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Hypoglycaemic and Hypocholesterolimic Efficacy of Horse Chestnut (*Aesculus indica)* using Rat Models

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Abstract

Indian Horse Chestnut is wildly grown at high altitudes and goes wastes due to lack of awareness, improper processing techniques for the development of value added products. That could be used to develop variety in products with high nutritional and medicinal values. The best known benefit is from the aescin inside the Horse Chestnut which cures several ailments. An attempt was made to see the hypocholesterolemic and hypoglycaemic efficacy of the flour by running animal trial. The blood glucose level was found to be decreased from 228 to 95 mg/dl in rats who were fed 75 per cent processed flour fed diet. The total blood cholesterol level was from 386 to 247 mg/dl. Triglycerides, VLDL and LDL level found lowered in all the different ratio of the processed flour from the control with 1 per cent cholesterol diet fed rats. However, HDL which is good cholesterol, among the test diet, was found maximum 39.41 mg/dl in processed flour (75 per cent) fed rats.

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Introduction

Horse Chestnut is a tree native to the Balkan Peninsula but found throughout the northern hemisphere. The seeds, leaves, bark and flowers of this plant have been used for centuries to help to relieve an array of health problems Horse Chestnut seeds are used as a traditional remedy for vascular problems like chronic venous insufficiency [1, 2].

Indian Horse Chestnut (Aesculus indica) belongs to the family Hipocastanaceae and is mainly grown at the high altitude (1000-3050 m) and is native to Himalayan region. In Himachal Pradesh, it is widely grown in some of the hill areas of Kangra, Shimla and Mandi districts. It is also distributed across North-eastern Afghanistan, Pakistan, Kashmir and Nepal.

This tree is a tall, deciduous, spreading tree with straight trunk and can grow up to 20-30 m height. All parts of the tree of the Indian Horse Chestnut have their own benefits and action in the biological system. It is used as food, feed and for wooden purposes as well as an ornamental tree in the home. It has tremendous medicinal as well as nutritional properties. Medicinally, the bark of the tree is used for lowering the intermittent fever and in the treatment of ulcer. On the other hand, the fruit is mainly used in the treatment of rheumatism, neuralgia, rectal complaints, skin disease, and hemorrhoids and to relieve headache. It is also given to the horses suffering from colic disorder. That's how; the term Indian Horse Chestnut is derived [3].

In the tribal area, people are highly dependent on the various plant species and Aesculus indica is one of them for curing various ailments. It contains most active chemical constituent called as Aescin , which is a mixture of triterpenoid saponin with excellent anti-inflammatory, anti-oxidant and -form which is βanti-oedema properties. Naturally, Aescin exists in water-insoluble and soluble in organic is therefore, used in the preparation of therapeutic agents required for oral administration [4]. All the species of Aesculus contain large amounts of toxin called Aesculin which is found in their seeds or nuts and is a simple coumarin aglycone (6, 7-dihydroxycoumarin, Aesculetin, cichorigenin). On the other hand, Aesculin has the properties against the dermal health and skin care. The treatment of aesculin is effective against age-related skin damage and to reduce alopecia (hair loss) by improving microcirculation and reducing oxidative stress. In addition; it also contains coumarin and flavonoids which include quercetin, rutin, and kaempferol [1].

Indian Horse Chestnut (Aesculus indica) is used in traditional Indian medicine for the treatment of skin diseases, rheumatism and headaches [5]. The seeds are toxic due to the presence of certain anti-nutrients like saponin and tannin. That's why it must be shelled, crushed and soaked overnight in water to remove its toxicity and dried in sunlight. The flour, which is known as tatwakhar is consumed by the local community in the form of halwa (Indian sweet). Sometimes it is blended with wheat flour and is taken in the form of chapatti by the tribal community of Himachal Pradesh [6]. The seeds of Aesculus indica have also been eaten during periods of famine after the removal of toxins [7]. Shubeena et.al made an effort to see the effect of acetlation on the physico-chemical properties of Indian Horse-chest nut starch [8].Keeping in view its medicinal value it was attempted to see the hypoglceamic and hypocholesterolemic efficacy of its flour.

