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EFFECT OF *MESUA FERREA* FLOWER AND *CARICA PAPAYA* SEED EXTRACTS ON BIOCHEMICAL CONSTITUENTS OF OVARY, LIVER AND KIDNEY IN FEMALE ALBINO RATS

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ABSTRACT

The effect of *Mesua ferrea (M.ferrea)* flower and *Carica papaya (C.papaya)* seed extracts on the levels of total carbohydrates, proteins and lipids in the ovary, liver and kidney of female albino rats was studied. In this study was used (48) good healthy adult female albino rats were divided into two experiments (Experimentation: 1 and Experimentation: 2), and each experimentation was divided into four groups (Group-I, group-II, group-III and group-IV), each group consist six animals. The 1st experimental groups (except group-I) were treated with M.ferrea flower extracts (100 mg/kg, 200 mg/kg, 300 mg/kg body weight) orally once daily. The 2nd experimental groups (except group-I) were treated with C.papaya seed extracts (100 mg/kg, 200 mg/kg, 300 mg/kg body weight) orally once daily. The experimental period was 21 days. The result showed that there was the total carbohydrate and cholesterol content were significantly increased and protein concentration was decreased in all groups of *M.ferrea* flowers extract treated rats when compared with the control. There was also increased in the physical activity of the test groups, while the control did not show any noticeable change. The serum levels of total cholesterol in treated groups were significantly higher than the control. From observation, on the test groups of 2nd experimentation, the levels of carbohydrate, cholesterol and proteins were higher in all treated groups. Thus the effect may be depending on the quantity of the seed incorporated into the feedstuffs. Generally, there were increased levels of cholesterol and protein due to the incorporation of M.ferrea flower and C.papaya seed extracts into the feed of the animals used in this study.

KEYWORDS: Carbohydrate, Protein, Cholesterol, *Mesua ferrea, Carica papaya.*

INTRODUCTION

Previous studies from the laboratory have examined the effects of hormonal deficiencies on the metabolism of glucose and pyruvate by rat liver slices.[1] Plants whether as a whole or their parts, juices, extracts etc have been an exclusive part of animal and human life on earth. Since ancient times, human are dependent on plants for their health and healing. Plants and plant products are not just a food product now, but are being used and explored for every possible opportunity. This has revoked the scientists to re-discover each and every plant with a fresh new approach towards its possible use. Primary metabolites i.e. proteins, carbohydrates, vitamins, hormones and lipids are essential for plants to live and reproduce. These primary metabolites provide the world with the food and feed stuffs and are the basis of nutrition for the entire living world.

Preparation of papaya seeds**:** The seed of rip pawpaw plant widely grown throughout the tropics for its tasty (*Carica papaya*) fruits weighing between 1-3 kg from edible fruit out of the 48 species of the genus *caricaceae* Narict pawpaw garden were removed, cleaned and sun known, only *Carica papaya* is of importance dried. The dried seeds were then dehulled and.[2] Nutritionally, the major components of papaya fruit pulp dry matter are carbohydrates. At the early stage of fruit development, glucose is the main sugar but the sucrose content increases during ripening and can reach up to 80% of the total sugars. The edible portion of the ripe papaya fruit contains both macro and micro minerals and these are Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn. *Carica papaya* is a source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B-6 and vitamin K.[3]

Papaya seeds have high oil content representing a potential source of oil rich in oleic fatty acid. The high proportion of unsaturated fatty acids would make the oil an acceptable substitute for other highly unsaturated oils. The seed has been shown to be a good source of oil (25.6%) that may be useful for medicinal, biofuel and industrial purposes.[4] The seeds are medicinally important in the treatment of sickle cell disease, poisoning related disorder.[5]

MATERIALS AND METHODS

Biochemical Assays

Selection and preparation of tissue for biochemical analysis

The tissues selected for the present study were the Ovary, Liver and Kidney. The rats were sacrified by cervical dislocation and the above tissues were quickly isolated and placed on ice cubes. The dissected organs were immediately homogenised in suitable media for later analysis. After isolation of tissues, they were stored at -20$^{O}_{C}$ till further use

Protein metabolism

Estimation of total Proteins

Total protein content was estimated by the method of Lowry et al.[9] Using bovine serum albumin (BSA) as standard. This method is designed based on the Folin reaction and is most sensitive even to about 0.01mg of protein/ml.It works on the principle of phosphomolybdo-tungstate of Folin-Ciocalteau reagent by amino acids, tyrosine and tryptophan present in the proteins resulting in the formation of strong blue colour due to the Folin reaction of the protein with alkaline cupric tartarate.

