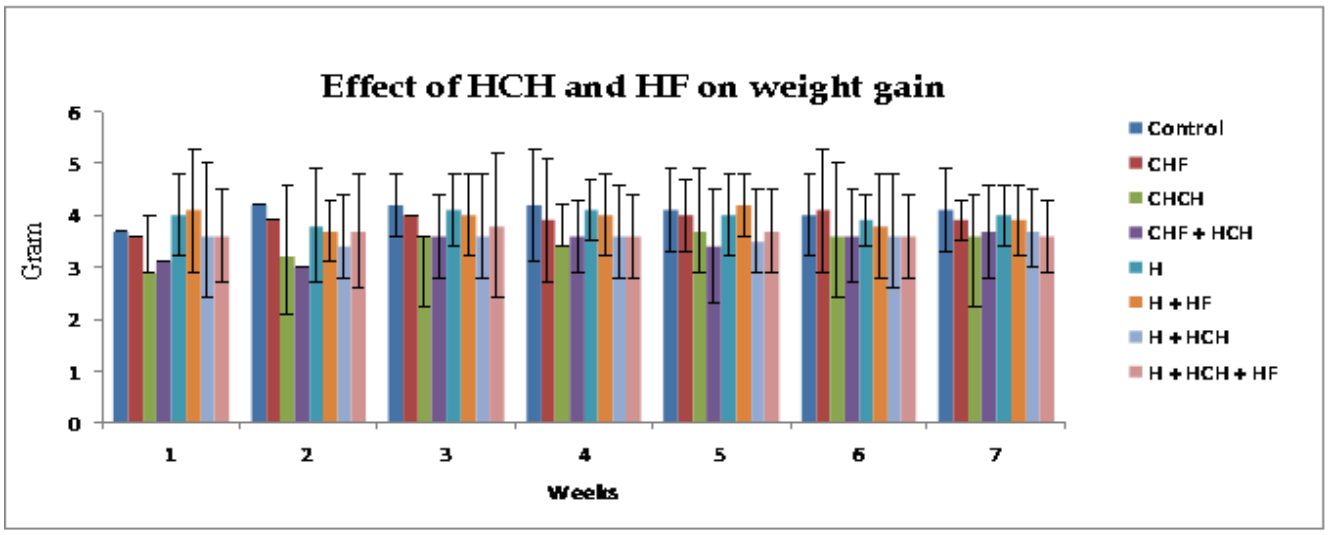
Table 5 : Effect of HF in rats exposed to HCH on organ wt.(g)

Dietary	Kidney		Heart		Liver	
	Normal	**Hyp**	**Normal**	**Hyp**	**Normal**	**Hyp**
Control	1.96 ± 0.23[a]	1.89 ± 0.20[a]	0.74 ± 0.11	0.77 ± 0.09	9.34 ± 0.55[a]	10.42 ± 0.72[a]
HF	1.81 ± 0.18[a]	1.94 ± 0.31[a]	0.69 ± 0.09	0.76 ± 0.07	9.82 ± 0.78[a]	9.86 ± 0.89[a]
HcH	3.64 ± 0.26[b]	3.42 ± 0.52[b]	0.71 ± 0.10	0.69 ± 0.08	13.26 ± 0.63[b]	13.48 ± 1.04[b]
HcH + HF	2.73 ± 0.34[c]	2.58 ± 0.46[c]	0.67 ± 0.14	0.75 ± 0.09	11.89 ± 0.88[c]	11.63 ± 1.18[c]

