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Effect of Clarifiers in Beer Brewing on the Lipid Profile of Consumers

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ABSTRACT

Background and Objective: Beer clarity remains a significant factor influencing consumer choice and preferences. Clarifying agents (gelatin, chill guard, isinglass, polyclar, Irish moss and activated charcoal) were deployed for clarification of produced beer and their influence on the lipid profiles of rats was investigated. The interrelationship between the consumption of study beers and obesity was also measured using physical parameters such as weight loss and gain. **Materials and Methods:** Sorghum beer was home-brewed using white maize and sweet potato as adjuncts while spicing with grains of selim and ginger. Seventy male Wister rats (150-170 g) were grouped into binge, moderate and mild alcohol drinkers using standard doses of administration for 21 days. **Results:** Filtered but not clarified beer served as the positive control group while unclarified beer served as the negative control. Beer clarified with isinglass gave the highest clarity while those of activated charcoal, Irish moss, polyclar, chill guard, gelatin and filtered beer gave lesser clarity in decreasing order. At $p \leq 0.05$, significant weight gain was obtained for all groups. Clarified beers under study showed a decrease in high-density lipoprotein activity but an increase in the levels of triglyceride, total cholesterol, very low-density lipoprotein and non-high-density lipoprotein. **Conclusion:** This study suggested that daily consumption of beer clarified with these findings even at moderate dosages, increases bad cholesterol and the likelihood of developing obesity which potentiates the development of type 2 diabetes hypertension, coronary heart diseases and stroke.

KEYWORDS

Fermentation, beer clarification, obesity, biochemical indices, lipid profile

INTRODUCTION

For thousands of years, beer has been a very common beverage and brewing is often branded as the oldest biotechnological process known to man[1]. Worldwide, beer is the fifth most consumed beverage besides coffee, tea, milk and carbonates like Coca-Cola[2]. According to Bokulich and Bamforth[3], the brewing sector holds a strategic economic position in the food industry with yearly world beer production surpassing 1.34 billion hL in 2002. The European Beer Guide as reported by Sawadogo-Lingani *et al.*[4] shows that beer is also the most widely consumed alcoholic beverage in the world.

Beer is a complex blend of more than 450 ingredients[5] with sorghum beer being the undisputed traditional "opaque" beer of Africa made both commercially and rurally with un-malted millet, maize and cassava root often used as adjuncts to produce different flavours[6,7]. Distinct from several alcoholic

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beverages, beer is characteristically unstable and its properties change with time[8]. Changes such as the presence of a light-struck character and foam collapse can occur in a very short period such as when drinking the beverage. And over longer periods, haze can also form alongside flavor decline[9].

The clarity of a beer has been a significant factor[10] influencing consumer choice and preferences and this is because the presence of haze[11] in beer is often linked with low quality. Therefore, the use of clarifying agents (also known as fining agents) to remove haze-forming compounds is very important.

Clarifiers or finings are substances that are typically added at or near the end of the brewing process to remove organic compounds thereby improving beer clarity or regulating its aroma/ flavor[8]. Generally, fining is the term used to describe the clarification stage/phase of beer making to brew a finished beer that is near perfect in terms of colour, taste and clarity.

The use of finings to assist in beer clarification has become a crucial process in the brewing industry. Having survived through the centuries to be part of our modern-day brewing technology, this indicates the efficiency of the fining process. In recent times, most beers are clarified by chilling followed by filtration. Fining is thus used as a pre-filtration treatment rather than its traditional application[12]. Nonetheless, it still plays a significant role in sedimenting suspended particles in beer.

This study investigated the use of isinglass, gelatin, chill guard, polyclar, Irish moss and activated carbon for clarification and their influence on the lipid profiles of rats.

MATERIALS AND METHODS

Study area: This study was carried out at the Department of Biochemistry, University of Port Harcourt Nigeria in July, 2019.

Equipment and apparatus: Analytical grade chemicals were used as received from standard commercial supplies without additional purification. The brewing materials used were also of food grade and obtained from reputable brewing stores.

Source of materials: *Sorghum bicolor* (red sorghum variety), *Zea mays* (white maize variety), *Ipomoea batatas* (off-white sweet potato), *Saccharomyces cerevisiae* (brewer's yeast), *Xylopia aethiopica* (grains of selim), *Zingiber officinale* (ginger) and the finings (gelatin, chill guard, isinglass, polyclar, Irish moss and activated carbon) used in this study were purchased from Mile 1 market in Port-Harcourt, Rivers State in Nigeria.