Materials & Methods

Preparation of samples

Matured seeds of the Indian Horse Chestnut (Aesculus indica) were procured; cleaned manually, washed to remove any adhering dirt, dust and foreign particles. The seeds were crushed and soaked in water for the removal of toxicity. For the removal of toxicity, the traditional practice which was used by the rural folks was followed .In this method, the crushed seeds were soaked in water for 6 days and daily soaked water was replaced with fresh water. This process was continued for 6 days.

Preparation of extracts

The seed sample were dried at 40 degree Celsius and then ground in to a powder. Then 100 g sample were weight precisely. Different methods/ treatments of extraction of crudes saponin/ aescin are given as follows:

Treatments

Fresh seed powder	T0
In Percolator	T1
Roasted seed powder	T2
Soxhlet extraction	T3
Processed flour	T4

Estimation of crude saponin/ aescin from the seeds

The crude saponin in the washed sample was estimated by doing necessary modification in the method of Sharma et.al. [9].Flow sheet is given in **Figure 1**.

Chemical Required

Methanol: Distilled water (80:20) TLC plate Capillaries

Hexane (650C B.P) n-Butanol Ethyl acetate Sulphuric acid (20 per cent)

System Solvent: n-Butanol: Distilled Water: Acetic Acid (40:50:10)

Processed flour was then dried after decanting water in a cabinet drier at 50-60 degree Celsius and ground to a fine powder in a grinder to get homogenous mass, packed in air-tight containers till further use for biological trial.

Biological evaluation

To see the hypoglycaemic and hypocholestroemic efficacy of processed flour of Indian Horse Chestnut, a biological trial by using albino rats was conducted. Male albino rats wistar strains weighing 25±5g aged 25 days were obtained from the germ free Small Animal House and divided into nine groups comprising of ten rats each. The mean weight was adjusted to be same for all the groups. All the rats were caged in metabolic cages which facilitates the urine collection in a beaker .There were three control groups, one was fed casein diet (Diet 1) and the second group was fed casein diet along with 1.0 per cent cholesterol (Diet 2). Whereas, third group was fed control diet with diabetes. Rests of the groups were assigned for test diet as explained (Animal Experimental design is given in **Figure 3**). Four groups of rats given alloxane subcutaneously (140 mg/kg body weight) after 48 hour fasting. Diabetes was induced in two days; the rats were left as such for 20 days without any changes in their diet (control diet) to make sure that diabetes was permanently induced. The induction of diabetes in the rats was checked by collecting blood and urine and analyzed by hand glucometer (Model GC Sense) and Benedict's method, respectively. In addition, blood sugar level was estimated by collecting blood after 21 days.

The diets were isoprotenious containing 10 per cent protein. The rats were caged individually in polypropylene cages. Weighed amount of feed was offered and water was given ad libitum. The composition of diet is given in **Table 1** and **Table 2**. The ingredients were mixed, homogenized and passed through 70 mesh sieves to ensure uniform distribution of vitamin and mineral mixture. The composition of mineral and vitamin mixture is as recommended by BARR (1972).

*Based on the NAS recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Analysis of blood sample

Collected blood was analysed by hand glucometer (Model GC Sense) to check the blood glucose level.

Analysis of urine sample

Urine sample were analysed for glucose by Benedict's method [10].This test was done to get the qualitative estimation of appearance of sugar in urine and results are interpreted as per the following **Table 3**.

Benedict reagent

Took 600 ml of water and boiled in a beaker and added 100 g of anhydrous Na2 CO3 in boiling water. 200 g of sodium citrate and 125 g of potassium thiocynate is then added. Dissolved it by warming on flame and filtered it. Added 18 g Cuso4 in 100 ml water and mixed with above solution slowly and added 5 ml of 5 per cent potassium ferricyanide and made the volume to 1 litre.

