Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.075

RESEARCH ARTICLE

# Determination of Lipid Lowering Effect of Corn Flour in Hyperlipidemic Albino Mice

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ARTICLE HISTORY (21-080)

Received: February 13, 2021
Revised: October 02, 2021
Accepted: October 05, 2021
Published online: October 27, 2021

**Key words:**
Albino mice
Corn flour
Efficacy
Hyperlipidemia
Lipid profile

**A B S T R A C T**

The current experimental study determined the lipid lowering effects of corn flour in albino mice. A total 180 adult albino mice having average body mass (30±9 g) were procured and kept in clean and aerated plastic cages. After 7 days of acclimatization, all the mice were randomly divided and kept in six equal groups consisting of groups A and B (untreated and treated control) and treated groups (C-F). Mice were fed diet containing cholesterol (400 mg/kg body weight) for induction of hyperlipidemia. All the groups were given atherogenic diet for 15 days except group A. Standard drug (Simvastatin) was given to control group @ 0.6mg/kg at day 15 till the completion of experiment. The mice were given corn flour in group C (1 gm/kg), D (2 gm/kg), E (4 gm/kg) and F (6 gm/kg) at day 15 up to day 60 of trial. Blood was collected for serum separation from each mice at days 0, 15, 30, 45, and 60 of trial. The results on serum biochemistry indicated that supplementation of different concentrations of corn flour significantly altered serum lipid profile (TL, TC, TG and LDL) in albino mice. The values of HDL were increased in mice of corn flour treated groups. Results showed that corn flour at 6 mg/kg body weight significantly lowered cholesterol levels in treated mice.

**To Cite This Article:** Iqbal Z, Aftab A, Swar SO, Hussain R and Mohiuddin M, 2022. Determination of lipid lowering effect of Corn flour in hyperlipidemic albino mice. Pak Vet J, 42(1): 112-116. http://dx.doi.org/10.29261/pakvetj/2021.075

## INTRODUCTION

Increased levels of lipids in the blood may result in abnormal lipid metabolism leading to dyslipidemias and development of the atherosclerosis ultimately causing multiple problems in heart (Iqbal *et al*., 2016; Abdel-Moneim *et al*., 2019; Austria *et al*., 2021; Uchendu *et al*., 2021). Dyslipidemia leads to onset of cardiovascular diseases (CAD) that have a strong correlation with increased cholesterol levels in different animals (Swamy *et al*., 2011; El-Newary, 2016; Solomou *et al*., 2019; Hussain *et al*., 2020). The dietary habits can play a key role in causing dyslipidemias. It is observed that physical, physiological and/or dietary interventions can be effective in lowering elevated lipid levels to avoid cardiovascular problems (Ahmad *et al*., 2018). Higher concentrations of LDL receptors enhance and improve the uptake of low-density lipoprotein cholesterol from blood circulation (Rajesh *et al*., 2012; Singh *et al*., 2013). Both animal and human models based investigations have highlighted that the functional food may be used for the control, management or treatment of various human health disorders and metabolic diseases (Ghaffar *et al*., 2017; Kian *et al*., 2019; Sindu *et al*., 2019). The functional foods can be used to decrease the high lipid profile that ultimately has various effects on sclerotic components and cardiovascular events (Farooq *et al*., 2019; Oguntibeju, 2019; Javed *et al*., 2021a). As the prescribed treatment regimens for dyslipidemia and CAD include synthetic drugs, of which mostly belong to Statin group of drugs that inhibit the enzyme catalyzing the rate-limiting step in cholesterol biosynthesis. Although these drugs have been found to impose adverse effects on humans, still they are widely recommended across the globe (Bhandari *et al*., 2002). It permits for searching into functional food and alternative herbal remedies, which are cheaper and might prove to be causing significant lowering of lipids as compared to their synthetic counterparts having adverse effects on the health of humans (Visavadiya and Narasimhacharya, 2005; Javed *et al*., 2006; Javed *et al*., 2009; Ping *et al*., 2019; Kuru *et al*., 2020).