10% tissue homogenate was prepared in 5ml of ice cold sucrose solution and centrifuged at 2500 rpm for 15min.To 0.5ml of supernatant, 4ml of alkaline copper reagent was added and allowed to stand at room temperature for 15min.Further, 0.5ml of dilute Folin-Ciocalteau reagent was added and colour developed was measured at 660nm after 30min in spectrophotometer against blank.

The standard graph of protein was prepared using BSA as standard and protein content of the tissue was calculated from the standard graph. The values are expressed as mg protein/g tissue.

Method of Calculation

Amount of proteins present in the sample (mg/gm wet wt of tissue) =

$$\frac{\text{O.D of the Sample x Con.of the Standard}}{\text{O.D of the Standard x Wet wt of the tissue (gm) x1000}}$$

Carbohydrate metabolism

Estimation of total Carbohydrates

Total carbohydrates content was estimated by the method of[10] using glucose as standard. This method is designed based on the Anthrone reaction and is most sensitive even to about 0.01mg of carbohydrate/ml.The sulphuric acid present in the anthrone reagent hydrolyses the polysaccharides into their individual monosaccharides. These monosaccharides react with the phenolic compound of anthrone. As a result a coloured complex is produced and the intensity of this colour is proportional to the concentration of the monosaccharides in the solution.

10% tissue homogenate was prepared in 5ml of TCA solution and centrifuged at 2500 rpm for 15min.To the blank, test samples and standards 4ml of anthrone reagent was added and kept in boiling water bath for 10 min, and cooled to the room temperature. The intensity of the colour was measured against blank at 630 nm in a spectrophotometer.

The standard graph of carbohydrate was prepared using glucose as standard and carbohydrate content of the tissue was calculated from the standard graph. The values are expressed as mg carbohydrate/gm tissue.

Method of Calculation

Amount of carbohydrates present in the sample (mg/gm wet wt of tissue) =

$$\frac{\text{O.D of the Sample x Con.of the Standard}}{\text{O.D of the Standard x Wet wt of the tissue (gm) x1000}}$$

Cholesterol metabolism

Estimation of total cholesterol:

The total cholesterol level was estimated by adopting the method given by Zak.[11] In strong acidic medium the cholesterol molecules first undergoes dehydration to from cholesta-3, 5-dien and then during oxidation and sulphonation reactions by conc H_2SO_4 (in the presence of Fe^{3+} ions as catalysts) form reddish purple colour. The colour formation is proportional to the cholesterol present in the sample and is measured optically at 540nm.

To 0.2ml of serum 4.9 ml of working $Fecl_3$ and Acetic acid reagent was added, mixed well and allowed to stand for 15 min and centrifuged. From this 2.5ml of supernatant was taken (2.5ml of working standard was taken as standard and 2.5ml of Fecl3 and Acetic acid was taken as blank) and 1.5ml of conc.H_2SO_4 was added to all the three tubes and each tube was mixed well and incubated in water bath at 60$^{o}_{C}$ for 10min, cooled to room temperature and then reddish purple colour was developed and measured at 560nm.

Method of Calculation

Amount of cholesterol present in the sample (mg/dl) =

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Con.of standard}$$

RESULTS AND DISCUSSION

Table 11: Effect of *M. ferrea* flowers extract on the levels of total Carbohydrates in ovary, liver and kidney of female albino rats was studied for 21 days.

Treatment groups	Dose mg/kg body weight	No. of days	Total Carbohydrates (mg/g)		
			Ovary	Liver	Kidney
Group-I	**(Control)**	------	168.04 ±9.85	98.86 ± 4.70	84.66 ± 2.98
Group-II	100	21	197.06 ±10.68	105.06 ± 4.90	96.73 ± 3.04
Group-III	200	21	240.08 ±11.05	126.08 ± 5.04	115.98 ± 3.19
Group-IV	300	21	275.09 ±11.09	134.09 ± 5.10	126.54 ± 3.99

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).