Brewing of sorghum beer: Sanitization was done using 1oz Star San Sanitizer, dissolved in 5 gallons of warm water and used to wash beer equipment[8].

Malting: The grains (sorghum and maize) were sorted by hand to remove debris. Sorghum grains (5 kg) were then steeped in distilled water (10 L) at room temperature (28-30°C) for 24 hrs. After rinsing with distilled water as described by Ezeogu and Okolo[13], the sorghum grains germinated at room temperature for 4 days. The resultant malted grains were sun-dried for 2 days and the malt formed was mixed with sorghum grains (5 kg), maize grains (2 kg) and sweet potato (1.5 kg) before milling to a consistent fine flour in a hammermill.

Wort production: Wort was produced by a decantation mashing technique developed for sorghum[14,15]. Milled malt weighing 9 kg was mixed with 22 L of distilled water at 45°C and left in decantation for 30 min. Afterwards, 10 L of the supernatant was removed while the mash residue was heated at 90°C for another

30 min to gelatinize the malt starch. The gelatinized malt starch was left to cool below 50°C before re-adding the supernatant to the brew pot. The resulting mixture was then brewed following this mashing program: 1 hr at 63°C, 10 min at 75°C and cooled to 30°C. After lautering, the filtrate (wort) was vigorously boiled for 1 hrs and a mixture of ground ginger and grains of selim (15 g) was added as flavor 10 min before the end of the boil.

Fermentation: The measured 20 L of wort was transferred into a fermenter equipped with an airlock bubbler and tap. It was then topped off with cold water until a 25 L mark. After cooling to about 24°C as described by Eßlinger[16], the cloudy wort was pitched at 1.037 specific gravity with 11.1 g of yeast. After pitching the yeast, the fermenter was sealed tight, the airlock and stopper were attached and the airlock was filled with water. The wort was then left to ferment at room temperature (25-28°C) for 72 hrs. After fermentation, 12 L of test beer was siphoned into six different glass bottles with caps (2 L each) and 5" headspace was provided for proper carbonation while awaiting clarification.

Physiochemical analysis: The pH was measured 15 min into the brew by taking clear wort from the top and allowed to cool to room temperature before measuring using a pH meter. The alcohol content of the beer was also determined by comparing the specific gravity of the beer before and after fermentation using a hydrometer[16].

Clarification of beer: Each fining was used according to the manufacturer's declaration. For the group clarified by filtration, muslin cloth of 1 mm was used to filter 2 L of test beer and preserved by refrigerating at a temperature below 10°C[8].

Experimental animals: Seventy male albino Wister rats, obtained from Animal House belonging to the Biochemistry Department of the University of Port Harcourt, were left to acclimatize for ten days at normal atmospheric temperature (25±5°C) and 12 hrs light/dark cycle before use. They were placed on standard feed and given access to water *ad libitum*.

Experimental design for animal study: A total number of 70 male albino Wistar rats weighing between 150-170 g were split into eight different groups and stained differently.

The perception of the average alcohol consumption is intended to help picture and estimate the absolute alcohol content of different beverage concentrations and serving sizes. Nevertheless, the definition of a standard drink and serving size differs from country to country[17]. According to the National Institute on Alcohol Abuse and Alcoholism[18], "moderate drinking" is defined as the consumption of approximately 30 g of alcohol. Hence, it is recommended for a 70 kg adult male to drink not more than 14 drinks (5% ABV) per week.

- If 70 kg (70,000 g) adult male = 1500 mL (5% ABV)
- 160 g albino rat = 3.42 mL (3% ABV)

To successfully extend this to humans, each group of 9 rats was subdivided into a sub-group of 3 each i.e., Groups A, B and C stained differently.

- Group A: 1.71 mL (3.42/2) daily (low alcohol dosage)
- Group B: 3.42 mL daily (moderate alcohol dosage)
- Group C: 6.84 mL (3.42×2) daily (high alcohol dosage)

Administration of clarified beer: After 10 days of acclimatization, the administration followed the experimental design outlined in Table 1 and lasted for 21 days. Group 1 served as the negative control group and was fed only on food and water. Group 2 was the positive control group and was administered T_0. Groups 3, 4, 5, 6, 7 and 8 were administered T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}, respectively at three dosages (1.71, 3.42 and 6.84 mL) equivalent to mild, moderate and binge alcohol drinkers.

