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Assessment and Biomonitoring of the Effect of Rapeseeds Oil on Wister Rat Organs

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ABSTRACT

Rapeseed oil is one of the important and cheapest vegetables oil in Bangladesh and many other countries. It is commonly used as cooking oil in Bangladesh particularly in rural areas and also used as other food items. This study evaluated the physiological effects of four type's rapeseeds namely Mustard (Wild), Mustard (hybrid), Rai (Wild), Rai (hybrid) oil on six strains of bacteria in addition to studying the effects of two varieties of rapeseeds oil Mustard (Wild) and Rai (hybrid) oil on Wistar rat's organs including liver, kidney, heart and skeletal muscles. Firstly, we examined the effects of these Rapeseeds oil on bacteria and found that these Rapeseeds oil possess antibacterial activities. Six bacterial strains such as *B. subtilis*, *S. lutea*, *X. campestris*, *E. coli*, *K. Pneumonia*, *P. denitrificans* was used to test the effect of these rapeseeds oil and observed that rapeseed oil showed inhibition against tested microorganisms in concentration-dependent manner. Finally, the effects of oil obtained from these two varieties were investigated after feeding rats for 8 weeks. Male Wistar rats were divided into three groups, each group contains 4 rats. Group A was considered as control diet group, while Group B was mustard (Wild) oil group and Group C was Rai (hybrid) oil group. Group A was containing 0.6 gram oil plus 14.4gm diet. We found that rats of both experimental groups exhibit weight loss, reduction of food efficiency ratio and increase cardiac and hepatic enzymes including CK-MB, ALP, SGPT and SGOT compared to the rats fed controlled diet. Furthermore, we also found that the bodyweight loss, food efficiency ratio markedly decreased and tested enzymes increased in rats fed Mustard (Wild) oil compared to rat fed Rai (hybrid) oil.

Keyword: Rapeseed, Erucic acid, Food efficiency ratio, Vegetable oil, *Brassica spp*, and Wister rat organs.

INTRODUCTION

Rapeseed is commonly known as mustered, *Brassica spp* in Bangladesh, is an annual, winter season crop a native to temperature regions of Europe (Bhuiyan *et al.,* 2008). Rape is a Latin word means turnip and rapeseed belong to the family *Cruciferae* is an important oil crop and currently it is the principal oil crop of Bangladesh. It contains 40-45% oil and 20-25% protein in seed (Mosyakin, 1996; Sarker *et al.*, 2019). Beneficial health effects also arise from the content of phenolic compounds, which are known to exert antimutagenic, anti-inflammatory activities (Cao *et al*., 2008; Vuorela *et al*., 2005). The antioxidant potential of phenolic compounds from rapeseed oil has been reported in the literature (Thiyam *et al*., 2006; Wakamatsu *et al*., 2005). This oil is a major source of vegetable oil in the world and contains many active biological compounds such as phyto-sterols, tocopherol, phenolics, β-carotene (Siger, 2017).

In Bangladesh, rapeseed and mustard are major sources of edible oils and are mostly grown on the residual soil moisture in Rabi season. Many different

parts of plants may yield oil in actual commercial practice oil is extracted primarily from the seeds of oilseed plants. Vegetable fats and oils are not only edible but also non-edible such as processed linseed oil and castor oil used in lubricants, paints, cosmetics, pharmaceuticals, and other industrial purposes. This fats and oils are used to describe concentrated energy sources found in many foods. Without fats and oils, our bodies would not function properly. The oil Rapeseed is one of the most valuable vegetable fats, due to its nutritional properties. It is a rich source of mono- and polyunsaturated fatty acids, while saturated fatty acids are minor constituents (Gunstone, 2011). The rapeseed oil extracted from the rapeseed top-quality edible oil, which contains rich acids, fast-soluble vitamins, phospholipids, and pigments but lacks cholesterol (Martin Hernandez, 2008). It also contains high-quality oil (38-46%) and protein (20-30%) as well as some antinutritional compounds, such as glucosinolates, phenols, and phytic acid (Zhang, 2007). Rapeseed processing in the food and chemical industry results in some by-products which can be used as animal feeds or as a protein component of the diet (Lomascolo *et al*., 2012).