Corn, in the Pakistan, India and Bangladesh (subcontinent) is frequently used as staple food from the past several decades. It has a very good taste having

highly nutritive and medicinal value as well (Salem, 2015; Abo Elnaga *et al*., 2016). Although corn oil has been found to decrease blood cholesterol as well as lipid profile levels (Maki *et al*., 2015) however, the antihyperlipidemic activity of corn flour has not been reported. In alternative medicine, it is known for lowering body fats but still no scientific data is available about this fact and its lipid lowering effect still remained to be properly investigated. Keeping in view the above facts, this study was designed to determine the lipid lowering effect of corn flour in albino mice.

## MATERIALS AND METHODS

**Experimental animals and treatment:** Adult albino mice (n=180) of both sexes having average weight 30±9 g were procured and kept at National Institute of Health (NIH), Pakistan in clean and aerated plastic cages under standard laboratory environment. After acclimatization for 7 days, the mice were randomly divided into six groups consisting of Treatment I, Treatment II, Treatment III, Treatment IV, Treatment control and untreated control groups. The mice had free access to standard mice feed and clean water. Atherogenic diet containing normal mice diet + cholesterol 400 mg/kg was fed to all the treatment groups for a period of 60 days.

**Induction of hyperlipidemia:** Cholesterol (Cholesterol 90% E, Applichem, Darmstadt, Germany) was given to mice (400 mg/Kg body weight). The hyperlipidemia was induced by mixing cholesterol in the diet of mice to make it atherogenic. This atherogenic diet was offered to all groups for 15 days, except group A, which was offered atherogenic diet till the end of experiment.

**Synthetic drug treated control group:** Simvastatin (standard drug) 10 mg, OBS Pharmaceutical, Karachi, Pakistan was chosen for lowering lipid in the treated control group. The drug was given to all the experimental mice from days 15 to 60 of trial (0.6 mg/Kg body weight). This group served as the standard control group.

**Corn flour treatment groups:** Corn flour was purchased from the commercial local market of Islamabad, Pakistan and was used to feed the mice of treatment groups. Corn flour equivalent to 1gm/kg, 2 gm/kg, 4 gm/kg and 6 gm/kg was offered to the treatment groups I, II, III and IV for 15-60 days. The feed and drug dosage protocol is mentioned in Table 1.

**Collection of blood samples:** Blood samples were taken at days 0, 15, 30, 45, and 60 of treatments respectively. Briefly, six albino mice on sampling day from each group were anesthetized using chloroform. Blood of each albino mice was collected in disposable sterilized gel tube directly from heart. Blood was centrifuged at 5000 rpm for 5 minutes to separate the serum and transferred to clean eppendorf tubes for further analysis (Mashkoor *et al*., 2013; Ghaffar *et al*., 2015; Iqbal *et al.*, 2016).

**Assessment of lipid profile:** Lipid profile was recorded using Spin Lab, a semi-automated chemistry analyzer (Spinreact, Girona, Spain). Total cholesterol (TC), Total lipids (TL), Triglycerides (TG) and High density lipoprotein (HDL) were analyzed using reagent kits (Spinreact, Girona, Spain). The concentrations of LDL in serum samples were determined according to previous protocol (Ghaffar *et al*., 2015; Ghaffar *et al*., 2017; Ahmad *et al*., 2018).

**Statistical analysis:** The significance of results was analyzed using t-test. Mean±SD (standard deviation) values of each parameter at post-treatment days 30, 45, and 60 were compared to the respective values at 15$^{th}$ day to check the significant difference. The value $P<0.05$ was taken as significant while $P>0.05$ indicated non-significant data.