Biochemical assays: On the twenty-first day, each rat was withdrawn from the cage and their weight taken five hours before sacrifice. The animals were then anaesthetized using a wet cotton wool moistened with chloroform and placed in a desiccator. The thoracic region was opened to expose the heart and blood was collected by cardiac puncture into well labeled EDTA bottles used for biochemical assays: Assessing levels of triglyceride, total cholesterol, low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein and non-high-density lipoprotein.

Statistical analysis: The triplicate data obtained from the experiment were expressed as Mean±Standard Error of Mean (SEM) and analyzed by One-way Analysis of Variance (ANOVA) at $p \leq 0.05$, using SPSS (Statistical Package for Social Sciences) version 20.0. Multiple comparisons were done using *post hoc* test.

RESULTS

Physicochemical analysis: The alcohol content of the test beer (T) was determined by comparing the specific gravity of the beer before and after fermentation using a hydrometer. The original and final specific gravity were 1.037 and 1.014, respectively producing a 3% alcohol by volume. The pH of the wort was also recorded as 5.4.

Sensory evaluation: After clarification, the different beer samples were generally assessed based on their degree of clarity and distinctive colour. All clarified beer samples appeared cloudy in the following order. Beers T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0, with the filtered beer being the cloudiest and Isinglass clarified beer showing the least cloudiness. Beer clarified with Irish moss also showed a distinctive reddish colour. Beers T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0 represent beer clarified with isinglass, activated charcoal, Irish moss, polyclar, chill guard, gelatin and by filtration, respectively.

Interrelationship between test beer and obesity: As shown in Table 2 and 3, there was approximately a 54% increase in the body weight of the negative control group between day one and twenty-one. However, the positive control group (T_0) showed approximately 30, 41 and 31% increase in body weight between day one and twenty-one for the head, back and tail, respectively. For the clarified beer samples, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} showed about (19, 43 and 33%), (22, 26 and 34%), (24, 30 and 33%), (27, 28 and 29%), (24, 19 and 25%) and (5, 20 and 25%) increase in body weight between day one and twenty-one for head, back and tail, respectively. When compared with the negative control group, T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0 all showed a significant increase ($p<0.05$) in body weight at day seven, fourteen and twenty-one for high and moderate alcohol dosages while day one showed an increase across all level of alcohol dosages. Finally, beer clarified with activated charcoal (T_{ac}) showed the least increase in body weight between day one and day twenty-one.

Table 1: Outline for administration of clarified beer, showing the grouping of animals per clarifier

Group	Number of rats	Food+Water	T_0	T_g	T_c	T_{ig}	T_p	T_{im}	T_{ac}
1	7	Yes	No						
2	9	Yes	Yes						
3	9	Yes		Yes					
4	9	Yes			Yes				
5	9	Yes				Yes			
6	9	Yes					Yes		
7	9	Yes						Yes	
8	9	Yes							Yes

T_0: Filtered test beer, T_g: Test beer clarified with gelatin, T_c: Test beer clarified with chillguard, T_{ig}: Test beer clarified with isinglass, T_p: Test beer clarified with polyclar, T_{im}: Test beer clarified with Irish moss and T_{ac}: Test beer clarified with activated carbon

Table 2: Body weight of male albino Wistar rats taken at day one and seven

	Day 1			Day 7		
	148.25±1.70			160.77±3.73		
Group						
Negative control	High	Moderate	Low	High	Moderate	Low
T_0	157.67±2.33	147.00±0.57	158.67±0.88	169.33±5.05	160.93±3.49	162.73±3.227
T_g	174.33±1.852	155.00±3.60	167.33±2.67	193.23±3.25	179.77±5.27	175.77±2.17
T_c	172.00±0.00	154.00±0.00	163.67±5.20	177.90±0.58	157.33±0.86	164.70±1.80
T_{ig}	170.67±3.48	160.00±3.60	168.33±3.28	184.43±2.77	166.67±4.69	175.90±6.26
T_p	171.33±1.20	161.00±4.00	167.00±4.04	177.70±2.84	172.27±6.99	162.20±3.46
T_{im}	174.33±5.89	162.00±4.00	170.00±3.05	183.77±7.49	157.33±8.10	164.63±1.69
T_{ac}	177.33±5.89	161.00±2.08	170.00±4.16	169.40±6.96	161.50±1.47	160.00±5.60