Vegetables fats and oils are derived from plants that are composed of mainly triglycerides. Rapeseed oil contains high levels of erucic acid that is damaging to the cardiac muscle of animals, and made it less nutritious in animal feed (O'brien, 2008). It helps to stay away from coronary heart disease and provide energy and transport fat-soluble vitamins A, D, E, and K. It also provides the essential fatty acids omega 3, 6 and 9 involved in many important functions in the body, including heart health. Help keep us warm and protect internal organs like the kidneys contain plant sterols (McKevith, 2005). However, the particular role of the component of these rapeseeds oil remains yet to be studied (O'brien, 2008). Therefore, the present study was undertaken with the aims and objectives is to determine the antimicrobial activities of these rapeseeds oil against some test pathogens to determine the body weight gain (growth rate) of rats fed with both oil supplements, to measure the amount of food consumed by rats, to calculate Food Efficiency Ratio (FER) of rats fed with rapeseeds oil, and to investigate the adverse condition of rats heart and liver by measuring the biomarker SGOT (AST), SGPT (ALT), CK-MB and ALP.

MATERIALS AND METHODS

Collection of Seeds - At first fresh, healthy and good quality Mustard (hybrid) seeds collected from BADC Amjhupi, Maherpur and Rai (hybrid) seeds collected from the farmers of "Shehala" Village, Daulatpur, Kushtia.

Then the seeds were stored at 4ºC in the refrigerator with the sealed plastic packet to avoid the microbial contamination.

Preparation and Storage of Oil - To preparation of the experimental oil, stored seeds were taken out from the refrigerator to the ghanni mil at Sheikhpara, Jhenidah. The bottle was also stored at -28ºC to prevent photo-oxidation. The bottle was preserved in a dark, cool and dry place.

Media

Nutrient Broth Media - The Nutrient broth is typically made of a powder beef or yeast extract that contains peptones (broken down proteins). The powder is dissolved in water, put in the conical flask and sterilized and incubated for 24 hours or more to encourage bacterial growth.

Nutrient Agar Media - Nutrient agar medium used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms.

Sample Used - Sample used for this purpose are four types of rapeseed oil. Oil samples are Mustard (wild) oil, Mustard (hybrid) oil, Rai (wild) oil, Rai (hybrid) oil. Oil directly extracted from seeds from an oil mill in a local market. Seeds are collected from farmers and local market in Kushtia, a general town in Bangladesh.

Preparation of Pure Culture and Sample Inoculation - To determine anti-microbial activity of oils, we had to prepare media for bacterial growth. Our experiment is divided into two parts. The first part includes the preparation of pure culture of each bacterial strain and the second part includes the spread of microbes in an agar plate and inoculate sample.

Disk Preparation - Make disks to soak sample oil by filter paper in the desired size. Take all disks in a bicker. Seal the bicker with aluminum foil paper to avoid contamination. Then sterilize the disk in the autoclave at 121 ºC for 30 min and open the bicker in front of laminar airflow.

Animal Care and Treatment

Experimental Animals (Wistar rat) - A rat of the species *Rattus norvegicus* which is bred and kept for scientific research is being used in the laboratory.

Animal Maintenance - Adult healthy male Wistar rats were purchased from animal house, Dept. of Pharmacy, Jahangirnagar University, Savar, Dhaka. With an average weight of about 80-100gm (4 weeks of rat). The rats were housed in a wooden cage with steel wire tops and wood-cob bedding (4 rats per cage) and had access to food and water at the BTGE laboratory.

Diet and Dosing of Oil - At first, the rats were adapted for five days. They were fed standard diet. The standard diet was purchased from animal house, Jahangirnagar University, Dhaka, Bangladesh. The a standard diet consisted of 7% protein source, 20% rice polish, 30% wheat bran and flour, 1.5% soybean oil and common salt, 0.5% vitamin mixture and 2.5% molasses. Each rat was fed about 15gm of diet/day. The appropriate doses of rapeseeds oil and diet given to the rats were carefully weighed using the electrical balance. Feeding was continued for 8 weeks. Amount of food consumed and the individual bodyweight of the rats of group A, B and C ware 89.33, 83.25 and 86.75g respectively.

Calculation of Food Efficiency Ratio (FER) - During the study, the weight of each rat was measured every day using the balance. The food efficiency ratio for each rat was calculated using the following formula: FER=Body weight gain/Food consume.

Sample Collection and Preservation - After 56 days of feeding, the 14 hours fasted rats were subjected to light anesthesia using chloroform. At first, the abdomen was washed using ethanol to avoid contamination. With the use of scissors and wearing gloves the abdomen was opened, blood samples were collected with 3 ml syringe from abdominal aorta or heart. Then the blood sample was transferred into a 1.5ml Eppendrof tube. For coagulation, blood was kept about 10 minutes at room temperature. After centrifugation at 3000 r.p.m for 15 minutes at 4 ^{o}C using Thermo scientific centrifuge, then the serum was carefully drawn off and placed in a 1.5 ml Eppendrof tube and preserved at -80 ^{o}C until the experiments were performed. Various organs namely heart, liver, kidney, tail, adipose tissue and skeletal muscle were collected, put onto Petridis and washed in 0.9% saline and then preserved. Heart sample was collected and preserved in Bouins Fluid. The liver, kidney, tail, adipose tissue and skeletal muscle were preserved in formaldehyde for further investigation. Bouins fluid contains 71.43% saturated picric acid, 4.76% glacial acetic acid, and 23.81% formaldehyde, which is known as a strong preservative.

