Article

Effect of dietary fatty acid on blood lipid profile and induced bacterial infection in rat

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Abstract: Ground nut is one of the commonly used decorative nutritious relish seed around the world. Ground nut have hypocholesterolamic, hypoglycemic, hypolipidemic, anti-atherosclerotic, immune-modulatory and bacterial counting effects. In Bangladesh, limited research has so far been performed on the action of ground nut in biological system and its comparative efficacy with commercialized drugs that reduce blood cholesterol. The aim of this study was carried on the effects of PUFA on blood total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) and against artificial inoculation (I/N) *Staphylococcus aureus* infection of experimental rat by feeding of ground nut. A total of fifteen long Evans rats (*Rattus norvegicus*) were used for this study. The rats were randomly divided into three equal groups (n=5) and numbered as A, B and C. Group A (control), Group B (50gm ground nut/ day/group), group C (100 gm ground nut/day/group). All groups were supplied with standard broiler pellet and fresh drinking water throughout the experimental period (January to June /2012). The blood samples were collected directly from the heart at the 1st and 60th day for biochemical test (TC, LDL, HDL and TG) and test was performed as per Memorial Diagnostic Centre, Charpara, Mymensingh. *Staphylococcus aureus* was cultured in nutrient broth and 100 µl of their culture was inoculated into the rats through intranasal route. Among all the treated groups, the rats of group C exhibited the lowest TC value, TG and LDL and reduced blood cholesterol significantly than (control group A) followed by group B. In bacteriological examinations, it was found that the number of bacterial colony lowest in group C in comparison with the group of A and B. From the present experiment, it can be assumed that PUFA has significant effect on blood lipid profile and against bacterial infection.

Keywords: ground nut; rat; blood lipid profile; bacteria

1. Introduction

Fat and oil are an important component in nutrition for animal and human being. Dietary fats and oils perform a variety of functions. Poly Unsaturated Fatty Acids (PUFA) has an affect many aspects of immune system including antibody production by B cell and better health of gut associated lymphoid tissue (Calder, 1998). Increasing the dietary supply of PUFA is one of the ways to enhance the magnitude and effectiveness of early immune responses against the challenge of staphylococci infection (Hilgemann, 2003). Hypercholesterolemia is a major risk factor for atherosclerosis that underlies the formation of coronary heart disease (CHD) (Marinetti, 1990; Wresdiyati 2006). The Occurrence of cardiovascular disease (CBD) can be reduced by decreasing the formation of atherosclerosis by lowering cholesterol levels in the blood and increase the concentration of high density lipoprotein (Nogrady, 1999). Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDL-C) is associated with decreased risk of atherosclerosis hypertension, diabetes and CHD (Brown *et al*., 1998; Grundy *et al*., 2004). Ground nut is one of the commonly used decorative nutritious relish seed around the world. Ground nut is known to contain a number of potentially bioactive substances, mainly crucial source of linoleic acid or w₃ fatty acid that is the PUFA that have hypocholesterolamic properties. It also was shown previously that long term dietary feeding of ground nut has hypoglycemic, hypolipidemic, anti-atherosclerotic, immunomodulatory and bacterial counting effects in rats and rabbits. In Bangladesh, limited research has so far been on the action of ground nut in biological system and its comparative efficacy with commercialized drugs that reduce blood cholesterol. PUFA is the product that is able to lower serum cholesterol levels and also provides the role of increasing the immunity for the clearance of bacteria from the body by immunomodulatory action. Initial studies of the mechanism of action of PUFA suggested that it acts mainly by reducing production of LDL-C and increasing the production of high density lipoprotein cholesterol (HDL-C). The presence of cholesterol, saturated fatty acids and trans fatty acids in fats and oils increase risk of coronary heart diseases by increasing the blood cholesterol (Lichtenstein, 1999). Studies have shown that long term diseased conditions decreases the effectiveness of immune system and some experts feel that reduced immunity is a key factor in most illness. With the advancement of civilization animals and humans are using some chemicals in the form of drugs to counter illness derived from commonest bacterial microorganisms and psychological affair. Unfortunately these drugs are losing efficacy and organisms are gaining resistance in much shorten time than it is expected to be. The predominant Staphylococci of the skin is the *Staphylococcus aureus* is often found colonizing in the nasopharynx. The presence of *Staphylococcus aureus* on the skin is common, but these are thought to be contaminants derived from nasal secretions (Free man, 1995). In addition to the skin and nasal cavities, *Staphylococcus aureus* may be found in the eyes, throat and in the intestinal tract. From these sources the organisms find their way into air, dust, and clothing and in other places from which they may contaminate foods (Rahman, 1990). The staphylococci are considered to be opportunistic pathogens. They are involved in disease production where host resistance is compromised or when the particular microbial strain possesses a combination of virulence factors that permit invasion and host damage (Nolan *et al.,* 1996). The study was carried on rats supplementing with ground nut to find out the objectives was to study the effect of PUFA on blood lipid profile and to examine the immuno-modulatory action of PUFA in case of bacterial infection.

