An Experimental Study of Coagulation Profile in Albino Rats Fed on Canola Oil Supplemented with Atherogenic Element for a period of 6 weeks

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An experimental study on albino rats was designed to estimate the coagulation parameters like Prothrombin time (PT), Activated Partial Thromboplatin Time (APTT) and fibrinogen level and coagulation parameters were compared in animals fed on low and high conc. of canola oil and canola supplemented with atherogenic element. Sixty albino rats were selected and divided into five different groups with twelve rats in each group. Group A was given synthetic diet (control) and other four groups were given low and high conc. of canola oil and canola oil+atherogenic elements for 6 weeks duration. At 6th week blood samples were collected. PT and APTT were reduced in groups taking different conc. of canola + atherogenic element when compared with those on pure canola oil. Groups on canola+atherogenic diet showed increased fibrinogen level when comparing with those on pure canola oil for six weeks.

Key words: Coagulation profile, canola oil

The activity of coagulation system can be assessed by different types of tests performed. PT shows the activity of coagulation factors involved in extrinsic and common pathways i.e. factors II, V, VII, X and APTT shows the activity of factors involved in the intrinsic and common pathways i.e. VIII, IX, XI, XII. Deficiency of these factors would lead to abnormal results in these tests[1].

There are three types of fatty acids, mono-unsaturated (olive oil, rapeseed oil, canola oil), polyunsaturated (corn oil, soyabean oil) and saturated (hydrogenated oil, butter oil). Chief fatty acid of canola oil is oleic acid. Saturated fats mainly contain palmitic acid and stearic acid. Botanical name of canola is the same as that of mustard i.e. brassica campestris. In Pakistan, it is called as sweet sarson. Thus canola is characterized by high level of oleic acid, intermediate level of polyunsaturated fatty acids and low level of saturated fatty acid. Garlic & omega-3 fatty acids can decrease the platelet aggregation and increase the fibrinolytic activity and are responsible to decrease thrombus formation[2,4,8].

Saturated fats and cholesterol in the diet caused rise in serum cholesterol while diet low in saturated fat decreased cholesterol level in human beings[5,6]. Canola oil contains 61% oleic acid and is second to olive oil in oleic acid contents. It contains high levels of PUFA than palm or olive oil i.e. 21% linoleic acid and 11% linolenic acid and only 7% saturated fatty acids[7,9]. Recent studies show that dietary alpha-linolenic acid (an Omega-3 fatty acid found in canola oil) alters the fat composition of cell membranes[10].

There is growing evidence that patients can improve lipid levels and decrease the rate of cardiovascular accidents by adding specific food to their diets and switching from saturated and polyunsaturated to monounsaturated fats and Omega-3 fatty acids. This dietary change decrease arteriosclerotic plaque formation reduces oxidation of LDL and enhance thrombolytic activity[11]

Subjects and methods

Sixty albino rats with equal number of males and females were selected for the study. Four different experimental diets were prepared and were given to albino rats for a period of 6 weeks. Diet 1, 2, 3, & 4 contained 2.9% canola oil, 2.9% canola+atherogenic elements, 20% canola oil and 20% canola+atherogenic element respectively. Mineral and vitamin mixture were mixed with diet according to the recommendation. The rats were grouped A to E according to the diets

Group A = Synthetic diet (Control)
Group B = 2.9% canola oil (diet 1)
Group C = 2.9% canola oil supplemented with atherogenic element (diet 2)
Group D = 20% canola oil (diet 3)
Group E = 20% canola oil supplemented with atherogenic element (diet 4)

Blood samples were taken after giving ether anesthesia to the albino rats at 6th week by heart puncture. 1.8ml blood was put in a test tube containing 0.2 ml of 3.8% sodium citrate. This plasma was used for prothrombin time, activated partial thromboplastin time and fibrinogen level. Student's 't'-test was used to analyze the results and data in this study.

Results

The present study was carried out on sixty albino rats. They were weighed at 6 weeks and divided into five groups, each group consisting of six male and six female animals.

The results of PT, APTT and fibrinogen levels are given in Table 1,2 and 3.