Data obtained from the experiment were expressed as Mean±Standard Error of Mean with significant difference ($p<0.05$), Negative control represents group fed with only food and water while T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0 represent beer clarified with isinglass, activated charcoal, Irish moss, polyclar, chillguard, gelatin and by filtration, respectively

Table 3: Body weight of male albino Wister rats taken at day fourteen and twenty-one

	DAY 14			DAY 21		
	172.57±3.65			202.13±4.80		
Group						
Negative control	High	Moderate	Low	High	Moderate	Low
T_0	178.06±5.96	176.70±3.03	172.57±4.74	194.33±5.66	195.50±3.26	192.50±8.65
T_g	205.57±4.36	182.27±7.58	191.93±0.86	202.67±3.61	209.53±10.45	206.73±1.90
T_c	188.80±1.53	171.93±3.59	191.83±2.80	204.10±4.99	187.30±5.98	202.13±5.69
T_{ig}	195.23±2.91	182.97±6.85	190.26±7.24	204.09±1.59	200.47±4.85	157.27±10.74
T_p	192.77±5.68	185.23±6.85	185.20±4.02	210.90±7.28	198.17±6.98	202.80±2.72
T_{im}	196.00±7.86	171.43±8.82	187.27±1.94	209.03±9.10	188.90±12.71	200.70±1.11
T_{ac}	175.53±5.51	174.63±3.56	179.57±4.56	185.77±7.08	188.93±0.63	200.00±1.88

Data obtained from the experiment were expressed as Mean±Standard Error of Mean with significant difference ($p<0.05$), Negative control represents the group fed with only food and water while T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0 represent beer clarified with isinglass, activated charcoal, Irish moss, polyclar, chillguard, gelatin and by filtration, respectively

Lipid profile study

Assessing variations in triglyceride level: In animal model studied in this marker, T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} all showed a significant ($p<0.05$) increase in triglyceride level when compared with the negative control. In groups 2, 7 and 8, the level of triglyceride decreased with a corresponding increase in alcohol dosage from low to high dosages. While in groups 3, 5 and 6, the level of triglyceride increased with a corresponding increase in alcohol dosage from low to high dosages. On the other hand, at moderate alcohol dosage, T_c showed a characteristically low level of triglyceride (Table 4).

Assessing variations in total cholesterol concentration: Analysis of albino Wister rats fed T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} all showed significant ($p<0.05$) rise in total cholesterol concentration when compared with the negative control with T_{im} showing the highest concentration for high, moderate and low alcohol dosages, respectively. Besides group 2, the level of total cholesterol increased with a corresponding increase in alcohol dosage from low to high dosages (Table 4).

Assessing variations in High-Density Lipoprotein (HDL) level: Analysis of albino Wister rats fed T_g, T_c, T_{ig} and T_{im} all showed a significant ($p<0.05$) decrease in high-density lipoprotein when compared with the negative control. In groups 3, 5, 6 and 7, the level of HDL decreased with a corresponding increase in alcohol dosage from low to high dosages. While group 2 showed an increase with a corresponding increase in alcohol dosage. At high alcohol dosage, T_c showed significantly low HDL activity when compared with the negative control while T_{im} showed generally low levels of HDL activity when compared to every other group (Table 5).

Table 4: Cholesterol profile (triglyceride and total cholesterol) of study animals