## RESULTS

**Hyperlipidemia induction in mice:** Hyperlipidemia was induced by offering high cholesterol diet to albino mice. 400 mg/kg body weight cholesterol was offered to the animals with routine rat feed (atherogenic feed). The atherogenic feed successfully increased the total lipid contents, triglycerides, total cholesterol and LDL levels by 75.62, 52.59, 84.53 and 126.73%, respectively and decreased the HDL cholesterol level to 30.28%.

**Antihyperlipidemic efficacy of Corn flour equivalent to 1 gm/kg:** Antihyperlipidemic efficacy of corn flour equivalent to 1 gm/kg body weight has been demonstrated in Tables 2 and 3. These results indicated that the values of lipid profile parameters were not altered significantly at days 30, 45 and 60 of post treatment.

**Antihyperlipidemic efficacy of Corn flour equivalent to 2 gm/kg:** The corn flour administered to mice at 2 gm/kg body weight lowered the lipid profile values (Table 2-3). The corn flour lowered the total lipids levels from 209±5.16 to 204±7.53, 200±5.72 and 191±5.87 at days 30, 45 and 60 of post treatment respectively. The percentage reduction values of total lipids at 30, 45 and 60th day post treatment was 2.39, 4.31 and 8.61%, respectively. The effect of corn flour was non-significant on mice at all post treatment days. A decreasing trend was observed in case of total cholesterol where Corn flour reduced the cholesterol levels to some extent from 152±7.90 to 143±8.81, 133±6.11 and 119±4.27 at 30, 45 and 60 respective days. The percentage reduction values of total cholesterol observed were 5.92, 12.50 and 21.71% showing a significant decrease at day 60 of trial. Corn flour lowered the triglyceride levels from 154±8.39 to 145±4.94, 128±6.43 and 114±9.27 at days 30, 45 and 60 of respective post treatment producing the percentage reduction values of triglyceride as 5.84, 16.88 and 25.97% respectively with a significant change at day 60 of post exposure. The results indicating LDL level showed that the corn flour decreased the raised levels of LDL from 81±8.05 to 65±4.73, 52±9.83 and 38±8.61 at days 30, 45 and 60 respectively. The respective percentage reduction values were observed 19.75, 35.80 and 53.09% at 30, 45 and 60 days. The change however, was significant at day 60 of treatment. The corn flour elevated the HDL levels from 28±4.52 to 34±6.76, 38±5.63 and 43±9.04 at 30, 45 and 60 days respectively. The percentage increase of HDL levels was 21.43, 35.71 and 53.57% after post medication days. Significant rise was observed at day 60 of treatment.

**Table 1:** Feeding and drugs administration plan in albino mice during the experimental

| Untreated Control (atherogenic diet) | Atherogenic Feed (Normal routine mice feed + Cholesterol 400mg/kg) 0 to 60 days |
|---|---|
| Treated Control (simvastatin 0.6mg/kg) | Atherogenic Feed 0 to 15 days, Atherogenic feed + Tablets survive® 15 to 60 days as cellulose replacement |
| Treated Group I (Corn flour @ 1 gm/kg body wt.) | Atherogenic Feed 0 to 15 days, Atherogenic Feed + Corn flour 15 to 60 days as cellulose replacement |
| Treated Group II (Corn flour @ 2 gm/kg body wt.) | Atherogenic Feed 0 to 15 days, Atherogenic Feed + Corn flour 15 to 60 days as cellulose replacement |
| Treated Group III (Corn flour @ 4 gm/kg body wt.) | Atherogenic Feed 0 to 15 days, Atherogenic Feed + Corn flour 15 to 60 days as cellulose replacement |
| Treated Group IV (Corn flour @ 6 gm/kg body wt.) | Atherogenic Feed 0 to 15 days, Atherogenic Feed + Corn flour 15 to 60 days as cellulose replacement |

