Original Article

DOI: 10.29054/APMC/19.760

Effects of Red Meat Consumption on Histology of Coronary Arteries in Adult Albino Mice

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ABSTRACT

Objective: To determine the effects of the red meat (Buffalo) on coronary arteries of adult albino male Mice. **Study Design:** Experimental study. **Settings:** Anatomy Department of Liaquat University of Medical and Health Sciences, Jamshoro in collaboration with animal house of Sindh Agriculture University Tando Jam. **Duration:** 6 months from January 2017 to July 2017. **Methodology:** Total 45 adult Albino male mice (laboratory male mice) of average 180–220 grams bodyweight was selected. Animals were housed (about 4-6 per cage) in a temperature-controlled room (22± 2°C) and at a humidity of 55%±5%, with a 12-hr light/dark cycle. Animals were divided into three groups as: group A (control), group B white meat (poultry) and group C (red meat). At the end of the experiment animals were sacrificed and hearts were taken from every animal as soon as possible and specimens were assessed for histopathology. **Results:** Mean cholesterol level was significantly higher among red meat consuming group as compared to control group (P- 0.001). Mean body weight was markedly higher among red meat consuming group in contrast to control group; p-values were quite significant. Total 6.66% mice suffered in red meat consuming group and out of them 3.34% died. **Conclusion:** It was concluded that red meat consumption is associated to a higher risk of cholesterol elevation and mortality, without any histological alteration.

Keywords: Albino mice, Coronary arteries, Red meat consumption.

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Submitted for Publication: 20-07-2019 *Accepted for Publication: 23-09-2019*

Citation: Wagan G, Bakhsh L, Mehmood A, Bano S, Memon S, Samo RP. Effects of Red Meat Consumption on Histology of Coronary Arteries in Adult Albino Mice. APMC 2019;13(4):287-91.

INTRODUCTION

The red meat (beef) diet, specially processed meat, positively correlates with total risk and results in different diseases like diabetes, colorectal cancer and CVD.[1] It has been expected that the positive correlation of beef consumption with the chronic diseases risk can possibly be attributed to highly saturated fatty acids and carcinogens or heme-iron substance, including *N*-nitroso compounds and heterocyclic amines.[2] In contrast, poultry is commonly supposed to be one of the healthy alternatives to beef and inversely correlates with the total risk of cardio vascular disease-associated mortality.[3,4] Contradictory studies on the connection amid mortality and meat consumption documented in earlier epidemiologic findings may be somewhat explained by several cooking and processing meat techniques; consumption of processed meat is more strongly linked to the mortality risk than consumption of non-processed meat. It has additionally been documented that sensitivity of insulin considerably augmented in rats when regimes of protein were contrasted to beef protein. Leptin is a derivative hormone of adipocyte, which promotes balance of negative energy. Rats fed on fats-rich and casein-rich regimes had lower concentrations of serum leptin in contrast to rats fed on a fat-rich and soy-rich diet.[5] In recent times, documented a fresh pathway in both laboratory-mice and humans concerning dietary phosphatidylcholine /choline microbiota metabolism to cardio vascular disease (CVD) pathogenesis.[6] Scrutinizing long-term potential statistics from two immense cohort findings, researchers established that people who ate unprocessed beef daily contained a 13% greater mortality risk during the period of research. An experimental research on histological assessment of coronary arteries in 6 wild-type and 12 ApoE (-/-) laboratory-mice, in comparison to the atherosclerosis division in coronary arteries of human, discloses that the chief lesions in the laboratory-mice were found in the valve sinus, as well as the coronary arteries origins.[7] These retro valvular lesions either terminate unexpectedly at the common coronary artery orifice or extend to the trunks of arteries. The first segment and first branch of all the major coronary arteries, the usual sites of disease in humans, are protected from disease. Although the arterial trunks and the first level branches are free of disease. Independent lesions are present in the heart in smaller intramyocardial vessels. These lesions are comprised predominantly of macrophages and proteoglycan and exhibit little extracellular lipid. In some cases, the independent lesions occlude the lumen without evidence of myocardial infarct in the surrounding tissue.[7] 9.3% of the seen mortalities in males and 7.6% of the mortalities in females may have been prevented by contributors utilizing below 0.5 per day servings of beef (42 g).[7] Beef consumption has been reported to be a risk factor for cardiovascular disease; as it effects the serum cholesterol; because beef comprises arachidonic acid, homocysteine, heme iron and high content of saturated fats.[8,9] Bacteria present in the digestive tract of individuals who consume meat have been observed to generate a spike in Trimethylamine N-oxide (TMAO) when given with carnitine-rich beef. TMAO is a metabolic agent which encourages atherosclerosis, the arteries thickening. The purpose of this study was to determine the effect

of red meat consumption on cholesterol level and histological alteration in coronary arteries of adult albino male Mice.