		Triglyceride (mg/dL)			Total cholesterol (mg/dL)		
	Group	188.00±24.16			96.50±4.94		
*	Negative						
a	control	High	Moderate	Low	High	Moderate	Low
b	T_0	291.00±19.21	$370.00±9.07_{ad}$	$423.33±15.34_{a}$	$116.00±2.08_{cdefgh}$	$125.67±1.45_{aefg}$	$123.33±0.67_{aeg}$
c	T_g	$393.00±6.00_{a}$	343.00±48.56	355.33±10.10	$199.00±10.00_{abgh}$	$124.67±2.40_{aefg}$	$113.33±0.88_{deg}$
d	T_c	$413.67±23.21_{ah}$	$185.67±4.70_{beg}$	350.33±42.21	$211.00±2.88_{abefgh}$	$147.67±3.17_{aeg}$	$138.66±1.20_{acfgh}$
e	T_{ig}	$412.00±9.60_{ah}$	$429.67±40.13_{ad}$	$403.33±63.33_{a}$	$183.67±2.33_{abdgh}$	$174.33±3.71_{abcdfgh}$	$148.00±2.88_{abcfgh}$
f	T_p	$467.00±6.65_{ah}$	338.67±30.77	324.00±79.77	$172.00±3.60_{abdgh}$	$151.00±4.61_{abcegh}$	$109.3±3.88_{deg}$
g	T_{im}	378.66±7.88	$387.50±3.50_{ad}$	$413.67±8.76_{a}$	$260.00±11.54_{abcdefh}$	$215.50±1.50_{abcdefh}$	$203.33±6.23_{abcdefh}$
h	T_{ac}	$232.00±33.24_{def}$	324.00±47.00	$387.33±40.91_{a}$	$139.33±2.72_{bcdefg}$	$121.50±1.50_{aefg}$	$110.67±1.85_{deg}$

Values are Mean±Standard Error of Mean, n = 3, Values found under each column labeled high, moderate and low with subscript letters (a, b, c, d, e, f, g or h) are significantly different when compared with the corresponding negative control and test samples (T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) labelled a, b, c, d, e, f, g and h, respectively under column*. Values without subscript letters are not significantly different ($p<0.05$) when compared with the negative control and test samples

Table 5: Cholesterol profile (high and low-density lipoprotein) of study animals

		High-density lipoprotein (mg/dL)			Low-density lipoprotein (mg/dL)		
	Group	60.75±3.94			13.75±5.56		
*	Negative						
a	control	High	Moderate	Low	High	Moderate	Low
b	T_0	$63.67±2.02_{cdefgh}$	$58.00±3.21_{g}$	$52.67±0.88_{g}$	$17.60±2.60_{cdefg}$	$23.67±2.96_{g}$	$21.33±1.20_{g}$
c	T_g	$33.50±1.50_{abfh}$	$53.33±3.28_{g}$	$52.67±1.20_{g}$	$109.00±8.00_{abfgh}$	$30.33±9.38_{g}$	$19.00±1.00_{g}$
d	T_c	$27.67±0.67_{abfh}$	$59.00±3.05_{g}$	46.00±1.52	$123.00±4.58_{abefgh}$	$61.00±0.57_{g}$	$48.00±0.93_{g}$
e	T_{ig}	$34.00±4.35_{abfh}$	51.67±0.67	$53.33±7.31_{g}$	$95.60±3.71_{abdgh}$	$49.00±16.77_{g}$	$44.67±10.92_{g}$
f	T_p	$42.33±2.84_{bcdeg}$	$43.67±1.76_{ah}$	$50.00±4.16_{ag}$	$70.00±6.50_{abcdg}$	$59.67±3.17_{g}$	$21.00±8.32_{g}$
g	T_{im}	$25.67±2.40_{abfh}$	$31.50±1.50_{abcdh}$	$34.00±1.73_{abcef}$	$173.33±12.46_{abcdefh}$	$127.50±2.50_{abcdefh}$	$110.33±7.17_{abcdefh}$
h	T_{ac}	$45.33±2.72_{bcdeg}$	$63.00±7.00_{fg}$	40.67±0.88	$61.00±2.08_{cdeg}$	$14.50±5.50_{g}$	$54.33±29.61_{g}$

Values are Mean±Standard Error of Mean, n = 3, Values found under each column labeled high, moderate and low with subscript letters (a, b, c, d, e, f, g or h) are significantly different when compared with the corresponding negative control and test samples (T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) labelled a, b, c, d, e, f, g and h, respectively under column*. Values without subscript letters are not significantly different ($p<0.05$) when compared with the negative control and test samples

Assessing variations in Low-Density Lipoprotein (LDL) level: All the clarified beer samples (T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) showed a significant ($p<0.05$) increase in low-density lipoprotein levels when compared with the negative control with T_{im} showing the highest level for high, moderate and low alcohol dosages respectively. At moderate alcohol dosage, T_{ac} showed a characteristically low amount of LDL activity. Besides group 4, where LDL levels reduced with an increase in alcohol dosage, the level of LDL in other groups increased with a corresponding increase in alcohol dosage from low to high-dosages in albino Wister rats studied (Table 5).