Biochemical Analysis of Serum

Determination of Serum Glutamate Oxalo-acetate Transaminase SGOT (AST) and Serum Glutamate Pyruvate Transaminase (SGPT) - SGOT and SGPT also have known as aspartate amino-transferase (AST) and alanine amino transferase (ALT), respectively. For the analysis of blood serum SGOT (AST) and SGPT (ALT) is used as a biomarker. Commercially available kits were used for the determination of SGOT and SGPT level in blood serum according to the Optimized UV-Test (Walter et al 1991). For the determination of SGOT and SGPT enzyme reagent (R1) and substrate liquid buffer (R2) were supplied ready-to-use. Prepared working reagent in the ratio of 4 parts enzyme reagent (R1) to 1 part substrate (R2).

At first, we were taken zero spectrophotometers at 340 nm with distilled water. For each sample and control, 200µl serum sample added with 1000µl mono reagent (R1+R2). Gently mixed and absorbance was recorded after 1 minute at 25^{o} C. Incubation continued at 25^{o} C and absorbance were recorded again at 2 and 3 minutes. Determined the average absorbance per minute (ΔA/min) and multiplied by the factor 952 for results in U/L.

Determination of Alkaline Phosphatase (ALP)- Kits were used in the determination of alkaline phosphatase according to the Optimized Standard Method (Weber et al., 1998). For the determination of ALP, alkaline phosphatase buffer and substrate liquid buffer were ready to use. Prepare working reagent in the ratio of 4 parts Buffer (R1) to 1 part Enzyme (R2) (i.e. 24 ml Buffer and 6 ml Substrate). At first, we were taken zero spectrophotometers at 405 nm with distilled water. For each sample and control, 20µl serum sample added with 1000µl mono reagent (R1+R2). Gently mixed and absorbance was recorded after1minute at 37^{o} C.

Incubation continued at 37^{o} C and absorbance's were recorded again at 2 and 3 minutes. Then determined the average absorbance per minute (ΔA/min) and multiplied by the factor 2757 for results in U/L.

Determination of Creatine Kinase (CK-MB) - For the quantitative determination of Creatine Kinase-MB in serum manual procedure was used. The procedures given by the manufacturers were followed without any modifications for the determination of these parameters. For the quantitative determination of Creatine Kinase MB (CK-MB) liquid buffer (R1) and enzyme (R2) liquid reagents are supplied ready-to-use. Prepare working reagent in the ratio of 4 parts Buffer (R1) to 1 part Enzyme (R2). At first, we were taken zero spectrophotometers at 340 nm with distilled water. For each sample and control, 1.0 ml working reagent added to cuvet and warmed to 37º for 4 minutes. Added 40 µl of serum to its respective tube and mixed gently. Incubation continued at 37º C and absorbance were recorded again at 6, 7, 8 and 9 minutes. Then determined the average absorbance per minute (ΔA/min) and multiplied by the factor 8360 for results in U/L.

RESULTS

Effects of Four Varieties of Oil on Micro-organisms.

Effects of Mustard (wild) Oil on *Xanthomonas campestris* - The experiment show that Mustard (Wild) oil plays a significantly inhibitory effect on *X. campestris* at the concentration of 1.5µl/disk compared to control plate.

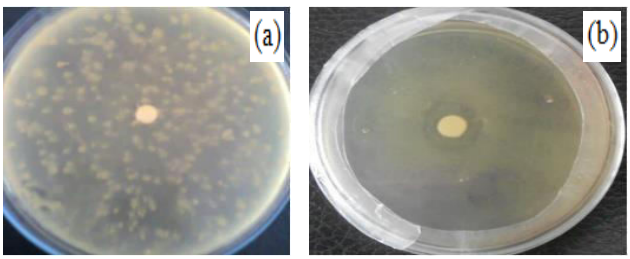


Fig. 1: (a) Control plate of strain 004 *(X. campestris*); (b) Highest inhibition of microbial growth at 1.5 µl.