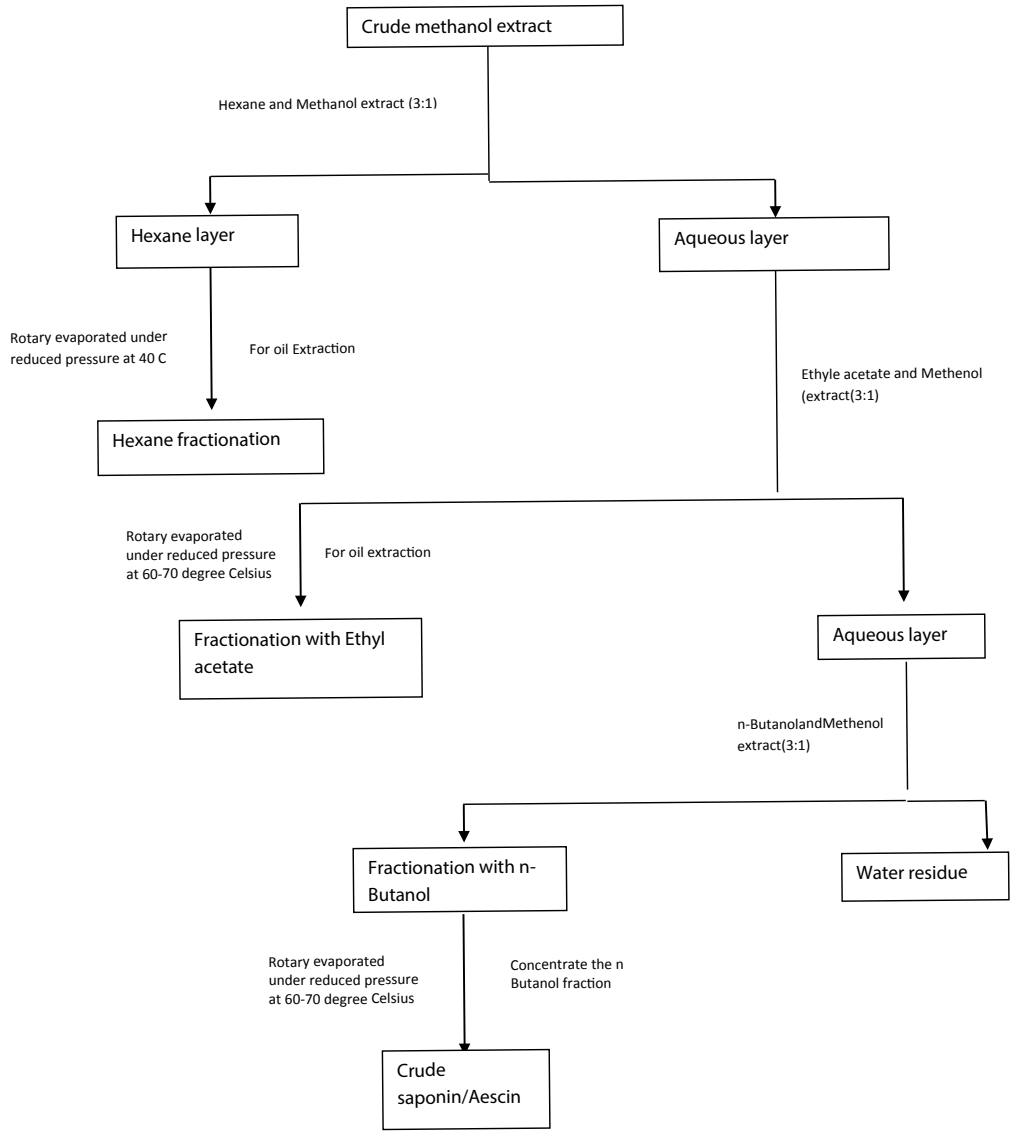

Figure 1 Extraction of Crude Saponin / Aescin from the seeds.

Procedure

Five ml of Benedict's solution were taken in the test tube and boiled for 1-2 minutes and then added eight drops of urine. Mixed well and then heated vigorously for 1-2 minutes and noted the colour changed.

Blood collection and analysis

Blood was collected from the tail of the rats after 40 days of feeding trial with the help of disposable syringe and used EDTA (Ethylene Diamine Tetra Acetic acid) to prevent coagulation. Then blood was centrifuged at 3000 rpm for 10 minutes for separation of plasma. Blood plasma was analyzed for the estimation of Blood cholesterol, High Density Lipoproteins and Triglycerides by using commercially available autopack kits. LDL (Low Density Lipoproteins) and VLDL (Very Low Density Lipoprotein) were calculated by using the formula:

LDL = Total Blood cholesterol - Triglycerides - HDL

———————

VLDL = Triglycerides / 5

Statistical analysis

The data was statistically analysed by using CRD.

Results & Discussion

Pertinent results thus obtained are discussed following.