METHODOLOGY

Study Design: Experimental study.

Settings: Anatomy Department, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, in collaboration with animal house of Sindh Agriculture University Tando Jam.

Duration: 6 months from January 01, 2017 to July 31, 2017.

Sample Size: Total 45 adult Albino male mice (laboratory male mice) of average 180 – 220 grams bodyweight were selected.

Inclusion Criteria: All the healthy adult Albino male laboratory-mice with average bodyweight of 180–220 grams were selected.

Exclusion Criteria: All the sick laboratory-mice, those not feeding well and female laboratory-mice were excluded.

Methods: All the animals were obtained from the Animal Resource Center of Sindh Agriculture University Tando Jam. After official approval, experiments were performed on adult male mice with average bodyweight of 180-220 grams. Animals were housed (about 4-6 per cage) in a controlled temperature room (22± 2°C) and at a humidity of 55%±5%, with 12-hr dark/light cycle. The animals were allowed to contact freely with food and tap water. All efforts were made to minimize the amount of meat consumed by animals and their distress.

Animals were divided in three groups as:

Animal Groups

1. Group A (n=15): Control laboratory-mice were fed normal diet.
2. Group B (n=15): Control laboratory-mice were fed a white meat (poultry).
3. Group C (n=15): laboratory-mice were administered on red meat (buffalo meat).

At the final stage of the trial, the animals were slaughtered at the suitable time. Major organs such as heart were immediately taken from each animal. Small pieces of the heart were fixed in 10% formalin and processed using a standard histological procedure. The tissue sections were evaluated for histological changes under compound light microscope and photomicrographs were taken using digital camera. All the histological effects of beef were analyzed on coronary arteries. Pre-approved proforma was used to collect and document data during research.

Sample collection and staining

The samples of heart tissue from male laboratory-mice were collected in 10% formalin for further histological processing.

For H & E sectioning:

Paraffin Tissue Processing for Sectioning: A series of processes were carried out starting from dehydration and clearing to wax impregnation before sectioned.

Dehydration: Dehydration was carried out by placing the tissue in increased concentration of alcohol ranging from 70% to 100%.

Clearing: After removing all the water from tissue sample, they were cleared of alcohol by means of xylene in order to mix wax into them.

Wax Impregnation: Following all the clearing, the sample was processed for changes of molten wax till thoroughly impregnated. At this point temperature was strictly controlled.

Automatic Tissue Processing: An automatic tissue processor, (Leica TP1020) was used to perform all the above-mentioned processes. The tissue was sectioned at 5µm by microtome and slides were prepared on charged glass slides.

Hematoxylin and Eosin Staining of Tissue Slides: Once all the slides were prepared, they were hydrated with water and then placed into the hematoxylin solution for 5 minutes. The hematoxylin was removed and tissues were washed with tap water. Tissues were fixed in acid alcohol to remove excess stain before bluing the nuclei in a saturated solution of lithium carbonate. The tissues were again washed in tap water then placed in to 1% eosin for approximately 1-2 minutes. Excess stain was removed and slides were washed in tap water. Finally, the tissues were dehydrated by passing through increasing concentration of alcohol and allowed to dry before being covered with cover slips.

Fig.1: Taking blood directly from mice heart

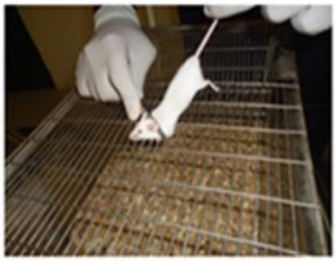


Fig.2: Merciful killing of the mice (cervical dislocation)

Fig.6: Dissection of mice

Fig.7: Dissection show (heart of mice)

RESULTS

Beef is a great cause of cholesterol elevation. Mean cholesterol was 200.39±8.14 mg/dL in group C which was significantly higher as compared to group A 180.66±23.5 mg/dL and control group B 195.39±8.14 mg/dL. Table 1