Effects of Mustard (Hybrid) Oil on *Klebsiella pneumonia* - The experiment also shows that Mustard (hybrid) oil significantly inhibits *K. pneumonia* at the concentration of 1.5µl/disk compared to the control plate

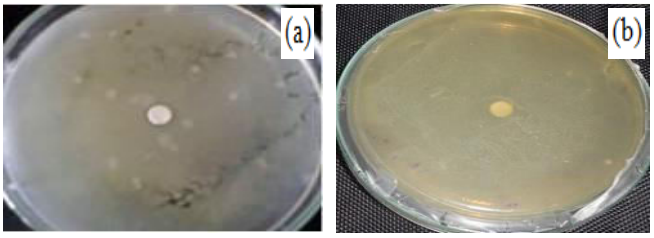


Fig. 2: (a) Control plate of *K. pneumonia*; (b) Highest inhibition of microbial growth at 1.5 µl.

Effects of Rai (Wild) Oil on *Sarcina lutea* - The experiment also show that Rai (wild) oil significantly inhibits *S. lutea* at the concetration of 1.5µl /disk compared to the control plate.

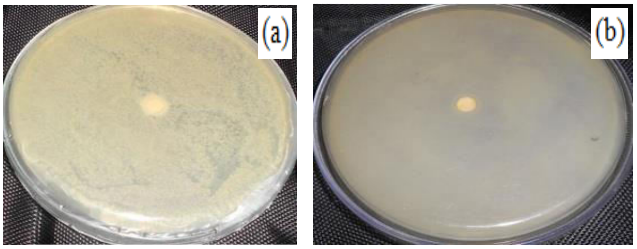


Fig. 3: (a) Control plate of strain 002 (*S. lutea*); (b) Highest inhibition of microbial growth at 1.5 µl.

Effects of Rai (Hybrid) Oil on *Pseudomonas denitrificans* - The experiment also show that Rai (hybrid) oil significantly inhibits *P. denitrificans* at the concentration of 2µl/disk compared to control plate.

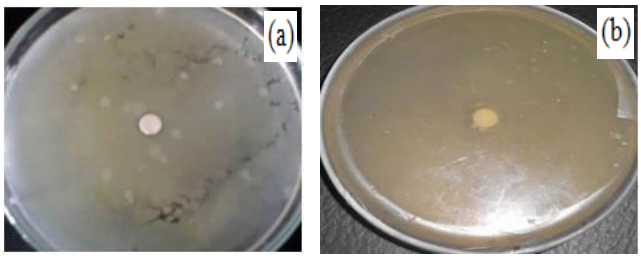


Fig. 4: (a) Control plate of strain 010 (*P. denitrificans*), and (b) Highest inhibition of microbial growth at 2 µl.

Table 1: Effects of four types of rapeseeds oil on microorganisms.

Serial No	Bacterial strains	Result of control (Disks without oil)	Amount of oil- 0.5µl Shows	Amount of oil- 1.0µl Shows	Amount of oil- 1.5µl Shows	Amount of oil- 2.0µl Shows
1.	001(*Bacillus subtilis)*	Positive growth	moderate inhibition	more inhibition than amount of 0.5µl.	greater inhibition than amount of 1.0µl	greatest inhibition among the 4 types of oil concentration

2.	002 (*Sarcina lutea)*	Positive growth	less inhibition	mild inhibition	high inhibition	high inhibition.
3.	004 (*Xanthomonas campestris)*	Positive growth	mild inhibition	moderate inhibition	high inhibition.	high inhibition.
4.	005 (*E. coli* iw 3310)	Positive growth	less inhibition	mild inhibition	high inhibition	high inhibition
5.	006 (*Klebsiella pneumonia)*	Positive growth	mild inhibition	mild inhibition but greater than amount of 0.5µl.	high inhibition	high inhibition
6.	010 (*Pseudomonas denitrificans)*	Positive growth	mild inhibition	high inhibition	high inhibition	greater inhibition

Table 2: Mustard oil (hybrid type seeds).

Serial No	Bacterial strains	Result of control (Disks without oil)	Amount of oil- 0.5µl Shows	Amount of oil- 1.0µl Shows	Amount of oil- 1.5µl Shows	Amount of oil- 2.0µl Shows
1.	001 (*Bacillus subtilis)*	Positive growth	less inhibition	mild inhibition	high inhibition	high inhibition
2.	002 (*Sarcina lutea)*	Positive growth	less inhibition	mild inhibition	moderate inhibition	moderate inhibition
3.	004 (*Xanthomonas campestris)*	Positive growth	mild inhibition	moderate inhibition but more than the amount of 0.5µl.	high inhibition	high inhibition
4.	005 (*E. coli* iw 3310)	Positive growth	moderate inhibition	more inhibition but more than of 0.5µl.	high inhibition	high inhibition
5.	006 (*Klebsiella pneumonia)*	Positive growth	moderate inhibition	more inhibition than the amount of 0.5µl.	higher inhibition	higher inhibition among the 4 concentrations of oil
6.	010 (*Pseudomonas denitrificans)*	Positive growth	less inhibition	mild inhibition	high inhibition	high inhibition

Table 3: Rai Oil (hybrid type seeds).