**Table 2:** Mean±SD values of total lipids (mg/dl) and total cholesterol (mg/dl) in hyperlipidemic mice (n=30)

| Groups | Parameters (mg/dl) | Treatment days | | | |
|---|---|---|---|---|---|
| | | 15 | 30 | 45 | 60 |
| Untreated control | Total lipids | 215±5.78 | 227±4.87 | 245±8.11 | 251±6.38 |
| | Total cholesterol | 149±6.94 | 156±3.10 | 169±7.83 | 176±4.54 |
| Treated control | Total lipids | 228±6.57 | 215±6.31 | 198±5.13 | 167±.44* |
| | Total cholesterol | 143±6.25 | 128±7.58 | 112±3.82* | 93±6.23* |
| Treated Group I | Total lipids | 218±4.23 | 215±7.19 | 208±6.68 | 205±6.91 |
| | Total cholesterol | 145±4.31 | 137±4.62 | 128±5.62 | 122±3.09 |
| Treated Group II | Total lipids | 209±5.16 | 204±7.53 | 200±5.72 | 191±5.87 |
| | Total cholesterol | 152±7.90 | 143±8.81 | 133±6.11 | 119±4.27* |
| Treated Group III | Total lipids | 212±4.38 | 209±6.42 | 204±7.83 | 176±4.73* |
| | Total cholesterol | 144±6.73 | 128±9.52 | 111±8.63* | 103±9.03* |
| Treated Group IV | Total lipids | 200±5.83 | 196±4.72 | 187±7.62 | 153±6.91* |
| | Total cholesterol | 138±7.62 | 115±5.37 | 104±6.27* | 93±5.34* |

**Table 3:** Mean±SD values of triglycerides LDL (mg/dl) and HDL (mg/dl) in hyperlipidemic mice (n=30)

| Groups | Parameters (mg/dl) | Treatment days | | | |
|---|---|---|---|---|---|
| | | 15 | 30 | 45 | 60 |
| Untreated control | Triglycerides | 144±6.10 | 155±8.73 | 166±8.51 | 178±9.33 |
| | LDL | 59±7.62 | 65±5.76 | 79±7.04 | 96±3.99 |
| | HDL | 31±5.53 | 28±8.70 | 25±7.83 | 19±9.76 |
| Treated control | Triglycerides | 152±7.78 | 126±6.82 | 103±6.89* | 80±8.22* |
| | LDL | 74±5.84 | 59±8.87 | 41±6.76* | 19±9.22* |
| | HDL | 30±6.74 | 37±9.17 | 44±8.74* | 55±7.13* |
| Treated Group I | Triglycerides | 148±9.18 | 141±8.73 | 126±7.21 | 119±7.68 |
| | LDL | 79±9.11 | 72±8.32 | 63±4.09 | 52±7.89 |
| | HDL | 23±8.27 | 25±8.55 | 29±7.61 | 32±7.38* |
| Treated Group II | Triglycerides | 154±8.39 | 145±4.94 | 128±6.43 | 114±9.27* |
| | LDL | 81±8.05 | 65±4.73 | 52±9.83 | 38±8.61* |
| | HDL | 28±4.52 | 34±6.76 | 38±5.63 | 43±9.04* |
| Treated Group III | Triglycerides | 145±5.89 | 129 ±7.74 | 110±6.71* | 89±5.46* |
| | LDL | 85±5.77 | 64±6.65 | 39±5.64* | 23±5.92* |
| | HDL | 33±7.38 | 41±5.23 | 47±8.44 | 52±6.92* |
| Treated Group IV | Triglycerides | 142±8.56 | 121±8.91 | 99±6.54* | 82±7.78* |
| | LDL | 80±8.27 | 58±8.90 | 33±7.54* | 14±6.32* |
| | HDL | 30±8.17 | 41±9.21 | 48±8.71* | 54±7.88* |