Table1 2: Effect of *M. ferrea* flowers extract on the levels of total proteins in ovary, liver and kidney of female albino rats was studied for 21 days.

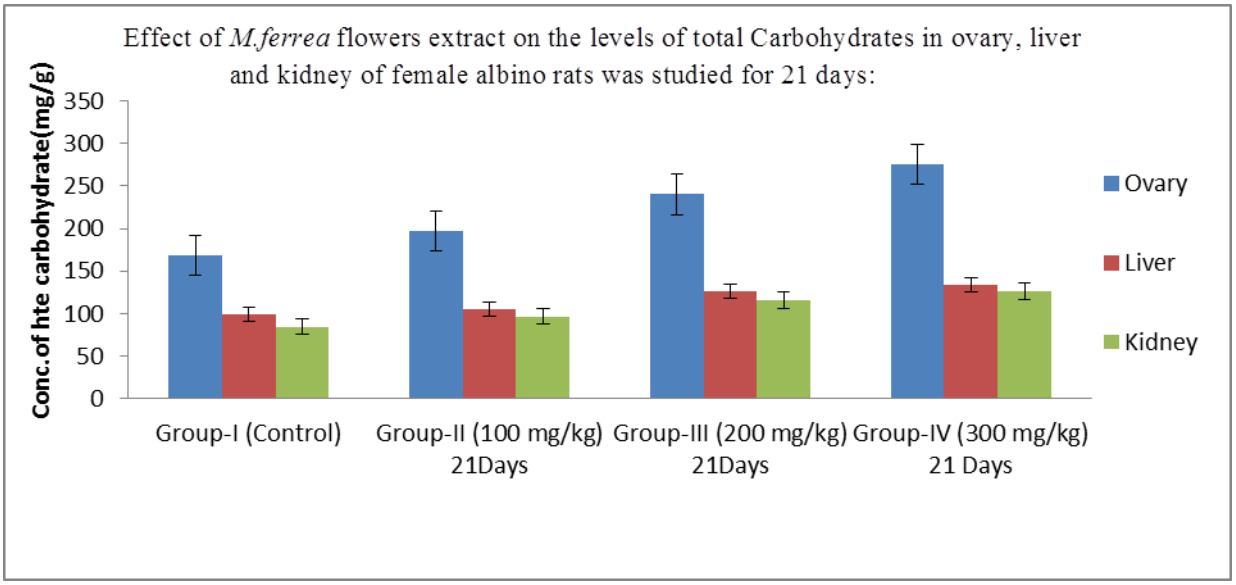
Treatment groups	Dose mg/kg body weight	No. of days	Total proteins (mg/g)		
			Ovary	Liver	Kidney
Group-I	**(Control)**	------	26.96 ± 0.14	96.4 ±1.05	126.12 ± 2.34
Group-II	**100**	**21**	24.23 ± 0.08	94.98±1.01	108.02 ± 2.07
Group-III	**200**	21	23.87 ± 0.05	91.79±1.02	99.01 ± 2.01
Group-IV	**300**	21	21.04 ± 0.02	88.68±1.04	85.06 ± 1.16