Extraction of saponins/aescin from different methods

From the **Table 4**, it is clear that the maximum aescin content (7.73±0.15g/100g) was obtained in the treatment T3 ie. obtained by Soxhlet exraction method. Aescin content in treatments T1 and

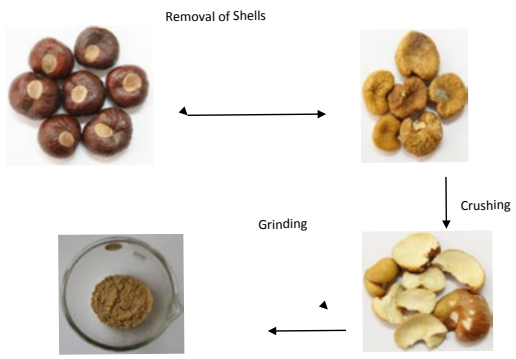

Figure 2 Plate 1: Preparation of sample.

T2 was estimated in the range of 5.00-5.20 g/100g. These values are statistically at par with each other. The seed powder which was roasted (T2) for 5 minutes at the low flame on the cooking gas so as to destroy the crude saponin of the flour contained 2.93 ± 0.15 per cent aescin. The lowest crude saponin/aescin content (0.08 per cent) was found in the treatment T4. To check the purity of aescin chemically, its melting point and pH was also determined. The aescin was acidic in nature having 212-224o melting point. The complete extraction was found in T3 because it might be due to the breaking of bonds or hydrolysis by the action of heat. Hostettmann and Marston (1995) reported 3.00 to 6.00 per cent aescin on dry weight basis in Aesculus hippocastanum seeds. Singh (2003) reported the yield of aescin as 2.00-3.00 per cent from Indian Horse chestnut seeds with shell.

Biological evaluation

Experiment with animals (albino rats) was conducted to see the efficacy of the flour on lowering blood sugar and lipid profile. For this the flour obtained by treatment T4 was used .The detailed results are discussed as:

Blood sugar level

The data obtained was given in the **Table 5**. The Blood glucose level in control group without diabetes was checked by testing sugars in the blood and it was found as 94.20 ± 12.56 mg/dl. On the other counterpart, the blood glucose level after test diet given to experimental group was obtained as 77.60 ± 10.78 mg/dl. There was a statistically significant difference CD (P ≤ 0.05) 17.07 observed. In the reference group (control diet with diabetes),the blood glucose level after inducing diabetes was obtained as 239.20 ± 9.15 mg/dl and after continuing the control the blood glucose level was found 204.60 ± 14.26 mg/dl. There was a significant difference (P ≤ 0.05)17.47 rather than the level of the glucose level. When the test diet was given to the group fed with diet containing 25 per cent processed flour, the blood glucose level was decreased from 219.40 ± 16.81 to 106 ± 39.89 mg/dl. The same hypoglycaemic effect was observed in groups which were fed with 50 and 75 per cent processed flour based diet. The values were recorded as a decrease from 231.60 ± 19.50 /dl to 119.20 ± 44.53 mg/dl and 228.80 ± 18.02 mg mg/dl to 95.20 ± 19.32 mg/dl respectively. It is apparent that with the increasing level of processed flour in the diet the level of blood sugar decreased proportionately. Earlier Yoshikawa et al. reported that the compounds isolated from the seeds of horse chestnut, Aesculus hippocastanum (Hippocastanaceae) L. escins, Ia, Ib, IIa, IIb, and IIIa Escins Ia, Ib, IIa, and IIb had an absorption-inhibitory effect and results in hypoglycemic activity in the oral glucose tolerance test in rats [11]. Later on Zhang et al. documented that the isolated compounds of escin Ia, Ib,IIa, IIb, deacetyl ascins Ia, Ib, IIa, IIb, and desacylescins I and II had inhibitory effect on the elevation of blood glucose levels and exhibited pancreatic activity thus helps in lowering the blood glucose levels [12]. The presence of such components might be responsible for the hypoglycaemic activity of the flour. This corroborates the present findings.

Blood lipid profile

Total blood cholesterol

Cholesterol is an essential component of mammalian cell membranes and is required to establish proper membrane permeability, fluidity, to produce hormones, vitamin D, and the bile acids that help to digest fat. But the body needs only a limited amount of cholesterol to meet its needs. When excess cholesterol is present, there may develop health problems such as Coronary Heart Disease. Cholesterol is carried through blood, attached to proteins. This combination of proteins and cholesterol is called a lipoprotein.