Assessing variations in Very Low-Density Lipoprotein (VLDL) level: Analysis of albino Wister rats fed T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} all showed significant ($p<0.05$) increase in the level of very low-density lipoprotein when compared with the negative control except T_c which showed no significant difference at moderate alcohol dosage. At high alcohol dosages, T_p showed the highest level of VLDL when compared with the negative control while T_{ac} showed the least level of VLDL at high dosages when compared with its moderate and low dosages for high, moderate and low alcohol dosages, respectively (Table 6).

Assessing variations in Non-High-Density Lipoprotein (Non-HDL) level: Groups 2-8 (T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) all showed significant ($p<0.05$) increase in non-high-density lipoprotein when compared with the negative control with T_{im} showing the highest level of non-HDL for high, moderate and low alcohol dosages, respectively. Besides group 2, where non-HDL levels decreased with an increase in alcohol dosage, the level of non-HDL in groups 3, 5, 6 and 7 increased with a corresponding increase in alcohol dosage from low to high dosages in albino Wister rats studied (Table 6).

Table 6: Cholesterol profile (very low and no high-density lipoprotein) of study animals

		Very low-density lipoprotein (mg/dL)			Non-high-density lipoprotein (mg/dL)		
	Group	37.50±4.79			30.00±3.34		
*	Negative						
a	control	High	Moderate	Low	High	Moderate	Low
b	T_0	57.67±3.71	$73.67±1.76_{ad}$	$84.33±3.17_{a}$	$52.33±3.71_{cdefgh}$	$68.00±4.35_{aefg}$	$71.00±1.15_{ag}$
c	T_g	$78.00±1.00_{a}$	68.33±9.49	70.67±2.02	$165.50±8.50_{abfgh}$	$71.00±4.93_{aefg}$	$60.67±1.76_{aeg}$
d	T_c	$82.67±4.70_{ah}$	$37.00±1.00_{beg}$	53.33±11.55	$183.00±2.64_{abfgh}$	$87.60±0.88_{aefg}$	$92.33±1.20_{ag}$
e	T_{ig}	$81.33±1.76_{ah}$	$85.66±8.19_{ad}$	$80.67±12.67_{a}$	$152.00±5.03_{abgh}$	$121.67±4.33_{abcdgh}$	$97.00±10.11_{acg}$
f	T_p	$92.67±1.76_{ah}$	66.00±6.80	64.67±15.89	$129.67±6.33_{abcdg}$	$107.33±6.35_{abcdgh}$	$59.33±3.48_{ag}$
g	T_{im}	$80.67±5.36_{ah}$	$77.50±1.50_{ad}$	$83.67±1.20_{a}$	$234.33±13.87_{abcdefh}$	$185.00±2.00_{abcdefh}$	$169.00±7.50_{abcdefh}$
h	T_{ac}	$46.33±6.88_{efg}$	74.00±1.00	$77.00±8.18_{a}$	$94.33±2.02_{abcdeg}$	$58.00±7.00_{aefg}$	$70.00±2.30_{ag}$

Values are Mean±Standard Error of Mean, n = 3, Values found under each column labeled high, moderate and low with subscript letters (a, b, c, d, e, f, g or h) are significantly different when compared with the corresponding negative control and test samples (T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) labelled a, b, c, d, e, f, g and h, respectively under column*. Values without subscript letters are not significantly different ($p<0.05$) when compared with the negative control and test samples

DISCUSSION

At 3% alcohol by volume, albino Wister rats in groups 2-8 all showed a significant increase in body weight between day one and twenty-one when compared with group 1 (negative control) as shown in Table 2 and 3.

Amongst the study beer, the unclarified beer (T_0) showed the highest increase in body weight between day one and twenty-one while beer clarified with activated charcoal (T_{ac}) showed the least increase in body weight within the same period. This result proposed that moderate to high daily consumption of alcohol (≥1.5L beer for a 70 kg adult male) poses a risk factor for obesity, which often leads to the development of other diseases[19]. Due to the presence of more carbohydrates per unit of ethanol compared to other alcoholic beverages like spirits and wines, beer consumption is generally thought to elevate the amount of available energy needed by the body thus increasing the risk of obesity[20]. Hence, for persons who choose to drink, factors such as body weight and body fat location should be considered when establishing daily calorie limits with the hope of reducing the possibility of abdominal obesity due to high energy content. Abdominal obesity (pot-belly) coupled with a sedentary lifestyle potentiates the development of type 2 diabetes hypertension, coronary heart diseases and high oxidized low-density lipoprotein cholesterol[21,22].