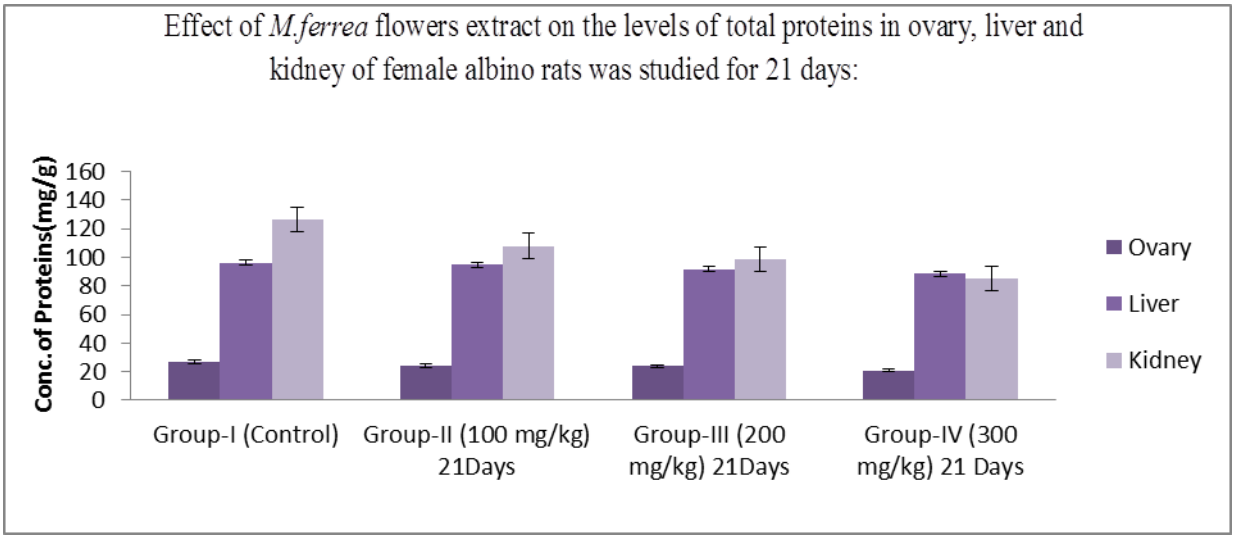
*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).

Table 13: Effect of *M.ferrea* flowers extract on the levels of total cholesterol in serum of female albino rats was studied for 21 days.

Treatment groups	Dose mg/kg body weight	No. of days	Total cholesterol(mg/dl) serum
Group-I	**(Control)**	------	3.07± 0.09
Group-II	**100**	**21**	3.56 ± 1.44
Group-III	**200**	21	3.84 ± 1.62
Group-IV	**300**	21	4.08 ± 1.97

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).





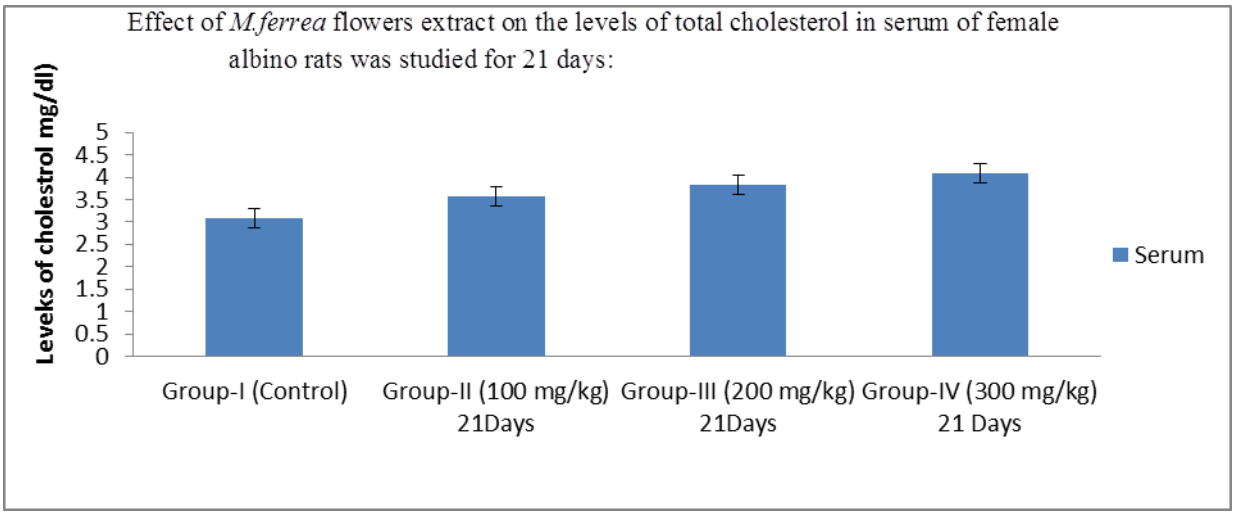


Table1 4: Effect of *C.papaya* seed extract on the levels of total carbohydrates in ovary, liver and kidney of female albino rats was studied for 21 days.