Table 1: Cholesterol distribution of the mice n=60

Groups	Cholesterol (mg/dL)		p-value
A vs B	180.66±23.5	195.39±8.14	0.057
A vs C	180.66±23.5	220.73±4.4	0.001
B vs C	195.39±8.14	220.73±4.4	0.001

No significant difference was seen amid bodyweight of the laboratory-mice after consumption of normal diet P- value 0.07. There was a significant difference was seen in initial and final body weights of laboratory-mice after consumption of white meat and red meat, (P- 0.001).Table 2

Table 2: Bodyweight distribution according to study groups

Study groups	Bodyweight		P-value
	Before	After	
Group A	190.91+10.13g	220.78+12.38g	0.076
Group B	215.97+13.13g	340.77+2.05g	0.001
Group C	208.10+1.53g	390.94+1.44g	0.001

Out of total laboratory-mice of group B and group C, 6 animals became suffered 2 from group B and 4 from group C. Out of these suffered animals 4 were died one from group B and 3 from group C. Table 3

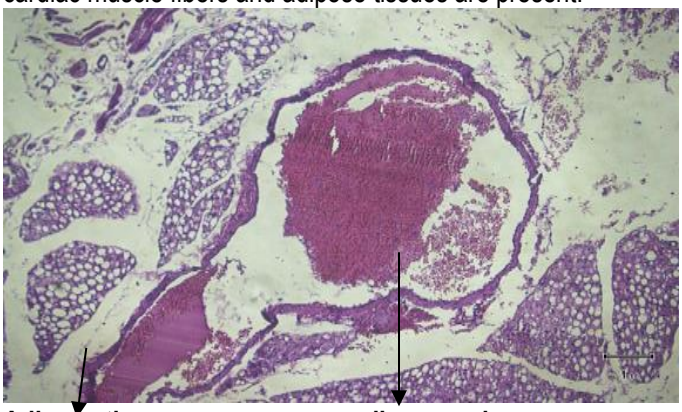
Table 3: Mice distribution according to illness and death

Variables	Study groups		
	Group A	Group B	Group C
Suffered			
Yes	00	02	04
NO	15	13	11
Death			
Yes	00	01	03
No	15	14	12

Histopathological findings of groups:

Group A (Control) Microscopic Examination:

The sections submitted from biopsy of heart that reveal normal architecture showing normal morphology of coronary arteries, cardiac muscle fibers and adipose tissues are present.

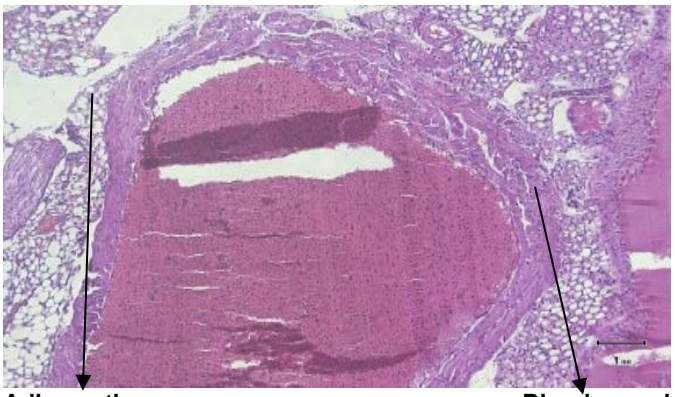


Adipose tissue cardiac muscle

Fig. 1: Section of control group

Group B (Chicken) Microscopic Examination:

The submitted section of mice heart revealed normal morphology of heart. Cardiac muscle fibers and adipose tissue have been shown. Wall of coronary artery revealed normal structure. No evidence of atherosclerotic changes or plaque formation was seen in the tunica intima of coronary artery.



Adipose tissue Blood vessel

Fig. 2: Section of chicken group

Group C: (red meat) microscopic examination:

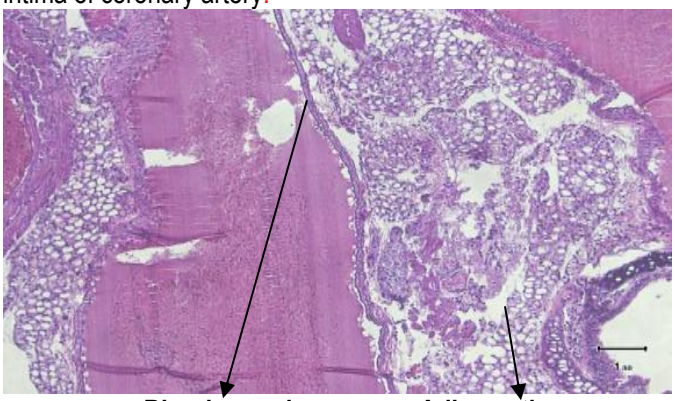
The sections submitted from heart of mice revealed normal heart. Morphology showing coronary arteries and cardiac muscle fibers, the coronary artery reveals no evidence of atherosclerotic changes in the tunica intima.