Table 1 Comparison of PT in control group and experimental groups in 6th week (n=12)

Groups	Mean±SD	Range
A (control)	11.8±0.87	11 – 13
B(Diet 1)	14.6±1.31	12 – 17
C(Diet 2)	13.9±1.16	12 – 16
D(Diet 3)	14.3±0.89	13 – 16
E(Diet 4)	12.5±1.17	10 – 14

Statistical Analysis

A Vs B ($p<0.001$) ***
A Vs C ($p<0.001$) ***
A Vs D ($p<0.001$) ***
A Vs E ($p<0.05$) ***
B Vs C ($p<0.05$) ***
D Vs E ($p<0.001$) ***
***(Highly Significant)

The results are expressed as mean±SD values (n=12)

Table 2 Comparison of APTT in control group and experimental groups in 6th week

Groups	Mean±SD	Range
A (control)	40±2.83	35 – 43
B(Diet 1)	42.4±3.33	35 – 46
C(Diet 2)	37.2±3.65	34 – 43
D(Diet 3)	42.7±3.23	33 – 39
E(Diet 4)	41.6±2.9	34 – 43

Statistical Analysis

A Vs B ($p<0.05$) **
A Vs C ($p<0.05$) **
A Vs D ($p<0.05$) **
A Vs E ($p<0.05$) *
B Vs C ($p<0.05$) **
D Vs E ($p>0.05$) *
*(Non-significant)
**(Significant)

The results are expressed as mean±SD values (n=12)

Table 3 Comparison of fibrinogen level in control group and experimental groups in 6th week

Fibrinogen (mg/dl)	Mean±SD	Range
A (control)	224.2±76.1	160 – 375
B(Diet 1)	220.6±58.2	155 – 350
C(Diet 2)	312.5±44.45	250 – 400
D(Diet 3)	221.1±44.45	200 – 400
E(Diet 4)	253.7±49.7	220 – 400

Statistical Analysis

A Vs B ($p<0.05$) *
A Vs C ($p<0.001$) ***
A Vs D ($p<0.05$) *
A Vs E ($p>0.001$) ***
B Vs C ($p<0.001$) ***
D Vs E ($p>0.05$) *
*(Non-significant)
***(Highly Significant)

PT in experimental groups was increased when comparing with control Group and the difference was highly significant statistically except in Group A Vs E. PT in groups containing pure canola oil is increased when compared with Canola + atherogenic Group and difference was highly significant in groups D Vs E.

APTT in 6th week was increased in experimental group when comparing with control group except group C and the difference was found to be significant statistically except in groups A Vs E. APTT in groups containing pure canola oil in different concentration was increased when compared with those of canola+atherogenic element but difference was significant statistically in groups B Vs C while non-significant difference was found in groups D vs E.

Fibrinogen level in 6th week was increased in experimental groups containing Canola + Atherogenic diet while decreased in Groups on Canola only when comparing with control group but the difference was non-significant in group A Vs B and A Vs D and highly significant in groups A Vs C & A Vs E. The experimental groups containing Canola + Atherogenic diet showed increased levels of fibrinogen when comparing with groups containing pure canola oil in different conc. and the difference was highly significant in groups B Vs C and non-significant in groups D Vs E.

Discussion

Prothrombin time

The mean PT of experimental groups B-D was prolonged significantly ($p<0.001$) as compared to control group. This rise in PT of the animals on canola oil diet (low and high conc.) may be due to effect of omega-3 on coagulation system. Our observations are in accordance with the results of Turner (1990)[3] who also observed similar findings in his experimental study. The mean PT of group D was found to be increased significantly ($p<0.05$) as compared to those on 20% Canola+atherogenic element at 6th week. It indicates that use of high conc. of canola oil is causing increased PT due to effect on coagulation pathways. The mean PT of group with atherogenic element was reduced significantly ($p<0.05$) than that of group on pure Canola. This finding can be due to inhibitory effect of atherogenic element on the coagulation system.

Activated partial thromboplastin time

At 6th week, the mean APTT of group C Vs B and group E Vs D was reduced and difference was found to be significant in groups C Vs B while comparable in groups E Vs D. This reduction in APTT may be attributed to the inhibitory effect of atherogenic element present in diets of groups C and E on coagulation system.

Fibrinogen level

The mean fibrinogen levels of groups on Canola oil+atherogenic element were raised significantly ($p<0.001$) as compared to that of groups on pure Canola oil. Such a rise in fibrinogen level in groups on Canola+atherogenic element at 6th week may be due to inhibitory effect of atherogenic element on fibrinolytic system.

The present study revealed that there was increase in PT and APTT while the levels of fibrinogen were reduced in experimental groups using low and high conc. of canola as compared to those on canola+atherogenic element in which there was decrease in PT and APTT while levels of fibrinogen were increased.

Thus it is concluded that dietary advice to patients with thrombotic tendency can be instituted towards the addition of monounsaturated fatty acids especially canola oil in exchange for saturated fats. This may help the

clinician to prevent the development of ischemic heart disease and other thromboembolic effects in individuals at increased risk by simple dietary interventions.

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