2. Materials and Methods

The experiment was conducted in the Department of Physiology and the Department of Microbiology and Hygiene at Bangladesh Agricultural University, Mymensingh. The following procedures were adopted for conducting the experiment.

2.1. Experimental design

A total of fifteen long Evans rats (*Rattus norvegicus*) 4 months of age irrespective of sex with average body weight 250-300g were used. The rats were randomly divided into there equal groups (n=5) and numbered as A, B and C. All groups were supplied with standard broiler pellet and fresh drinking water through out the experimental period. Group A was considered control and fed with normal feed, Group B was supplemented with 50gm ground nut/ day (for group B). And group C was fed with 100 gm ground nut / day (for group C) in addition to normal feed. All three groups were reared under this feeding trial for 3 months after the start of the experiment. This was done by allowing the animal in usual protected cages in laboratory under quiet environment with temperature range of 22-25⁰C. During this trial animals were allowed for natural breeding among themselves.

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2.2. Management practices

The cages were kept on a cleaned and ventilated room. The feed was supplied daily to the rats with available fresh drinking water. Rat cages were cleaned regularly and proper hygienic and sanitary measures were also adopted during the experimental period. The feeds were distributed to the rats at the rate of 50g/group (For Group B) and 100g/group (For Group C) treated diet with normal ration daily for 90 days.

2.3. Collection of blood

On the 1st day and the last days (60th day) of treatment, the 5 ml of blood samples were collected directly from heart. This blood was then taken in the test tube containing anticoagulant (3.8% Na citrate) for bacterial culture. Same amount of blood (5 ml) was taken into other test tubes without anticoagulant for collecting serum.

2.4. Biochemical test

The biochemical parameters of blood total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) were performed as per company instruction (Reflectron® -Humalyzer, Humantype, Germany) at Memorial Diagnostic Centre, Charpara, Mymensingh. Bangladesh.

2.4. Determination of total serum cholesterol

The cholesterol was determined using the procedure described by Trinder (1969); 10 ul ready serum sample was taken in each cuvette (1cm light path) with the help of micropipette. Then 1000 µl reagent was taken to each cuvette and mixed thoroughly by shaking. The cuvettes were incubated at 37^{0}C for 5 minutes. After incubation, each mixture was placed in the Reflectron® Humalyzer (Humantype, Germany) against the blank reagent at 500 mm, Hg 546 mm wave length. Then result was recorded from display. The result was expressed in mg/dl.

2.5. Determination of HDL cholesterol

Two hundred µl serums were mixed with 500 µl diluted precipitant in the test tube. Then the mixture was allowed to sit for 10 minutes at room temperature and then centrifuged for 10 minutes at 4000 rpm. 100 µl clear supernatant was separated within two hours in such way 20 supernatant was from 20 serum sample. 100 µl supernatant was taken in the curette (1cm light path) by micropipette. Then 1000 µl reagent (cholesterol) was mixed with supernatant by shaking. After mixing the mixture was incubated in Reflectron® Humalyzer (Humans type, Germany) for 5 minutes at 37^{0}C. Then the mixture was placed in the Reflection ® against the blank reagent at a wave length of 500 nm. Then the result was recorded in mg/dl which displayed in Reflectron® Humalyzer (Human type, Germany).

2.6. Determination of triglycerides

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quiononeimine formed from hydrogen peroxide, 4-aminophenazone and 4- chlorophenol under the catalytic influence of peroxidase. The triglyceride of blood serum is determined by Reflectron® Humalyzer (Human type Germany) according to the described technique by Trinder (1969). This procedure is similar to total Serum cholesterol. The result was expressed in mg/dl.