Values are mean ± SD for 8 rats

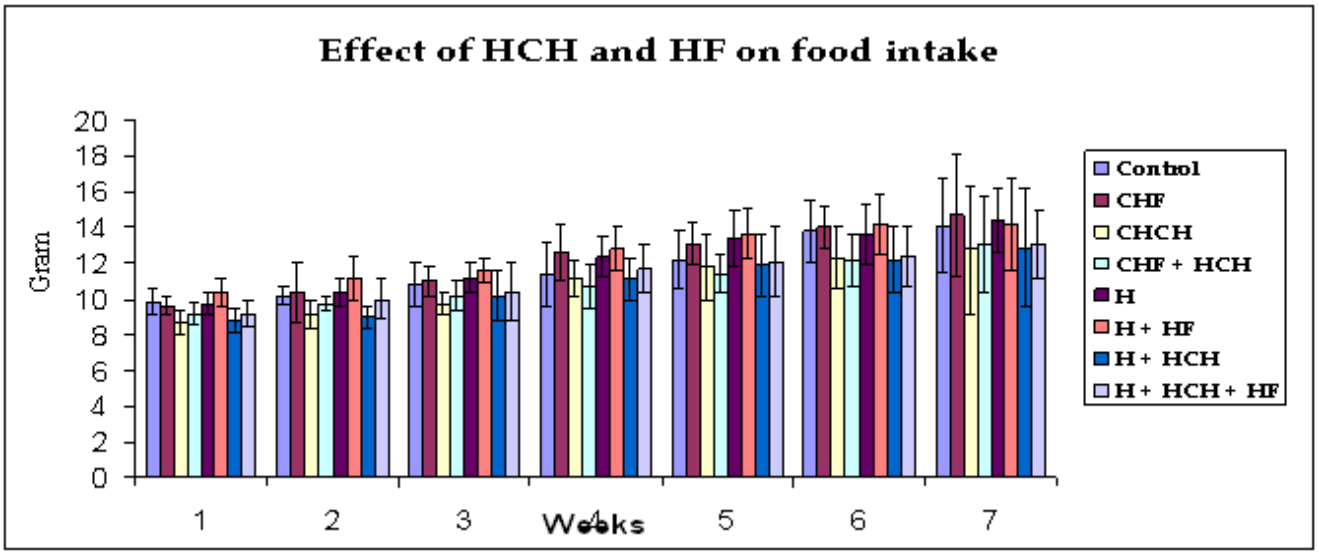
Values bearing different superscript in a column are significantly different at $p<0.005$

Fig 1: Effect of HCH and HF on weight gain (g/wk)



C: Control, H : Hypercholestremic, Values are mean ± SD for 8 rats in each group

Fig 2: Effect of HCH and HF on food intake (g/wk)



C: Control, H : Hypercholestremic, Values are mean ± SD for 8 rats in each group

Table 6 : Effect of HCH & HF on blood lipids and lipid peroxidation in normal & hypercholestremic rats (mg/dl)

Normal	TC	HDL	LDL + VLDL	TG	TBARS M mols/min
C	118.7 ± 8.4[a]	47.6 ± 3.2[a]	73.8 ± 5.5[a]	118.2 ± 10.2[a]	0.11 ± 0.02[a]
CHF	121.3 ± 6.9[a]	46.9 ± 1.7[a]	77.3 ± 6.6[a]	117.9 ± 8.9[a]	0.05 ± 0.02[a]
CHCH	162.4 ± 7.5[b]	33.6 ± 2.9[b]	122.1 ± 8.3[b]	151.3 ± 10.6[b]	0.28 ± 0.03[b]
CHF +HCH	114.3 ± 7.6[a]	40.3 ± 1.31[c]	79.4 ± 5.7[a]	115.3 ± 9.9[a]	0.16 ± 0.03[c]
H	222.8 ± 14.7[c]	46.8 ± 2.1[a]	184.2 ± 10.6[c]	119.3 ± 9.3[a]	0.14 ± 0.03[a]
H + HF	159.3 ± 11.6[b]	46.9 ± 2.5[a]	108.9 ± 8.4[b]	115.7 ± 7.2[a]	0.06 ± 0.00[a]
H+HCH	269.8 ± 13.5[d]	35.2 ± 2.1b[b]	219.4 ± 14.1[d]	158.4 ± 8.7[b]	0.26 ± 0.05[b]
H+HCH+HF	189.2 ± 10.7[e]	41.7 ± 1.9[c]	169.4 ± 13.7[c]	113.7 ± 10.7[a]	0.15 ± 0.03[c]

C = control, H = hypercholestremic, values bearing different superscripts in a column are significantly different at $p<0.005$

Determination of vitamins A, E and C in liver

Total fat in liver was determined by Folsch method. Fat obtained was saponified by alcoholic KOH and extracted with petroleum ether. The extracted fraction was evaporated to dry ness under nitrogen, redasolved in ether chloroform (2:1). Vitamin A was determined by antimony trichloride method [29]. Vitamin E was determined by reacting with α-α bipyridyl and ferric chloride [30]. Vitamin C was determined by colorimetriy [31].

Statistical analysis- Student`s't' test was used to compare the data and all tests were considered statically significant at $p<0.05$.