Total blood cholesterol and serum triglycerides were also estimated to see the efficacy of processed flour (tatwakhar) on the experimental animals. The mean serum level of total blood cholesterol of the experimental group data was presented in the **Table 6**. It is apparent from the table that in rats which were fed on Diet 1 (control group), the total cholesterol level was estimated as 86.60±2.86 mg/dl whereas, in the other group which fed on Diet 2 (control with 1% cholesterol) contained maximum cholesterol level with the value (386.20±7.68 mg/dl). The blood cholesterol was gradually decreased with increasing levels of processed flour which was given to the rats. When 25 per cent flour was given with 1 per cent cholesterol, the level was found to be 336.44 ± 9.61 mg/dl; further increasing the proportion of flour to 50 per cent, the total blood cholesterol was decreased to 273.04 ± 5.98 mg/dl. The diet which contains 75 per cent processed flour; cholesterol was lowered up to the 247.73 ± 6.00 mg/dl. Statistically, significant difference was found by the use of different proportion of processed flour. It can be concluded from the above data, that all the percentage of processed flour exhibited cholesterol lowering effect. But among all, the 75 per cent processed flour was relatively significant lowered than the cholesterol containing group. The cholesterol might be decreased due to the effect of the aescin. As the isolated compounds from the aescin, inhibited the lipase activity. So that it causes lowering the effect of cholesterol level in the blood. Dworschak et al. reported in a preliminary animal study that 1(%) Horse Chestnut Seed Extract reduced elevated blood cholesterol [13]. Zhang et al. documented that the isolated compounds derived from edible seeds of Aesculus turbinata exhibited inhibitory effect on lipase activity [12]. That's how lowering the triglyceride and cholesterol level. This gives credence to the present findings.