2.7. Determination of LDL cholesterol

The LDL was determined by subtracting the triglyceride value that was divided by five and HDL cholesterol value from total serum cholesterol value.
LDL -C = Total serum cholesterol – Triglycerides/ 5-HDL-C

2.8. Inoculation of bacteria

S. aureus were used for this study. Bacteria cultured in nutrient broth at 37°C for 16 hours. 100 µl of their culture was inoculated into the rats through intranasal route. After 24 hours of inoculation rats were sacrificed by cervical dislocation and lung sample was collected after dissection. After collection of the samples, the lung was homogenized by pastle and morter in 10 ml of PBS. Then 50 µl lung homogenate was plated on nutrient agar plates and plates were incubated at 37°C for 24 hours. Then number of colonies was enumerated manually. All the experiments were repeated at least twice.

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2.9. Statistical analysis

All data were expressed as the mean ± SE, and differences among the groups of animals were compared using one-way ANOVA with Post-hoc Turkey's test. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software Version 12 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

This study was performed to investigate the countering role of PUFA on blood biochemistry and bacterial infection in rat.

Table 1. Effects of groundnut on Total cholesterol (TC) (mg/d1) in rats.

Group	Treatment	1st day	60th day
A	Control	102.50 ± 2.50	147 .50 ± 2.50
B	Ground nut (50g nut/day)	115.00 ± 5.00	97.50 ± 2.50
C	Ground nut (100 g nut/day	107.50 ± 2.50	92.50 ± 2.50

Values given above represent the Mean ± Standard Error (SE) of 2 rats
　　*=Significant at p<0.05　　**= Significant at p<0.01

Table 2. Effects of groundnut on high density lipoprotein (HDL) (mg/d1) in rats.

Group	Treatment	1st day	60th day
A	Control	37.00 ± 1.00	41.00 ± 1.00
B	Ground nut (50g nut/day)	41.00 ± 3.00	47.00 ± 2.50
C	Ground nut (100 g nut/day	44.50 ± 3.50	51.00 ± 1.00

Values given above represent the Mean ± Standard Error (SE) of 2 rats
　　*=significant at P<0.05　　　　**=significant at P<0.01

Table 3. Effects of groundnut on low density lipoprotein (HDL) (mg/dl) in rats.

Group	Treatment	1st day	60th day
A	Control	81.50 ± 5.00	88.50 ± 1.50
B	Ground nut (50g nut/day)	79.50 ± 0.50	67.00 ± 1.00
C	Ground nut (100 g nut/day	77.00 ± 1.00	61.00 ± 1.00

Values given above represent the Mean ± Standard Error (SE) of 2 rats
　* =significant at P<0.05　　　　** =significant at P<0.01

Table 4. Effects of groundnut on triglyceride (TG) (mg/dl) in rats.

Group	Treatment	1st day	60th day
A	Control	77.00 ± 1.00	83.00 ± 1.00
B	Ground nut (50g nut/day)	74.50 ± 0.50	75.00 ± 1.00
C	Ground nut (100 g nut/day	71.00 ± 1.00	71.00 ± 1.00

Values given above represent the Mean ± Standard Error (SE) of 2 rats
　* =significant at P<0.05　　　** =significant at P<0.01

Table 5. Effects of groundnut on bacterial colony in rats.

Group	Pre and Post Treatment (CFU/lung)	
A	Control	65000.00 ± 5.00
B	Ground nut (50gm nut/day)	56600.00 ± 1.00
C	Ground nut (100 gm nut/day	55000.00 ± 5.00

Values given above represent the Mean ± Standard Error (SE)
　*=significant at P<0.05　　　** =significant at P<0.01

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3.1. Total cholesterol in blood