RESULTS

Effect of HF and HCH on food intake and body weight gain

Fig 1 and 2 shows food intake and body weight gain of various groups of animals. HCH in the diet has not affected neither food intake nor body weight gain significantly. Calculating the food intake for 42 days the average intake of HCH was found to be 14.8 ± 1.2 mg / rat / day.

Effect of HCH and HF on organ weights

Significant increase in kidney and liver was observed with no change in the weight of heart. A 30% increase in weight was observed in kidney weight on HCH and was reduced to 18% increase with HF. A 40% increase in liver weight observed with HCH which was reduced to 20% increase with HF. The percent of increase or decrease was same both in normal and hypercholestremic animals.

Effect of HF & HCH on blood lipid profiles of rats

Effect on cholesterol in hypercholestremic group

Supplementation of cholesterol and bile salts increased plasma cholesterol from 118mg to 222mg. (85%). It increased further with HCH to 252mg and reduced significantly by HF from 220mg to 159mg (29%). In HCH and HF group the reduction was approximately 250mg to 189mg (25%).

Effect in normal animals

HCH elevated cholesterol level by 31% (120mg to 158mg). HF normalized the cholesterol level

Effect on LDL + VLDL in normal animals

LDL + VLDL were calculated by difference (total cholesterol – HDL cholesterol). Significant increase was observed with HCH (60%) which was normalized on HF.

Effect in hypercholestremic animals

LDL + VLDL was elevated by 145% on HCH reduced by 41% with HF. HCH increased further the levels by 165% but the reduction was approximately 34%.

Effect on HDL

HCH reduced the HDL in both normal as well as hypercholestremic animals. The decrease was by approximately 25% and the increase due to HF was between 16 to 18%. Similar observation was made in hypercholestremic animals.

Effect on TG

Elevated TG (27%) in HCH group and was brought back to normal on HF in both control and hypercholestremic group. Supplementation of cholesterol has not affected the TG levels.

Effect on TBARS in normal animals

TBARS values decreased significantly in control groups fed with HF in both dietary treatments**.** Lipid peroxidation increased by 109% with HCH, was inhibited to 80% on HF. Similar observations were made in hypercholestremic animals also.

Effect of HCH and HF on antioxidant vitamins

Significant reduction in vitamin A and E levels were observed with HCH (25% and 49%). Whereas increased vitamin C content was noticed (53%) in normal as well as in hypercholestremic animals. HF inhibited the oxidation of vitamin A & E. Vitamin A oxidation was completely inhibited with HF whereas the oxidation of vitamin E was inhibited to 28%. Vitamin C concentration did not increased in animals fed with HF and HCH.

Effect of HCH and HF on lipid peroxidation antioxidant enzymes and glutathione in liver

Effect on MDA levels in normal animals

MDA levels increased by 121% 138% in normal and hypercholestremic animals with no significant difference among the groups. HF inhibited the MDA formation by 42% and 39% respectively.

Effect on GSH

Glutathione concentration was decreased by 48% with HCH. Animals fed with both HCH and HF decreased the glutathione from 48% to 27%. The effect was same in hypercholestremic animals.

Effect on SOD, Catalase and GSH-Px

Pesticide decreased the activity of SOD, Catalase and GSH-Px (67%, 50% and 64% respectively). The decrease in the activity of these enzymes due to HF with HCH was 35%, 24% and 28% only. Decrease or increase in the activities of enzymes was same in hypercholestremic group.

DISCUSSION

Oxidative stress imbalances the pro and antioxidant system through formation of excess of free radicals affecting the cellular function if not stabilized causes many diseases such as cardio vascular disease, cancer, diabetes, aging, cataract etc. Under these circumstances the supplementation of antioxidant may be essential in maintaining the balance. In the present study the SBT leaves were blended with herbs and spices to enhance the sensory attributes for consumption in humans if found beneficial in animals. The results obtained are discussed here.