Serial No	Bacterial strains	Result of control (Disks without oil)	Amount of oil- 0.5µl Shows	Amount of oil- 1.0µl Shows	Amount of oil- 1.5µl Shows	Amount of oil- 2.0µl Shows
1.	001 (*Bacillus subtilis)*	Positive growth	moderate inhibition	high inhibition	high inhibition	high inhibition
2.	002 (*Sarcina lutea)*	Positive growth	mild inhibition	mild inhibition	moderate inhibition	high inhibition
3.	004 (*Xanthomonas campestris)*	Positive growth	less inhibition	mild inhibition	mild inhibition	mild inhibition
4.	005 (*E. coli* iw 3310)	Positive growth	moderate inhibition	high inhibition	high inhibition	high inhibition
5.	006 (*Klebsiella pneumonia)*	Positive growth	mild inhibition	moderate inhibition	high inhibition	high inhibition
6.	010 (*Pseudomonas denitrificans)*	Positive growth	less inhibition	mild inhibition	moderate inhibition	high inhibition

Effects of Two Varieties of Oil on Wistar Rats Organs

Table 4: Rai Acid value, Iodine value, Saponification value and Unsaponifiable Matter of mustard (wild) and Rai (hybrid) rapeseeds oil.

Name of oil	Acid value	Iodine value	Saponification value	Unsaponification value
Mustard wild	1.33±.068	111.055±1.82	178.11±2.42	1.395±.037
Rai hybr	0.84±.080	85.67±5.49	169.7±.81	1.00±.12

Determination of Acid Value of Rap Seeds Oil

- In this data mustard wild and Rai hybrid shows acid value 1.33, 0.84. Here the acid value of mustard wild is greater compare to Rai hybrid. The standard error of mustard wild and Rai hybrid includes the value 0.68,0.080 respectively that have been shown in the column.

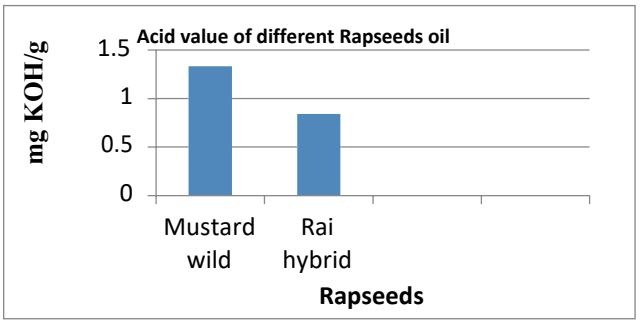


Fig. 5: Graphical Representation of Acid value of rapeseed oil.

Determination of Saponification Value of Rapeseeds Oil - In this data mustard wild and rai hybrid shows acid value 178.11, 169.7. Here the acid value of mustard wild is greater compare to rai hybrid. The standard error of mustard wild and rai hybrid includes the value 2.42, 0.81 respectively that have been shown in the column.

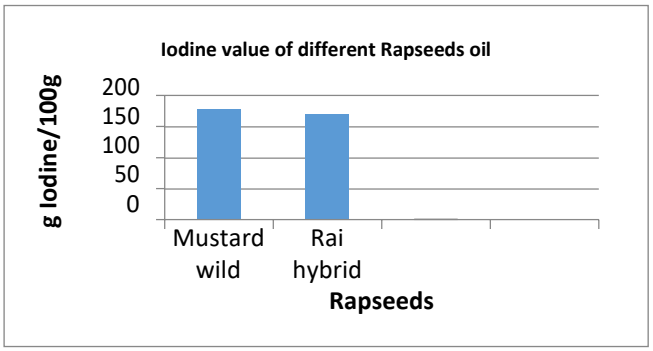


Fig. 6: Graphical representation of Iodine value of rapeseed oil.

Body Weight Gain - The average weight body gain of the rats was measured for 6 weeks. The **Table 5** illustrates the mean values of bodyweight gain of the rats fed diet with Mustard wild; Rai hybrid illustrates the graphical presentation of body weight gain of rats for the eight weeks. One group was considered as control rat which fed only normal diet. And another seven groups of rat induced the normal diet with 0.6 gm Mustard wild, a normal diet with 0.6 gm Rai hybrid. The average body weight gain of rats was calculated into weekly and plotted on the graph weight gain vs. week.