HDL cholesterol

High Density Lipoprotein (HDL) cholesterol is considered as good

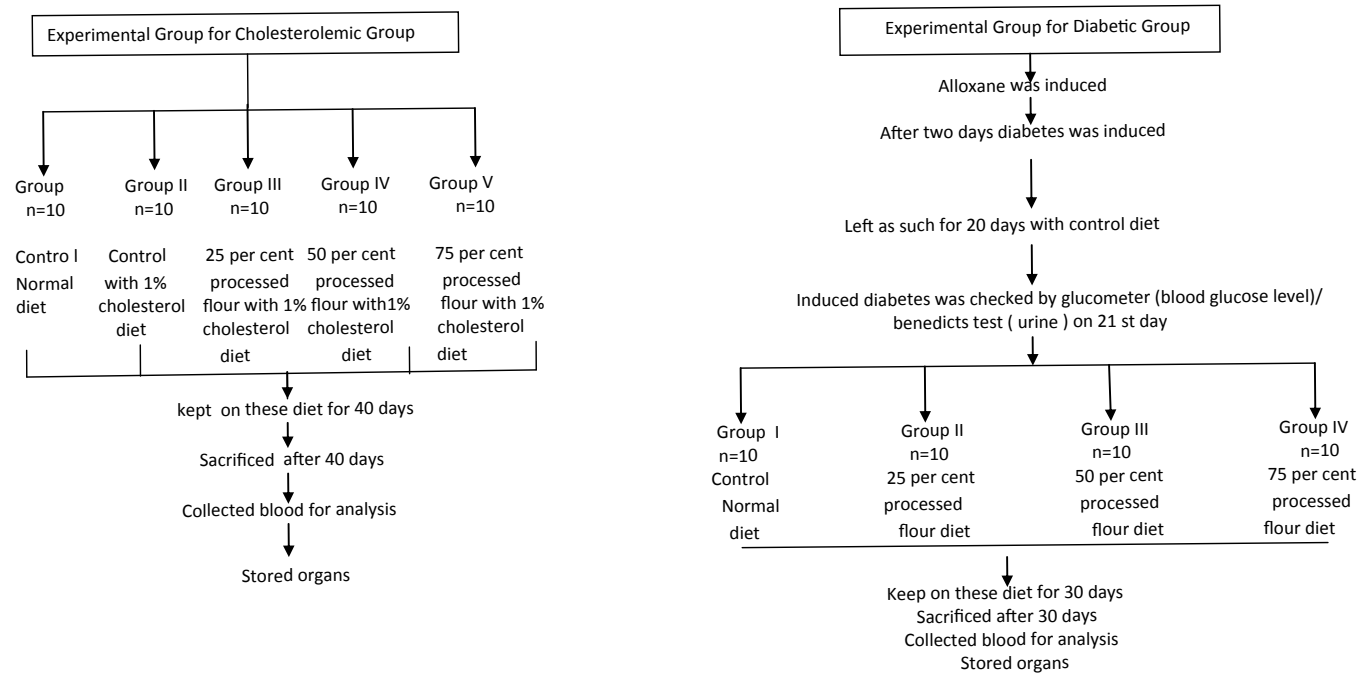

Figure 3 Animal Experimental Design

Table 1 Composition of Experimental Diets (g/100 g) for Glycaemic group.

Ingredients	Control diet	25 per cent processed flour	50 per cent processed flour	75 per cent processed flour
Casein	10.00	8.18	6.37	4.55
Ground nut oil	10.00	9.90	9.80	9.70
Sucrose	10.00	10.00	10.00	0.35
Cellulose	5.00	5.00	5.00	5.00
Mineral mixture	4.00	3.79	3.58	3.38
Vitamin mixture	1.00	1.00	1.00	1.00
Choline chloride	0.02	0.02	0.02	0.02
Starch	59.98	37.11	14.23	1.00
Processed flour	-	25.00	50.00	75.00

* All diets contained 10% protein including the crude protein from processed flour source
** All diets contained 10% fat including the crude fat from processed flour source
*** All diets contained 5% fibre

cholesterol among all cholesterol. This cholesterol picks up the excess cholesterol and takes it back to the liver. The data presented in the same **Table 5** depicts the blood HDL cholesterol levels of rats that were fed on different percentage of processed flour of Indian Horse Chestnut. As is evident from the data that the value estimated for HDL in rats fed on Diet 1 was more (41.47 ± 1.46 mg/dl) than other group which fed with Diet 2 attained less value (34.72 ± 1.88 mg/dl.). The HDL level of 75 per cent processed flour dependent diet was 39.41 ± 1.6 mg/dl. On the other hand, the HDL level of 25 and 50 per cent supplemented processed flour was given to the rats were 31.37 ± 3.38, 38.35 ± 1.25 mg/dl. A significant ($P \leq 0.05$) difference was there in HDL cholesterol when these groups were compared to each other. It can be concluded that the maximum flour given to the rats exhibit the increasing HDL level. HDL might be increased due to the optimum level of Aescin present in 75 per cent flour as the isolates of Aescin inhibit the lipase activity and decrease the total cholesterol content and thus increasing the good cholesterol.

Triglycerides

Triglycerides (TG) are another type of fat that is carried in the blood by very low density lipoproteins. Excess calories, alcohol, or sugar in the body are converted into triglycerides and stored in fat cells throughout the body. From the **Table 5**.16, it reveals the feeding effect of processed flour on triglycerides level of the blood profile of the rats. By just go through the table, the triglyceride level was significantly highest in Diet 2 fed group (121.64 ± 2.95 mg/dl) and lowest in 75 per cent processed flour fed group i.e. 54.54 ± 4.22 mg/dl. The rats fed with Diet 1 level as 52.69 ± 4.05 mg/dl. There was also significant lowering the triglycerides level in the blood when 25 per cent (95.70 ± 1.49mg/dl) and 50 per cent (94.27 ± 1.68) processed flour was given to that group of rats. A significant ($P \leq 0.05$) difference was there when Diet 2 fed group was compared with Diet other fed group. Zhang et al. reported the same isolates derived from edible seeds of Aesculus turbinata exhibited inhibitory effect on lipase activity and lowering the triglycerides level. Thus study supports the findings [12].

Table 2 Composition of Experimental Diets (g/100 g) for Cholesterolemic group.