**Antihyperlipidemic efficacy of Corn flour equivalent to 4 gm/kg:** Corn flour given at 4 gm/kg body weight decreased the lipid profile indicators as demonstrated in Tables 2 and 3. The Corn flour lowered the total lipids levels from 212±4.38 to 209±6.42, 204±7.83 and 176±4.73 at post medication days 30, 45 and 60 respectively. The percentage reductions of total lipids were observed to be 1.42, 3.77 and 16.98%, showing the insignificant difference at post treatment days 30 and 45 while significant decrease at day 60 of experiment. In total cholesterol, a decreasing trend was found when the value 144±6.73 of total cholesterol was reduced to 128±9.52, 111±8.63 and 103±9.03 on 30, 45 and 60 days, after the Corn flour administration. The percentage reduction of total cholesterol was observed to be 11.11, 22.92 and 28.47% showing a significant decrease at 45th and 60th day of the experiment. Corn flour treatment lowered the triglyceride levels from 145±5.89 at post cholesterol feeding day (15th) to 129±7.74, 110±6.71 and 89±5.46 at 30, 45 and 60 days, respectively. The percentage reduction values 11.03, 24.15 and 38.62% produced by Corn flour at 30, 45 and 60 days showed that the change was significant at 45th day and final (60th) day of experiment. The LDL levels were also changed after the administration of Corn flour from 85±5.77 at 15th day of experiment to 64±6.65, 39±5.64 and 23±5.92 at 30, 45 and 60 days. In this case statistically significant changes were observed at 45 and 60 post treatment days. HDL levels increased from 33±7.38 to 41±5.23, 47±8.44 and 52±6.92 at post treatment days 30, 45 and 60, respectively. The percentage increase was observed as 24.24, 42.43 and 57.58% showing a significant rise in the value at final day of treatment.

**Antihyperlipidemic efficacy of Corn flour equivalent to 6 gm/kg:** The administration of 6 gm/kg body weight corn flour significantly reduced the lipid profile values in hyperlipidemic albino mice (Tables 2- 3). The levels of total lipids 200±5.83 at day 15 decreased to values 196±4.72, 187±7.62 and 153±6.91 on 30, 45 and 60 days respectively after post treatments. The decrease was non-significant at sampling day 30 while at post treatment

days 45 and 60 of trial, a significant reduction was recorded when compared to day 15 of the experiment. The resultant percentage reduction values were measured to be 2.00, 6.50 and 23.50%, respectively. Total cholesterol level of 138±7.62 at day 15 of post cholesterol feeding reduced to 115±5.37, 104±6.27 and 93±5.34 at days 30, 45 and 60 respectively after the corn flour administration. The respective percentage reduction levels were 16.67, 24.63 and 32.61%. The reduction in total cholesterol levels after treatment was statistically similar with standard drug simvastatin at respective sampling days. The levels of triglyceride after corn flour administration were reduced when this level 142±8.56 was changed to 121±8.91, 99±6.54 and 82±7.78 on 30, 45 and 60 days. The percentage reduction levels were 14.79, 30.28 and 42.25% on 30, 45 and 60 days respectively. The levels of percentage reduction at post medication days 45 and 60 induced by corn flour and simvastatin both were significant and statistically similar to each other. After the administration of corn flour, LDL levels at day 15 post cholesterol feeding were 80±8.27, which became 58±8.90, 33±7.54 and 14±6.32 at 30, 45 and 60 days respectively. It provides the respective percentage reduction values as 27.50, 58.75 and 82.50% showing statistically significant reductions at 45th and 60th days. In hyperlipidemic mice, the treatment with Corn flour substantially elevated the HDL levels from 30±8.17 to 41±9.21, 48±8.71 and 54±7.88 at 30, 45 and 60th day post medication. The respective percentage increase observed was 36.67, 60.04 and 80.20% (Tables 2-3). The percentage increase at post treatment days 45 and 60, induced by corn flour was significantly different from the reference value at 15th day and was statistically comparable to the respective increase by simvastatin.