The effect of groundnut on total cholesterol in blood varied significantly in the treated groups (Table 1 and Figure 1). The group A (control) showed the highest total blood cholesterol (147 .50 ± 2.50 mg/d1) where it increased significantly (P<0.01) compared to group B and C where it decreased significantly (p<0.05). In group B it was (97.50 ± 2.50 mg/d1). The lowest value was recorded in group C i. e, (92.50 ± 2.50 mg/d1). Where as many researchers demonstrated that saturated fatty acid increase total blood cholesterol and decrease HDL cholesterol in rats (Ascherio *et al.,* 1997; Stender *et al., 2003*). PUFA effectively reduce the cardiovascular events of patients with hypercholesterolemia (Levine *et al*., 1995; Shepherd *et al.,* 1995) and PUFA is also helpful to lower hyperlipidemia including total blood cholesterol in hypercholesterolemic rats of wister strain (Newall *et al*., 1996; Gujaral *et al.*, 1978; Bhandari *et al*., 2005).
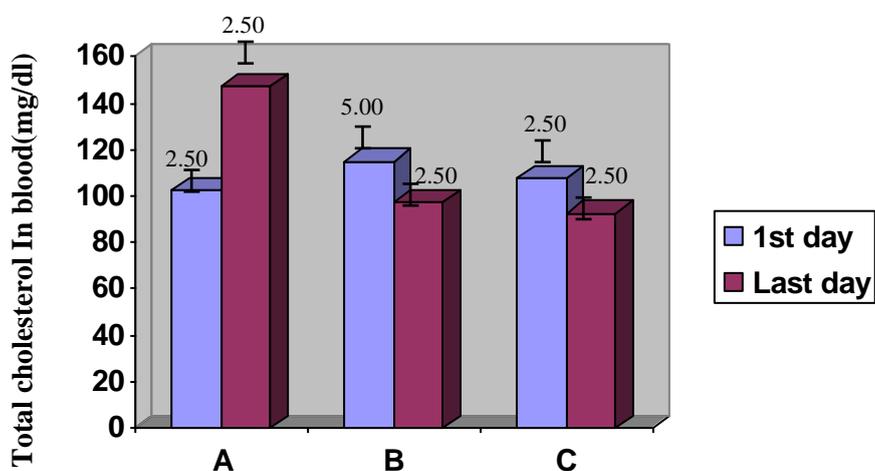
Figure 1. Effects of groundnut on Total cholesterol (TC) (mg/d1) in rats.

3.2. High density lipoprotein (HDL)

The effect of the different treatment groups on blood HDL of rats were varied significantly (Table 2 and Figure 2). The HDL values also showed the significant fluctuation over control. The treatment with ground nut in group A showed a significant decrease (p<o.o5) on blood HDL level (41.00 ± 1.00 mg/d1). The values were increased in group B and C. In group B the value of HDL was found in group C i. e (51.00 ± 1.00 mg/d1) and significantly increase (p<0.01). The result of HDL revealed that the application of groundnut significantly increased the value of HDL in blood of rats where as in case of untreated group a significant decrease on blood HDL level. A significant increased of HDL cholesterol was observed in the rats given 100g of nut/day/group. These results are in agreement with the study of *Gotto and Brinton (2004),* who found that intake of PUFA diet significantly increased the plasma lipid profile including HDL cholesterol and decreased LDL cholesterol as well. La Rosa *et al.* (2004) revealed that the consumption of PUFA raises HDL and reduces LDL a powerful counter of the risk of CHD.
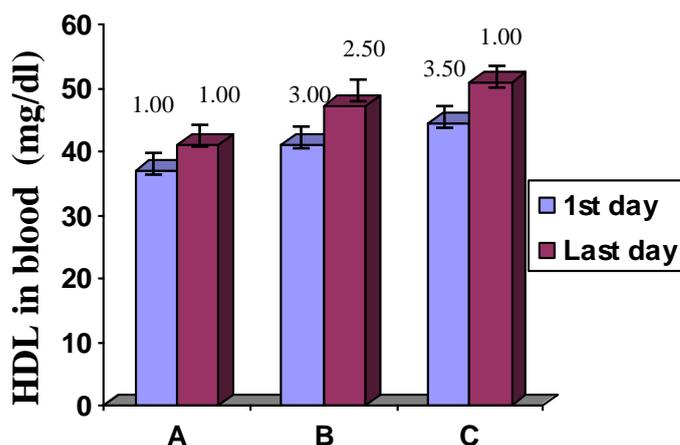
Figure 2. Effects of groundnut on high density lipoprotein (HDL) (mg/d1) in rats.