Ingredients	Control diet	Control diet with cholesterol	25 per cent processed flour	50 per cent processed flour	75 per cent processed flour
Casein	10.00	10.00	8.18	6.37	4.55
Ground nut oil	10.00	10.00	9.90	9.80	9.70
Sucrose	10.00	10.00	10.00	10.00	0.35
Cellulose	5.00	5.00	5.00	5.00	5.00
Mineral mixture	4.00	4.00	3.79	3.58	3.38
Vitamin mixture	1.00	1.00	1.00	1.00	1.00
Cholestrol	-	1.00	1.00	1.00	1.00
Choline chloride	0.02	0.02	0.02	0.02	0.02
Starch	59.98	58.98	36.11	13.23	-
Processed flour	-	-	25.00	50.00	75.00

* All diets contained 10% protein including the crude protein from processed flour source
** All diets contained 10% fat including the crude fat from processed flour source
*** All diets contained 5% fibre

Table 3 Interpretation of Benedict's test.

Colour	Report	Urine g %	Blood mg %
Green discolouration	0 to trace	-	≤ 200
Green precipitate	+	0.25	200-250
Greenish yellow purple	++	0.5	250-300
Yellowish orange deep	+++	1.0	300-350
Brick red precipitates	++++	≥200	≥350

Table 4 Quantitative estimation of aescin content from different treatment of seed flour.

Treatment	Melting point	pH	Aescin (g/100g)
Fresh seed powder(T_0)	224	4.3	5.20±0.1b
In Percolator (T_1)	223	4.5	5.00±0.1b
Roasted seed powder (T_2)	222	4.5	2.93±0.15c
Soxhlet extraction(T_3)	221	4.1	7.73±0.15a
Processed flour(T_4)	-	-	0.08d
CD (P≤0.05)		0.21	

*Control =Aescin content of fresh seed flour (5.2g/100 g)
Each value represents mean of three replicates. In the same column, significant differences according to CRD are indicated by different letters. Same letter represent that their values are at par

Table 5 Glycaemic effect of feeding processed flour of *Aesculus indica* on blood glucose level of rats (mg/dl).

Attribute	Control diet without diabetes	Control diet with diabetes	25 per cent processed flour	50 per cent processed flour	75 per cent processed flour
Blood glucose level after induced diabetes	94.20^{a}± 12.56	239.20^{a}± 9.15	219.40^{a}± 16.81	231.60^{a}± 19.50	228.80^{a}± 18.02
Blood glucose level after test diet given to experimental group	77.60^{a}± 10.78	204.60^{b}± 14.26	106.00^{b}± 39.89	119.20^{b}± 44.53	95.20^{b}± 19.32
CD (P≤0.05)	17.07	17.47	45.47	50.13	28.78

*Diet 1- Control without cholesterol, **Diet 2- Control with 1% cholesterol
In the same column, significant differences according to CRD are indicated by different letters. Same letter represent that their values are at par

LDL cholesterol

Low-density lipoprotein (LDL) or "bad," cholesterol transports cholesterol particles throughout body. LDL cholesterol builds up in the walls of arteries, making them hard and narrow. A glance at **Table 6** reveals the effect of feeding different processed flour proportion on the blood HDL level of rats. It is clear from the **Table 6** that the blood LDL cholesterol level of rats fed on Diet 1 was 34.30 ± 3.83 mg/dl and for Diet 2 fed group was 327.16 ± 6.89 mg/dl whereas the rats depends on the 25, 50 and 75 per cent processed flour, there LDL level was 274.95 ± 3.83, 222.82 ± 8.73 and 197.41 ± 6.24 mg/dl was found. Statistically, a significant (P ≤ 0.05) difference was observed in LDL cholesterol when all groups were compared with each other. There was decreasing in the blood LDL cholesterol level was observed. The LDL lowering properties were might be due the optimum level of aescin or saponin content in the flour.

VLDL cholesterol

Very low density lipoprotein (VLDL) contains the most triglycerides,

Table 6 Chlolesterolemic effect of processed flour (tatwakhar) on blood lipid profile of rats.