Treatment groups	Dose mg/kg body weight	No. of days	Total Carbohydrates (mg/g)		
			Ovary	Liver	Kidney
Group-I	(Control)	------	168.04 ±9.85	98.86 ± 4.70	84.66 ± 2.98
Group-II	100	21	186.74 ±10.67	100.53 ± 4.83	85.47 ± 3.24
Group-III	200	21	198.87 ±11.01	102.68 ± 4.95	87.63 ± 3.51
Group-IV	300	21	247.96 ±11.09	104.72 ± 5.02	89.76 ± 3.89

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).

Table 15: Effect of *C.papaya* seed extract on the levels of total proteins in ovary, liver and kidney of female albino rats was studied for 21 days.

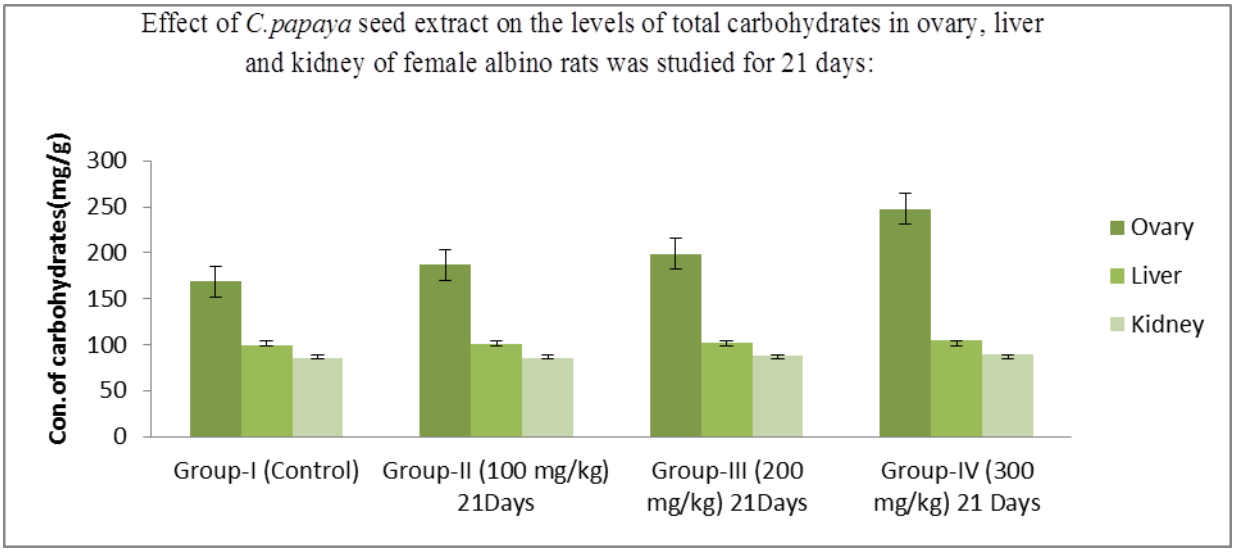
	Dose mg/kg body weight	No. of days	Total proteins (mg/g)		
			Ovary	Liver	Kidney
Group-I	**(Control)**	------	26.96 ± 0.14	96.4 ±1.05	126.12 ± 2.34
Group-II	**100**	**21**	27.2 ± 0.21	96.98±1.08	126.92 ± 2.87
Group-III	**200**	21	28.87 ± 0.56	97.29±1.15	127.81 ± 3.04
Group-IV	**300**	21	30.99 ± 0.86	98.81±1.43	128.39 ± 3.46