Table 5: Body weight of different groups.

Duration (week)	Group A (Control diet)	Group B Mustard (wild)	Group Rai (hybrid)
1week	10.33±1.83	8±0.55	8.25±1.75
2week	31.44±0.92	24.05±.81	24.54±0.87
3week	47.33±1.09	37.95±1.84	36.45±1.98
4week	59.81±.68	45.2±0.44	46.75±1.26
5week	67.67±2.00	53.1±0.76	59.35±1.28
6week	78.00±0.29	61.25±1.65	64.53±0.76
7week	86.55±.43	70.42±0.23	81±1.68
8week	94.22±0.17	77.33±0.87	91.33±1.11

It was observed that the average weight of the rats in group A linearly increased. From 1st to last weeks the point of Group A is showed weight gain linearly. At the last week (8th week) group A showed the highest weight gain than the other two groups. Group B showed weight gain linearly from the 1st to 3rd week except the 4th and 5th week. In this point slightly prohibited the continuous weight gain of rats. Group B leading to the lowest weight gain compared to group A and C. Group C showed weight gain linearly from the 1st to 4th week except for the 5th and 6th week. In this point slightly prohibited the continuous weight gain of rats.

Table 6: Food efficiency ratio.

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Food Intake	Food efficiency ratio (FER)
Group A	89.33±3.79	183.55±3.55	94.22±0.14	1045.48±2.79	0.09±0.05
Group B	77.5±2.13	165.25±2.32	80.25±0.19	1094.5±3.24	0.07±.05
Group C	86.75±4.495	178.083±4.61	91.33±.12	1064.89±2.61	0.08±.04

Rats fed for 8 weeks and Food Efficiency Ratio (FER) was measured. Group A fed normal diet, Group B for diet combined with Mustard (wild) oil and Group C for a diet with Rai (hybrid) oil. The table indicates that when rats were fed normal diet combine with Mustard (wild) and Rai (hybrid) oil, there is highly decrease of FER as compare to control Group A. The FER of Group B Mustard (hybrid) oil that is exhibited lower than Rai (hybrid) group.

Estimation of ALP in Rat Serum - ALP levels of group A includes the value 125.2 U/L. whereas group B and group C include the value 201.96 and156.23 respectively compare with control group B and group C had increased ALP level. ALP level of group B value is the highest point compares to group C.

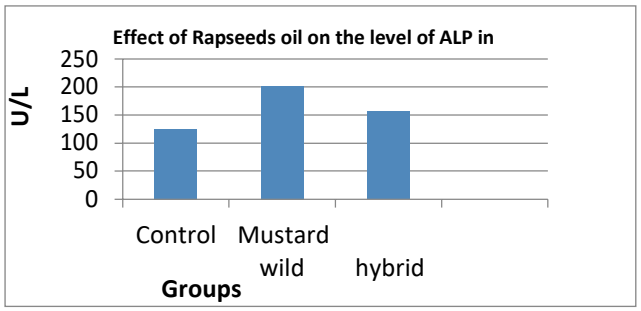


Fig. 7: Graphical representation of rapeseed oil on the level of ALP activity in rats.

Estimation of CK-MB Group A - Group A rat of CK-MB include the value 33.72 and 32.24 respectively compare with control group B and group C had increased CK-MB level. CK-MB level of group, B value is the highest point compare to group C.includes the value 31.36 U/L. whereas group B and group C

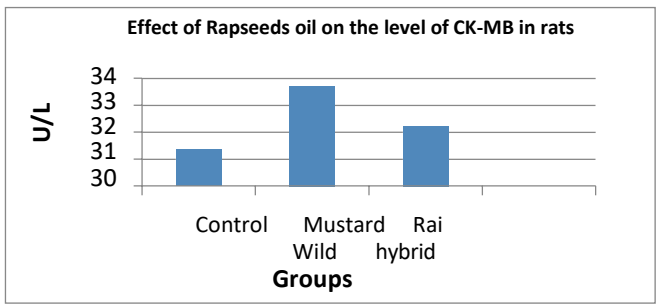


Fig. 8: Graphical representation of rapeseed oil on the level of CK-MB activity in rats.

Estimation of SGPT (ALT) in rat serum group A - Group A rat of SGPT (ALT) includes the value 35.06 U/L. whereas group B and group C include the value 42.345 and 39.93 respectively compare with control group B and group C had increased SGPT(ALT) level. SGPT (ALT) level of group B value is the highest point compare to group C.