Attribute	Diet 1*	Diet 2**	25 per cent processed flour	50 per cent processed flour flour	75 per cent processed flour flour	CD (P≤0.05)
Total blood Cholesterol (mg/dl)	$86.60^{e} \pm 2.86$	$386.2^{a} \pm 7.68$	$336.44^{b} \pm 9.61$	$273.04^{c} \pm 5.98$	$247.73^{d} \pm 6.00$	6.52
HDL Cholesterol (mg/dl)	$41.47^{a} \pm 1.46$	$34.72^{c} \pm 1.88.00$	$31.37^{c} \pm 3.38$	$38.35^{bc} \pm 1.25$	$39.41^{ab} \pm 1.60$	2.44
Triglycerides (mg/dl)	$52.69^{c} \pm 4.05$	$121.64^{a} \pm 2.95$	$95.70^{b} \pm 1.49$	$94.27^{b} \pm 1.68$	$54.54^{c} \pm 4.22$	4.09
LDL Cholesterol (mg/dl)	$34.30^{e} \pm 3.83$	$327.16^{a} \pm 6.89$	$274.95^{b} \pm 3.83$	$222.82^{c} \pm 8.73$	$197.41^{d} \pm 6.24$	8.17
VLDL Cholesterol (mg/dl)	$10.54^{c} \pm 0.81$	$24.33^{a} \pm 0.59$	$19.14^{b} \pm 0.30$	$18.85^{b} \pm 0.34$	$10.91^{c} \pm 0.85$	0.82
Blood LDL:HDL Cholesterol Ratio	$0.83^{d} \pm 0.11$	$9.44^{a} \pm 0.46$	$7.18^{b} \pm 0.33$	$7.19^{b} \pm 1.01$	$5.02^{c} \pm 0.32$	0.71

a type of fat, attached to the proteins in the blood. VLDL cholesterol makes LDL cholesterol larger in size, causing blood vessels to narrow. Data presented in **Table 6** reveals the efficacy of feeding different proportion of processed flour on VLDL (Very Low Density Lipoprotein) cholesterol. It is clear that the highest VLDL (24.33 ± 0.59 mg/dl) volume was in the group of rats that fed on control with cholesterol. And lowest was found in the control group (10.54 ± 0.81 mg/dl). When the diet was supplemented with 25 per cent processed flour, the VLDL level was 19.14 ± 0.3 mg/dl was analyzed. Likewise in 50 per cent processed flour, it was 18.85 ± 0.34 mg/dl. The diet which contains 75 per cent processed flour, the VLDL level was considerably lower 10.91 ± 0.85 mg/dl than the rest of the diet. Statistically there was significant ($P \leq 0.05$) difference was observed in VLDL cholesterol when all the groups were compared with each other. The decrease in VLDL content in 75 per cent processed flour, might have been due to the presence of certain chemical constituents such as, aescin, flavonoids etc. which leads to lowering the VLDL level in rats.

Blood LDL: HDL cholesterol ratio

Data shown in **Table 6** depicts the effect of feeding processed flour on the blood LDL: HDL cholesterol ratio in rats. As it is clear from the data that the maximum LDL: HDL cholesterol ratio was in the rats that were fed on Diet 2 (9.44 ± 0.46 mg/dl) whereas minimum value (0.83 ± 0.11) was obtained by the control group. Significant ($P \leq 0.05$) difference was found in the blood LDL: HDL cholesterol when all groups were compared to each other. From the same table, it is apparent that the ratio of the LDL and HDL cholesterol in the group that were fed on 25 per cent processed flour, they got the 7.18 ± 0.33, whereas 50 per cent had 7.19 ± 1.01 and 75 per cent flour got 5.02 ± 0.32 cholesterol ratio. From the above, it can be concluded that the rats who were fed on control with cholesterol diet they increased ratio whereas the ratio was significantly and gradually decreases when diet 25 per cent, 50 per cent and 75 per cent flour was given, respectively.

Conclusion

As is evident from the biological experimentation there is increase in the good fat ie.HDL and decrease in the values of bad fats .So from the aforesaid discussion it is inferred that utilization of processed flour in the diet can definitely improve the blood lipid profile. All these changes might be due to the active component “aescin” of Indian Horse Chestnut flour. The isolates derived from the other species of Aesculus. Dworschak et al. found after animal experiment that 1(%) Horse Chestnut Seed Extract (aescin) reduced the elevated blood cholesterol level whereas, Zhang et al. reported that the isolated compounds derived from edible seeds of Aesculus turbinata exhibited inhibitory effect on lipase activity responsible to decreased level of triglycerides and cholesterol [12,13]. It is inferred that the presence of saponin in diet might be responsible for improving the blood lipid profile in experimental rats.

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