HF normalized TC in HCH fed animals however the effect in reducing the same in hypercholestremic animals was limited suggesting HF was effective with moderately elevated cholesterol levels. The percent of decrease in hypercholesterolmic animals with HCH which elevated the cholesterol level further was similar to that of normal animals exposed to HCH (24-29% table 6). TG levels increased with HCH in both dietary treatment agrees with the findings reported [32]. Cholesterol in the diet did not influence the TG levels. HF normalized TG levels suggesting hypolemidemic effect. Significant reduction in TBARS fed with HF suggest reduced oxidative stress could be attributed to antioxidant present in HF. Hypolipidemic effect of antioxidants has been reported by many workers and could be due to reduced activity of hydroxyl methyl glutaryl CoA (HMG CoA) [33-34], The rate limiting enzyme in addition to aceto acetyl CoA. Reports are also there the effect could be due to altered rate of excretion as bile acids or reduction in absorption or increased sterol excretion [35-39]. The animal with HF has consumed approximately 30-35 mg fiber which might have also exerted synergistic effect. Assembly of HDL molecules takes place in plasma which might have been affected by the metobolites of HCH causing lipid peroxidation. This could be one of the reasons for decrease in HDL in HCH group which was corrected significantly to increase HDL level in animals fed with HF which reduced lipid peroxidaiton significantly. Complete inhibition of lipid peroxidation was not observed with antioxidant present in HF which may be one of the possible explanations of HDL not reaching the control values. Hypercholestramia has resulted in increased levels of LDL + VLDL. One of the functions of VLDL before converting itself into LDL is to deliver TG molecules to tissues. Increased lipid peroxidation might have oxidized the VLDL molecules might have resulted in increased TG levels by impairing the transportation of the molecule. Inhibition of oxidation of VLDL has made efficient transportation of the TG molecule was observed in the present study. Thus the developed HF may be having hypolipedemic effect and also may be helpful in preventing coronary heart diseases. Reduced lipid peroxidation TG, LDL and VLDL with increased HDL observed in the present study may have positive impact on cardiac function [40-43].

Oxidative damage at the cellular and the sub cellular level is considered to be an important disease causing event. Antioxidant vitamins namely A, E and C may play vital role in preventing the damage. Reduction in the levels of the vitamins A & E with HCH was reverted completely and partially with HF suggesting positive effect (T**able 7**). Increased synthesis of vitamin C a strong reducing agent was observed in the animals exposed to oxidative stress may be an adaptive defence mechanism but not in animals fed with both HCF and HF could be due to reduced oxidative stress. Ascorbic acid has been reported to prevent the oxidation of vitamin E or otherwise recycling of the same [44]. It is surprising that increased levels of vitamin C has not prevented the oxidation of both the vitamins, where as HF inhibited the oxidation effectively. Decreased levels of vitamin A and increased levels of vitamin C with HCH has been reported supports our findings [11, 44-45]. Decreased levels of fat soluble vitamins could be due to increased levels of MDA. HF by inhibiting MDA elevated the fat soluble vitamin concentration. However, complete recovery was not attained and could be attributed to partial inhibition of lipid oxidation.

Table 7 : Effect of HCH and HF on hepatic antioxidant vitamins

Groups	VITAMIN A μ g/g	VIT AMIN E mg/g	VITAMIN C mg/g
C	10.07 ± 1.32[a]	13.42 ± 0.13[a]	0.43 ± 0.02[a]
CHF	9.74 ± 0.72[a]	12.98 ± 0.89 [a]	0.42 ± 0.05[a]
CHCH	7.34 ± 0.66[b]	6.69 ± 0.44[b]	0.59 ± 0.07[b]
CHF +HCH	10.58 ± 0.89 [a]	9.57 ± 0.75 [c]	0.44 ± 0.03[a]
H	9.78 ± 0.77[a]	14.01 ± 0.93[a]	0.41 ± 0.07[a]
H + HF	10.59 ± 0.62 [a]	12.69 ± 1.08 [a]	0.46 ± 0.05[a]
H+HCH	7.68 ± 0.82 [b]	7.38 ± 0.46 [b]	0.61 ± 0.06[b]
H+HCH+HF	10.21 ± 0.55[a]	10.12 ± 0.59[c]	0.41 ± 0.03[a]

C = control, H = hypercholestremic, values bearing different superscripts in a column are significantly different at p<0.005

Another line of defence in inhibiting the oxidative stress is through liver antioxidant enzymes SOD, Catalase, GSH-Px and glutathione a tripeptide antioxidant. These enzymes scavenge harmful superoxide, hydroxyl radicals to hydrogen peroxide and than to water. Decrease or increase in the activities of these enzymes and glutathione may suggest the degree of oxidative stress and also the potency of antioxidants. HCH decreased drastically the activities of all the enzymes and the level of glutathione which was reversed significantly increased **(table 8)** on HF. Decreased activity of these antioxidants enzymes, increased lipid peroxidation with reduced glutathione concentration has been reported in rats exposed to oxidative stress and agrees with the present findings [46-48]. Reduced glutathione, an another known antioxidant the concentration of which was found decreased could be due to scavenging the free radicals generated through HCH resulting in oxidized GSH. HF has increased the concentration of GSH may be attributed to its antioxidant content which might have neutralized the free radical generation due to HCH may be a substitute for GSH. Increase concentration of GSH in tissue may be helpful in inhibiting the oxidation of vitamin E. It has been shown increased activity of glucose – 6 – Po_4 dehydrogenase has been observed in animals injected with HCH and supplemented with antioxidants which might have increased NADPH concentration through hexosmonose phosphate shunt which scavenges free radicals and maintains glutathione levels [11]. This explanation could be possible in the present study in which herbal formulation increased glutathione concentration.