Generally, samples from T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} animals all showed significant increases in triglyceride and total cholesterol concentrations when compared with the negative control group (Table 4). Total blood cholesterol measures the level of HDL-cholesterol, LDL-cholesterol and other lipid components. This information is used to determine patients' risk of developing heart disease and the best way to manage it. On the other hand, triglycerides store unused calories and provide energy. Hence, it constitutes the main body fat in humans and plays a key role in aiding the bidirectional transference of blood glucose and adipose fat from the liver[23]. In human bloodstreams, high levels of triglycerides have been linked to obesity which potentiates the development of type 2 diabetes hyper-tension, coronary heart diseases, high oxidized low-density lipoprotein cholesterol[21,22] and stroke[24]. High levels of triglycerides observed in this study may have contributed to the increase in body weight shown in Table 2 and 3. The risk posed by triglycerides is associated with a strong inverse relationship with high-density lipoprotein (good cholesterol) cholesterol and its levels increasing the quantity of Low-Density Lipoprotein (LDL)[25]. The progressive increase in total cholesterol concentrations (with a corresponding increase in alcohol dosage) shown in Table 4 further suggests a risk of developing coronary heart diseases in rats fed with study beers especially rats fed T_{im} which showed the highest concentration of total cholesterol (378.66±7.88, 387.50±3.50 and 413.67±8.76) across all dosages.

In Table 5, samples from T_g, T_c, T_{ig} and T_{im} groups showed notable decrease in HDL levels from the negative control with T_{im} showing the least (25.67±2.40, 31.50±1.50 and 34.00±1.73) when compared with every other group. High-density lipoprotein reduces, reuses and recycles low-density lipoprotein cholesterol from other parts of the body and transports it back to the liver. At low HDL levels, the endothelium of blood vessels stands a risk of being damaged thereby increasing the danger of atherosclerosis, which leads to several heart diseases and stroke[26].

All clarified beer samples (T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac}) in Table 4 showed a significant rise in the level of low-density lipoprotein (bad cholesterol) from the negative control with Tim showing the highest values (173.33±12.46, 127.50±2.50 and 110.33±7.17). Low-density lipoprotein supplies fat for receptor-mediated endocytosis by transporting the fat molecules around the body in the extracellular fluid[27]. High levels of low-density lipoprotein potentiate the development of cardiovascular diseases[28] when they invade the endothelium and become oxidized, as they are more easily retained in oxidized form by the proteoglycans.

Animals fed T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} all showed significant ($p<0.05$) increases in Very Low-Density Lipoprotein (VLDL) as well as Non-High-Density Lipoprotein (Non-HDL) when compared with the negative control (Table 6). The VLDL enables the movement of fats and cholesterol within the water-based component of the bloodstream. It also serves in transporting hydrophobic intercellular messengers such as the morphogen Indian hedgehog (protein) across long-range[29]. Extra triglycerides stored in fat cells are later released when needed for energy. High levels of VLDL can be linked with the development of plaque deposits on the walls of the artery and this narrows/restricts the passage of blood. Lowering triglyceride levels is the best approach towards lowering VLDL cholesterol. Lately, non-HDL cholesterol has been used as a marker to monitor blood lipid patterns and their relationship with heart diseases. Hence, the highest levels of non-HDL cholesterol seen in T_{im} (234.33±13.87, 185.00±2.00 and 169.00±7.50) when compared with other groups further suggests the negative relationship that exists between beer clarified with Irish moss and cholesterol.

Although the level of bad cholesterol and triglyceride generally increased with a corresponding increase in alcohol concentration across all groups, T_{im} was most prominent thus posing the most increased risk of heart-related diseases.

In recent times, many critics have made different propositions. Some say that certain advantageous antioxidant flavonoids are removed by some finings while others target polyphenolic compounds thus potentiating carcinogenesis. Others claim that key proteins, electrolytes, charged polysaccharides and other vital nutrients are drained out after clarification thus altering protein intake and some neurological activities. However, the validity of such claims remains vague. Unaware of any previous attempt to empirically address these questions, there was a need to research selected clarifying agents of beer production and their influence on selected biochemical profiles of albino Wistar rats with the hope of understanding their effect in humans.