## DISCUSSION

Increased levels of lipids in the blood may result in abnormal lipid metabolism leading to dyslipidemias and development of the atherosclerosis, which eventually lead to multiple heart problems (Onyeike *et al*., 2012; Iqbal *et al*., 2016). Dyslipidemia leads to onset of cardiovascular diseases including CAD, which has been proven and is shown to have a strong correlation with increased cholesterol levels in several studies (Solomou *et al*., 2019). As dietary habits play a key role in leading to dyslipidemias, therefore, physical, physiological and/or dietary inventions can be effective in lowering elevated lipid levels to avoid cardiovascular problems (Salem, 2015; Abo Elnaga *et al*., 2016). Sufficient evidence is available in both animal and human models highlighting that the functional food may be used for the control, management and/or treatment of various human health disorders and metabolic diseases (Kian *et al*., 2019; Sindu *et al*., 2019; Javed *et al*., 2021b). In other way, functional food can be used to decrease the high lipid profile which ultimately has effect on sclerotic components and cardiovascular events (Farooq *et al*., 2019; Oguntibeju, 2019). In this study, we have used cholesterol mixed feed to increase the lipid profile in mice. Different studies have employed the same approach to experimentally induce hyperlipidemia in animal models (Zulet *et al*., 1999) and supported the dosage regimen followed in this study. Dhulasavant *et al*. (2010) used similar approach in rabbits while Iqbal *et al*. (2016) reproduced it in rat model explaining that diet containing high cholesterol can increase the lipid levels in experimental animals.

It is evident from our findings that 1 and 2 gm/kg body weight corn flour did not able to induce lipid lowering effect (Tables 2 and 3). However, 4 and 6 gm/kg corn flour significantly reduce the values indicating lipid profile i.e., total lipids by 176±4.73 and 153±6.91, total cholesterol by 103±9.03 and 93±5.34, triglyceride by 89±5.46 and 82±7.78 and LDL by 23±5.92 and 14±6.32, respectively at 60th day after medication. Moreover, this 4 and 6 gm/kg Corn flour significantly increased the HDL levels by 52±6.92 and 54±7.88 respectively at day 60 post treatment. Therefore, dosage levels i-e, 4 to 6 gm/kg corn flour have been proven more efficacious as compared to lower levels of corn flour offered via diet. These results were also supported by Farooq *et al*. (2019), where olive oil (Omega-3 fatty acids) supplementation in diet of growing goats under harsh environmental conditions lowered ALT, AST, total Cholesterols and triglycerides levels in blood of experimental goats. The findings were further supported by Oguntibeju (2019), where he reviewed data on different traditional medicinal plants and proposed that garlic was proved to be a remarkable remedy for lowering lipid profile, thus making it a good choice to be used as alternative medicine. In this study, simvastatin was used as a standard control drug for comparison with different doses of corn flour and finding their efficacy against high lipid levels in albino mice. Similar studies were carried out by Javed *et al*. (2009) in albino rabbits using cinnamon versus standard drug (simvastatin) and Iqbal *et al*. (2016) using pure cinnamon powder in albino rats to study antihyperlipidemic efficacy of these compounds in laboratory/experimental animals. In the present study, treatment using corn flour (6 gm/kg) or simvastatin has improved/lowered the lipid profile in experimental animals.

**Conclusions:** The outcomes of the present study have proven corn flour as a significant remedy which had reduced the serum lipids including TL, TC, TG and LDL and enhancing HDL levels in treatment groups. Furthermore, 6 gm/kg corn flour was found to induce significant reduction in cholesterol levels. Additionally, these findings provide some biochemical basis for the use of corn flour as a good antihyperlipidemic alternative, which could prevent and cure hyperlipidemia. However, more detailed studies are required to understand the explicit role of corn flour and its possible mechanism of action.

**Authors contribution:** ZI and AA planed, designed and executed the experiments. ZI, AA, RH and MM involved in data collection and statistical investigation. RH and ZI involved in interpretation of results and manuscript preparation.

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