Table 8 : Effect of HCH and HF on liver MDA antioxidant enzymes, and GSH

Groups	MDA[1]	GSH[2]	SOD[3]	Catalase[4]	GSH-Px[5]
Control	1.19 ± 0.17[a]	14.86 ± 0.82[a]	2.42 ± 0.13[a]	1.47 ± 0.16[a]	3.82 ± 0.31[a]
CHF	1.08 ± 0.14[a]	13.59 ± 1.04[a]	2.48 ± 0.26[a]	1.44 ± 0.14[a]	3.71 ± 0.41[a]
CHCH	2.63 ± 0.32[b]	8.03 ± 0.62[b]	1.33 ± 0.18[b]	0.76 ± 0.09[b]	1.77 ± 0.18[b]
CHF +HCH	1.68 ± 0.16[c]	10.21 ± 0.59[c]	1.74 ± 0.21[c]	1.12 ± 0.11[c]	2.71 ± 0.26[c]
H	1.32 ± 0.13[a]	13.91 ± 0.55[a]	2.38 ± 0.30[a]	1.52 ± 0.14[a]	3.67 ± 0.44[a]
H + HF	1.02 ± 0.08[a]	14.11± 1.13[a]	2.36 ± 0.11[a]	1.54 ± 0.13[a]	3.69 ± 0.41[a]
H+HCH	2.72 ± 0.08[b]	7.82 ± 0.76[b]	1.23 ± 0.09[b]	0.82 ± 0.01[b]	1.89 ± 0.27[b]
H+HCH+HF	1.64 ± 0.11[c]	10.78 ± 0.82[c]	1.84 ± 0.16[c]	1.24 ± 0.09[c]	2.64 ± 0.39[c]

C: control, H: hypercholestremic, values bearing different superscripts in a column are significantly different at p<0.005.

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1: nano moles/g; 2: µ moles/g; 3: mg/min/mg protein; 4:Δ A /min / mg protein; 5: µ moles NADP reduced / min / mg protein.

Domination of pro-oxidants in HCH fed animals may play a role in intra cellular signaling thus decreasing antioxidant defence [49]. These uncontrolled ROS formation may lead to chain reaction which indiscriminately target proteins [50] and lipids [51]. These lipids oxidation by ROS or protein maturation may release toxic metabolities may also cause changes in the antioxidant defence. Studies have shown ROS over production lowered antioxidant defence and alteration in the enzymatic path way [52-53].The decreased activity of antioxidant enzyme namely GSH-Px, SOD and catalase, hence, may be attributed to impaired intra cellular signaling which may affect the synthesis of the enzyme or could be due to changes in protein or lipid oxidation or toxic products formed due to oxidation of lipids and proteins. HF in the present study HF enhanced the antioxidant status by increasing the activities of enzymes and glutathione concentration.

HCH has not affected the intake of food or weight gain significantly (F**ig 1 & 2).** Hyperplasia of liver and kidney observed in the present study correlates with the reported literature (T**able 5**) [54] and was reduced significantly by feeding HF. Histopathological examination of liver with HCH feeding for 7 week showed, localized accumulation of dense, sudonophallic lipids in the periportal hepato cyst and hepatic lipid accumulation with no histo chemically demonstratable changes in the liver of HCH treated rats [54].

It is an interesting observation made in the present study that hypercholestremic diet increased only the cholesterol levels and not affected the over all biochemical changes in blood,liver, food intake or weight gain.

CONCLUSIONS

The present study suggests that it may be needed to supplement natural antioxidant products to inhibit the toxic effects of HCH. The developed seabuckthorn leaf based herbal formulation may be helpful in reducing blood lipids and enhances defence mechanisms. These properties of HF may be attributed for its photochemical contents.

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