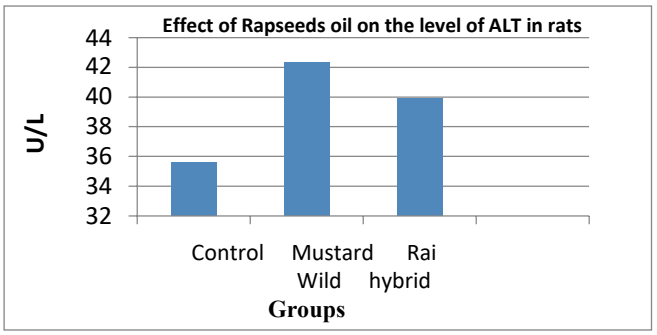


Fig. 9: Graphical representation of rapeseed oil on the level ALT of activity in rats.

Estimation of SGOT (AST) in Rat Group A - Group A rat of SGOT (AST) includes the value 35.06 U/L. whereas group B and group C include the value 42.345 and 39.93 respectively compare with control group B and group C had increased SGOT (AST) level. SGOT (AST) level of group B value is the highest point compare to group C.

DISCUSSION

Rapeseed oil is grown as an edible oilseed crop in Bangladesh, India and Pakistan. Rapeseed oil has a pungent smell and a sharp taste. The edible rapeseed oil contains the lowest amount of saturated fatty acids as compared to other vegetable oils. It contains nutritionally desired oleic acid, which gives stability to the oil, along with two essential fatty acids, linoleic and linolenic. However, in addition, it is characterized by high levels of monounsaturated fatty acids (62%) and significant levels of polyunsaturated fatty acids 32%, and it contains very little saturated acids 6% (Alander et al., 2007). There are basically four types of rapeseed oil namely Mustard (Wild), Mustard (hybrid), Rai (Wild) and Rai (hybrid) were used to finding out the activity against microorganisms.

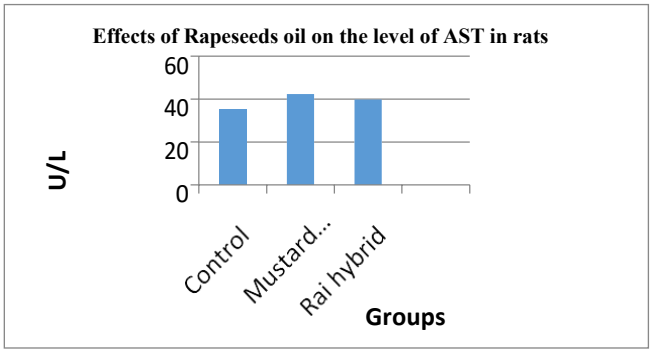


Fig. 10: Graphical representation of rapeseed oil on the level of AST activity in rats.

We observed that all types of tested oil possessed toxic effects against bacteria which are shown in **Fig. 1-4** and **Table 1-6** This result might be due to rapeseeds oil contains erucic acid, a 22 carbon long-chain monounsaturated fatty acid, which is harmful to health as well as living organisms. It also contains high amounts of erucic acid, which is nutritionally undesired. There is a report that erucic acid-rich oil may cause cardiac lipidosis by the accumulation of substantial fat in the heart muscle (Kramer *et al*., 1992). After examination of antimicrobial activities of oil, adult male Wistar rats have been treated with the control diet, Mustard (wild) and Rai (hybrid) oils at 8 weeks (56 days). Then the physiological effects of oil were tested.

In this study, we observed that the average weight of the rats in the control group increased linearly from the first week to last week at each time point. Mustard (hybrid) oil group showed weight gain linearly from 1st to 4th week, 7th week and 8th week but except the point 5th and 6th weeks. It reflects that the reduction rate of body weight of rats is highest in mustard Wild oil-treated group compared to control and rai hybrid groups. Mustard (Wild) oil group has growth retarding activity compared to both the control group and Rai (hybrid) group caused by erucic acid. Several mechanisms may be responsible for diminishing weight by mustard (Wild) oil. It has been shown that high body weight gain for rats fed diet containing low erucic acid rape oil than the high (Badawy, 1994, Renner, 1979). Because of these injuries rats tend to consume a lower amount of diet, leading to ultimate weight loss or it may also happen that though rats ate diet, but due to these metabolic disorders, they could not able to digest it. So it could be decided that reason for lower weight gain is the development of metabolic disorders in Mustard oil (erucic acid) induced rats. Rai (hybrid) group C showed weight gain linearly without 6th week. This may be due to fewer intakes of foods because of the physiological condition of rats. Rats fed for 8 weeks and FER was measured and results summarized in **Table 6**.