Blood vessel Adipose tissue

Fig 3: Section of beef

Group C: (Red meat) Microscopic examination:

Histopathology examination of submitted sections from heart of mice revealed normal coronary artery, cardiac muscle fibers and adipose tissue. The wall of coronary artery showed normal structure. No evidence of plaque formation seen in the tunica intima of coronary artery.



Blood vessel Adipose tissue

Fig 4: Section of chicken and beef

DISCUSSION

Red meat (beef) is a major source of proteins and fats. Considerable data from epidemiological studies exhibits that consumption of meat is linked to high risk of mortality.[10] In a cohort study with ten years of follow-up reported that a greater consumption of red meat is linked to raised mortality risk.[6] Though, in this finding a big association was seen among beef and cholesterol and CHD as compare poultry.

In this study, it was observed that beef is a great risk for cholesterol elevation, because mean cholesterol level was significantly higher in red meat consumed group as compared to white meat and normal diet groups.

Pan *et al*[12] documented that adjustment for dietary cholesterol, saturated fats, and heme iron explained a few although not all of the beef consuming risk. In other studies of Hu FB *et al*.[13] and Bernstein AM *et al*[14] also documented that consumption of beef was associated to a raised coronary cardiac disease risk and cholesterol and saturated fats from beef can partially account for this relationship. In an experimental research of Zhang WJ *et al*[15], the intracellular iron chelator desferrioxamine reserved atherosclerosis and inflammation in mice, which proposes a part of iron in atherogenesis.

In this series 6 animals became unhealthy, out of them 4 from red meat consumed group. Similarly it was reported that low-carbohydrate, high-protein, high fats regimes (which are generally elevated in beef) can possibly speed up atherosclerosis via mechanisms which are unassociated with the risk factors of classic cardiovascular disorders.[16] Mice which were offered a high-protein, low-carbohydrate diet had approximately double the arterial plaque level than the mice offered with a Western diet; although the classic risk factors were not considerably varying amid groups.[16] The mice which were offered with the low-carbohydrate and high-protein regimes had markedly less circulating endothelial progenitor cells and higher non esterifies FA levels (encouraging inflammation) as compare to the mice which were offered with the Western diet.[16]

In this study body weight was significantly raised in animals those were on red meat diet. As projected by published "gained bodyweight graphs" in associated findings, timing is an aspect, which must be considered when investigating the whey-reduced bodyweight gain mechanisms. For instance, female whey-offered C57BL/6J mice gain no bodyweight all through initial 10 days of dietary consumption comprising high-fats involvement, whereas the high-fats managed mice without delay gained bodyweight.[17]

In one more mice study, whey decreased bodyweight gain slowly throughout the diet involvement.[18] In one rat finding, the most deep decrease in bodyweight gain via whey was observed throughout the diet intervention's initial week as soon as whey protein was contrasted to beef protein[19]. Whereas, Royle *et al*.[20] documented decreased bodyweight gain via whey in contrast to casein throughout both later and early weeks of intervention.

RECOMMENDATION

Numerous studies as well recommend an elevated mortality risk associated to consumption of beef.

CONCLUSION

We came to the conclusion that red meats (beef) consumption is correlated to a higher risk of *Hypercholesterolemia* and mortality. No adverse histological events were found among all study groups. Further studies should be conducted for long time red meat consumption to assess the histological alteration.

CONFLICT OF INTREST

There is no conflict of interest in this study.

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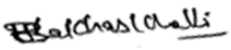
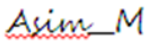
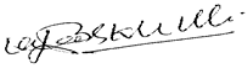
Dr. Lal Bakhsh Associate Professor, Anatomy Bilawal Medical College Jamshoro, Pakistan	Contribution in manuscript writing	
Dr. Asim Mehmood Assistant Professor, Anatomy Bhitia Dental and Medical College Mirpur Khas, Pakistan	Contribution in data analysis	
Dr. Shamshad Bano Lecturer, Anatomy Bilawal Medical College Jamshoro, Pakistan	Contribution in manuscript writing	
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