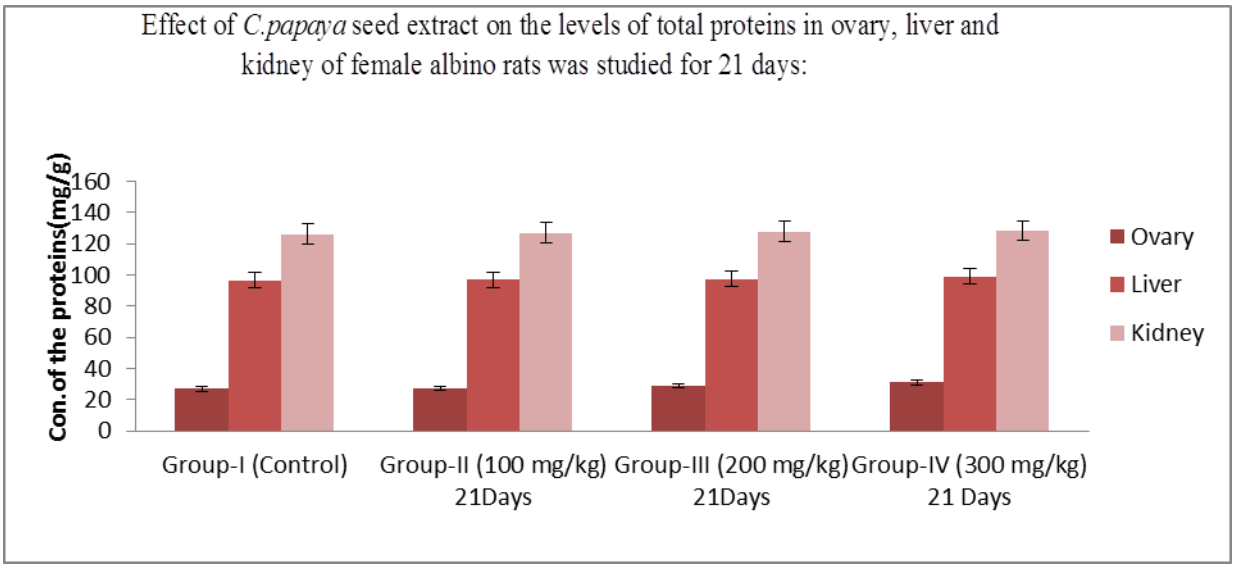
*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).

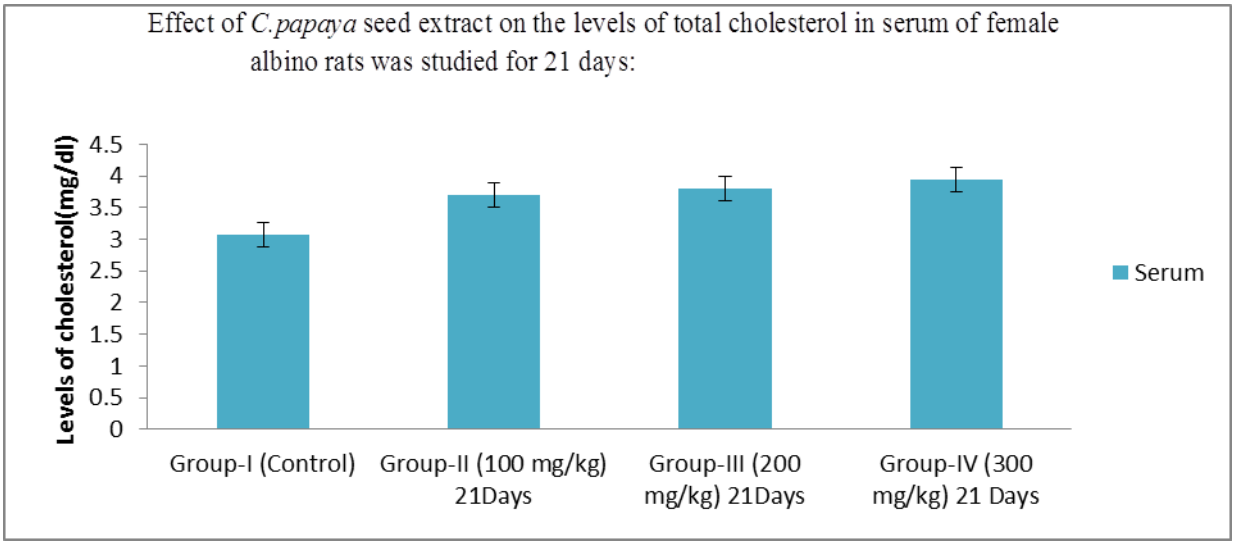
Table1 6: Effect of *C.papaya* seed extract on the levels of total cholesterol in serum of female albino rats was studied for 21 days.