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3.3. Low density lipoprotein (LDL)

Impacts of different treated group of LDL presented in the Table 3 and Fig 3 was statistically significant at 5% level of probability. Most of the treated group showed fluctuation over control. The highest LDL was found in the group A (88.50 ± 1.50mg/dl) which was significantly increased (P<0.01). The values were decreased significantly (p<0.05) in group Band C. In group B the value is (67.00 ± 1.00 mg/dl). The lowest value was recorded in group C *i. e* (61.00 ± 1.00 mg/d1). A significant augmentation in levels of LDL cholesterol was found in the rats given normal nation. The reduced level of LDL in rats fed with groundnut might occur due to the counter action of PUFA that inhibits the accumulation of fat in the liver which resulted in an reducing number of acetyl co-A in liver cells to produce cholesterol (Guyton 1991). In the present study, the post treatment of rats with PUFA caused reduction in the levels of LDL cholesterol. This result in the consistent with the study of Unger (2003), Who found that dietary feeding of PUFA has hypocholes terolemic effects in rats. Several lines of evidence stated that the hypocholesterolemic effect of ground nut could have possibly resulted from the inhibition of cellular cholesterol biosynthesis after the consumption of the ground nut extract (Fuhrman *et al.,* 2000). Furthermore, the reduction of cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhance removal of LDL from plasma, resulting in reduced plasma cholesterol concentration (Ness *et al.,* 1996).
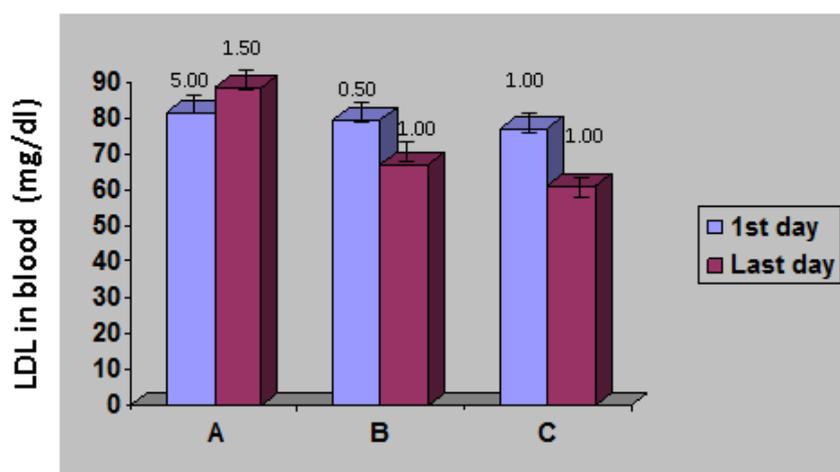
Figure 3. Effects of groundnut on low density lipoprotein (HDL) (mg/dl) in rats.

3.4. Triglyceride (TG) in blood

Different treatments regimen caused significant and non-significant alterations in blood TG level (Table 4 and Fig. 4). Most of the treatment group showed significant difference over control. The group A produced the highest level of TG (83.00 ± 1.00 mg/dl) and significantly increased (P<0.01). The values were reduced significantly (p<0.05) on group B and C. In group B the value was (75.00 ± 1.00 mg/d1). The lowest value was found in group c i. e (71.00 ± 1.00 mg/d1). Such findings are in agreement with the study of Rinaldo *et al.,* (2002) who reported that PUFA reduces blood cholesterol and triglyceride. The result revealed that, the treatment of PUFA decreased the TG in blood of rats significantly. Haemmerle *et al.* (2003) demonstrated that PUFA, show one of the most potent action against total cholesterol, LDL and triglyceride.

3.5. Confirmation of *S. aureus*

Next I tested whether the organism in the lung homogenate was *S. aureus* or not. The colonies those were grown from lung homogenate culture in nutrient agar media were subjected for Gram's staining. In Gram's staining the organisms were found spherical, cluster forming and violet colour indicating the characteristics of *S. aureus*.

3.6. Bacteriological study

To assess the ability of 50 gm and 100 gm groundnut feeding raised immunity to eliminate *S. aureus* infection, the number of viable bacteria in lung homogenate was examined after intranasal challenge with *S. aureus*. Feeding of 50 gm and 100 gm ground nut resulted in a significantly reduced number of bacteria in the lung compared with control feeding rats (Figure 5).