We observed that food intake was lowest in the control group but FER is highest in controlled animals, whereas rats fed normal diet combined with Mustard (Wild) and Rai (hybrid) decrease FER as compared to the control group. This may be due to the fact that they are unable to digest it due to metabolic disturbance caused by erucic acid which is toxic and lower the FER by creating metabolic problem in rats. Our results indicate that control diet group improved the digestion power of rats. Alkaline phosphatase is an enzyme found throughout the body. It is a hydrolyzing enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins, and alkaloids. Diseases that damage and destroy the cells of organs lead to the release of alkaline phosphatase into the blood, which raises the blood level of alkaline phosphatase.

Large scale infections can also increase the enzyme. Thereby, we checked the level of ALP in rat serum of experimental groups. Compared with control group (A), Mustard (Wild) Group B and Rai (hybrid) Group C had increased ALP levels shown in **Fig. 4**. Usage of a higher dosage of erucic acid (38.2%) in Mustard oil group is responsible for inflammation. It is also found elevated ALP level in erucic acid-induced rats like present study (Badawy, 1994). ALP levels of group B Mustard (Wild) value is higher ALP compared to the control and Rai (hybrid) oils. CK-MB is a form of the enzyme that is found mainly in the heart. It helps cells to perform their normal functions but it rises in blood level when specifically heart cells are injured. A high CK-MB level suggests that there is disease or damage to the heart muscle specifically (Panteghini, 1998).

The CK-MB level in rat serum was estimated after 8 weeks. On compared with the control group (A), Mustard (Wild) Group B and Rai (hybrid) Group C had increased CK-MB levels shown in **Fig. 5**. The increase in CK-MB level of Mustard (hybrid) oil group may indicate damage to heart muscle specifically. It is reported that erucic acid is poorly oxidized by the mitochondrial beta-oxidation system, especially by the myocardial cells, which results in an accumulation of erucic acid, producing myocardial lipidosis which has been reported to reduce the contractile force of the heart (Mori, 1998). Control group has the lowest CK-MB level compare to other rapeseed oil groups. ALT is also known by the name of SGPT. It is normally con-centrated in the liver.

The moment there is an injury in the liver, it is released into the bloodstream. Therefore, the ALT level in rat serum was examined in rats. On compared with control group (A), Mustard (Wild) Group B and Rai (hybrid) Group C had increased ALT levels shown in **Fig. 6**.

The ALT levels of group B mustard (Wild) value are the highest compared to Rai (hybrid) oil. The elevation of ALT indicates injury of liver function in rats fed Mustard oil. The enzyme AST is also known by the name of SGOT is a more specific indicator that primarily aids to the diagnosis of liver disease but also aid to the diagnosis of heart injury. So, we checked the AST level in rat serum. On compared with the control group (A), Mustard (Wild) Group B and Rai (hybrid) Group C had increased AST levels shown in **Fig. 7** AST levels of group B mustard (Wild) value is a higher compared to Rai (hybrid) oils.

This elevation of AST might be due to higher dose of erucic acid in Mustard wild groups because erucic acid is responsible for inflammation. It might due to its high erucic acid content.

CONCLUSION

Rapeseed is an important oil crop in Bangladesh which can contribute to a large extent in the national economy. The people of Bangladesh and many other countries consume this edible oil. Especially in the rural area of our country use more this edible oil as a chief source of nutritionally required fatty acids in the human diet. Rapeseed oil is one of the oldest vegetable oils that contain erucic acid which is harmful to health. Our study investigated the antimicrobial activity of rapeseeds oil in addition to the physiological effects of two varieties of oil namely Mustard (wild) and Rai (hybrid) on Wistar rat organs. Results showed that erucic acid of Mustard (wild) and Rai (hybrid) causes the weight loss of rat to compare to control diet groups of rats. In addition rapeseeds, oil of Mustard (wild) and Rai (hybrid) reduced Food Efficiency Ratio (FER) compare to control diet groups. It also elevated blood serum enzymes SGOT, CK-MB, SGPT, ALP level. Mustard (wild) was shown more effective compare to Rai (Hybrid) rapeseed oil. Both types of oil contain a high amount of erucic acid that has adverse effects on cardiac and hepatic enzymes. So human should not be consumed rapeseed oil an excess amount that contains high erucic acid but consumes only that rapeseed oil that contains a low amount of erucic acid that has no adverse effect on health.

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CONFLICTS OF INTEREST

The author (s) declared no potential conflicts of the interest with respect to the research work.

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