Treatment groups	Dose mg/kg body weight	No. of days	Total cholesterol(mg/dl) serum
Group-I	**(Control)**	------	3.07± 0.09
Group-II	**100**	**21**	3.69 ± 1.04
Group-III	**200**	21	3.80 ± 1.52
Group-IV	**300**	21	3.94 ± 1.70

*Values are means and standard errors for 6 rats per treatment. Means along the row with different superscript are significantly different (P > 0.05).







DISCUSSION

In the present investigation effect of the flower of *M.ferrea* extract on the levels of carbohydrate, protein and cholesterol in female albino rats were studied for 21 days. There were changes in the body weight of rats in all groups. The rats in all treated groups (group-II, group-III and group-IV) gained more weight than the group-I. During the period of feeding, the animals treated with the flower of *M.ferrea* extract showed increase in physical actives, food and water intake. The actual biochemical mechanism responsible for these observations was clearly known at this state of the research. However, they may be attributed to a disruption of normal metabolic pattern by the chemical constituent of the extracts.

In the present study the total carbohydrates and cholesterol content were significantly increase and protein concentration was decrease in all groups (control and treated) of *M.ferrea* flowers extract treated rats when compared with the control rats. (Table.1)

From the result, the Serum concentration of Cholesterol and total carbohydrate were significantly higher (>0.05) and protein concentration low (<0.05) in the test groups (II, III and IV) then the control. The exact reason for this increase in level of Cholesterol and total carbohydrate was obscure but could indicate the presence of carbohydrate, cholesterol and the flower of *M.ferrea*.

In this work, the effect of the seeds of *C.papaya extract* on the levels of carbohydrate, protein and cholesterol in albino rats was studied for 21 days. There were changes in the body weight of rats in all groups. The rats in all treated groups (group-II, group-III and group-IV) gained more weight than the groups. During the period of feeding, the animals treated with the seeds of *C.papaya* extract showed increase in physical actives, food and water intake. The actual biochemical mechanism responsible for these observations was clearly known at this state of the research. However, they may be attributed to a disruption of normal metabolic pattern by the chemical constituent of the extracts.

Generally there was increase in body weight of animals in the test groups compared to the control group. This increase in body weight may be due to the reported increase in food intake[6] or the weight increase may be referred to as an index of toxicity.[7]

In the present study the total carbohydrates, proteins and cholesterol content were significantly increase in all groups (control and treated) of *C.papaya seeds* extract treated rats when compared with the control rats. (Table.1)

From the result, the Serum concentrations of Cholesterol, total protein and carbohydrate in the test groups (II, III and IV) were significantly higher (>0.05) then the control. The exact reason for this increase in level of Cholesterol, total protein and total carbohydrate was obscure but could indicate the presence of carborhydrate, lipid and the seeds of *C.papaya*. According to[8], the Seeds of *C.papaya* from Nigeria were analyze and found to contain 316g/kg carbohydrates, 364g/kg proteins and 52g/kg fixed oil on dry weight basis. The result in table: 6 shown that the level of carbohydrate, proteins and cholesterol in group-IV and group-III were higher than that of group-I and group-II. This result suggests that the level of cholesterol increases with an increase in the concentration of the seeds.

CONCLUSION

The amount of total Carbohydrates, proteins and cholesterol levels in the female reproductive rats exposed to M. ferrea and C.papaya was quantitatively determined. A significant increased in ovary, liver and kidney. Carbohydrates, proteins and cholesterol contents were observed in all treated groups when compared with control groups. Hence, it can be concluded from this study that both low and high dose of M.ferrea and C.papaya treatment enhanced carbohydrates, proteins and cholesterol levels compared to control group of ovary liver and kidney. This study unambiguously demonstrates that one of the mechanisms of M.ferrea and C.papaya action on fast proliferating reproductive cells is

by inhibiting the source of energy, thereby controlling carbohydrate, proteins and cholesterol metabolism in the female reproductive tract.

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