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Feeding of 50gm ground nut per day in rats raised moderate level of immunity resulting in the decreased number of bacteria in the lung when compared with control group ($P<0.05$, Figure 5). Feeding of 100g nut per day raised sufficient level of immunity resulting significantly reduced number of bacteria in the lung when compared with control. Similar findings were also observed by Osterud *et al.,* (2003) and Spriet *et al,.* (2003). they stated that PUFA has the immunomodulatory effect against bacterial infection and also showed that decreased bacterial load in PUFA feeding animals.
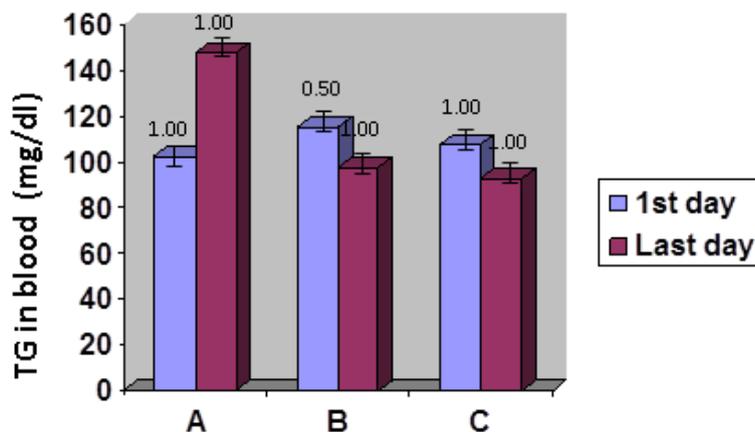
Figure 4. Effects of groundnut on triglyceride (TG) (mg/dl) in rats.
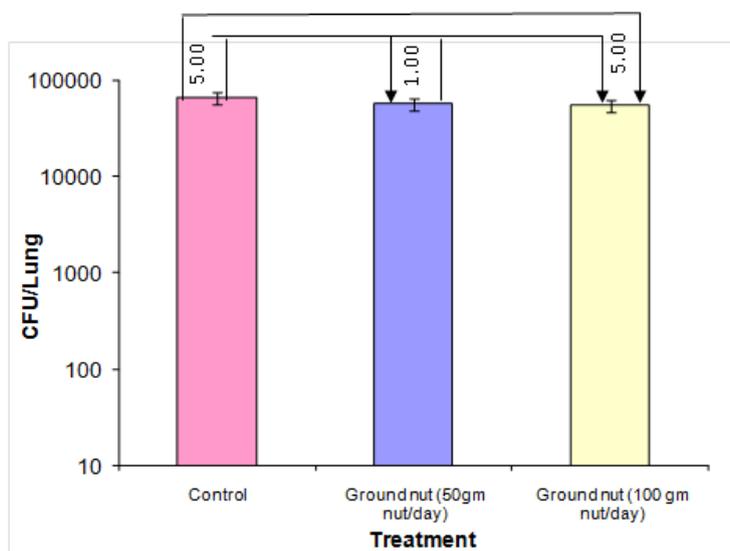
Figure 5. Effects of groundnut on bacterial clearance in rats. Rats were given 50gm or 100gm ground nut or normal feed per day for a period of 90 days. *S. aureus* **was given intranasally both in control and treatment groups as described in materials and methods. After 24 hours of inoculation lungs were collected, homogenized and cultured for CFU of bacteria. The figure shows the Mean ± SE (n=3 rats/group) * *P*<0.05 by ANOVA test.**

4. Conclusions

The study was performed to investigate the role of ground nut as source of PUFA on blood lipid profile and bacterial infection in rat under different conditions for a period of 60 days. A total of 15, 4 months of age Long Evans rats (250-300 gm) were randomly assigned into three treatment groups, namely: Group A, normal control; Group B, treated with 50 gm ground nut/group; Group C, treated with 100 gm ground nut/group in addition to normal ration. Among all the treated groups, the rats of group C exhibited the lowest TC value, TG and LDL and reduced blood cholesterol significantly than (control group A) followed by group B. In bacteriological examinations, it was found that the number of bacterial colony lowest in group C in comparison

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with the group of A and B. From the present experiment, it can be assumed that PUFA has significant effect on blood lipid profile and against bacterial infection.

Conflict of interest
None to declare.

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