Beer (3% alcohol by volume) brewed from a blend of sorghum, white maize and sweet potato was grouped, labelled and clarified with isinglass, activated charcoal, Irish moss, polyclar, chillguard, gelatin and by filtration (T_{ig}, T_{ac}, T_{im}, T_p, T_c, T_g and T_0, respectively). All study beers appeared cloudy in the following order $T_{ig}<T_{ac}<T_{im}<T_p<T_c<T_g<T_0$, with the filtered beer (T_0) being the cloudiest and Isinglass clarified beer (T_{ig}) showing the least cloudiness.

Amongst the study beer, unclarified beer (T_0) showed the highest increase in body weight between day one and twenty-one while beer clarified with activated charcoal (T_{ac}) showed the least increase in body weight within the same period.

In relating these clarifying agents with lipoproteins and fat, T_0, T_g, T_c, T_{ig}, T_p, T_{im} and T_{ac} showed significant increases in the levels of triglyceride, total cholesterol, very low-density lipoprotein as well as non-high-density lipoprotein when compared with the negative control. Animals fed T_g, T_c, T_{ig} and T_{im} all showed significant ($p<0.05$) decreases in observed levels of high-density lipoprotein.

Ethanol, when consumed in excess, often leads to neurotoxicity and if taken continuously over a long period of time, may lead to hepatotoxicity. Ingested ethanol is often metabolized to acetaldehyde which potentiates the development of reactive oxygen species, leading to cellular damage. The acetaldehyde produced is metabolized to acetic acid, before acetyl-CoA. Acetaldehyde stimulates the enzyme Sterol Regulatory Element-Binding Protein 1 (SREBP-1c) which is responsible for activating enzymes of de novo lipogenesis[30].

Malonyl-CoA is produced in excess thus leading to the inhibition of carnitine palmitoyl transferase-1 which inhibits the β-oxidation of fatty acids[31]. Fatty acid β-oxidation is also blocked by ethanol by inhibiting adenosine monophosphate-activated protein kinase and peroxisome proliferation-activated receptor-α. Consequently, amplified de novo lipogenesis hinders fatty acid β-oxidation in the liver, leading to the accumulation of intrahepatic lipid[30].

Export of Very Low-Density Lipoprotein (VLDL) is the key lipoprotein in reducing intrahepatic lipid and VLDL synthesis needs correct apoB100 protein folding before export which rests on microsomal triglyceride transfer protein (MTP). Still, ethanol reduces hepatic peroxisome proliferation-activated receptor-α which downregulates MTP[32] leading to alterations in VLDL particle size and succeeding rate of blood clearance, consequently potentiating hypertriglyceridemia alongside obesity besides recorded high nutrient content[33]. Purification and clarification were as good as reported by Pinguli *et al.*[34] and could benefit from catalyzed steps using immobilized enzymes.

The study highlights the potential impact of clarifiers in beer brewing on the lipid profile of consumers, raising concerns about the nutritional composition of the beverage.

These findings imply that beer drinkers should be mindful of the potential effects on their lipid levels and consider moderation in consumption. Brewers and manufacturers can utilize the study's findings to develop alternative clarifying agents or techniques that minimize the potential alteration of lipid profiles in beer.

Health-conscious consumers can make informed choices by selecting beer brands that employ clarifiers with minimal impact on lipid profiles. Further research should be conducted to investigate the specific clarifying agents used in the brewing process and their precise effects on lipid profiles.

Regulatory bodies and industry organizations should consider establishing guidelines or standards for the use of clarifiers in beer production to protect consumer health.

The study's findings may not fully represent the broad range of clarifiers used in beer brewing, as the research focused on specific clarifying agents.

The study might not account for variations in individual responses to the altered lipid profiles, as genetic and lifestyle factors can influence lipid metabolism differently.

CONCLUSION

The study indicates a significant rise in body weight among albino Wistar rats with 3% alcohol by volume, suggesting a potential risk of obesity linked to moderate to high daily alcohol intake. The type of beer and its clarifying agents play a role, with unclarified beer showing the highest weight gain. The research also reveals heightened levels of triglycerides, total cholesterol, very low-density lipoprotein and non-high-density lipoprotein in rats fed with study beers. The findings emphasize the need to consider body weight and fat distribution when setting daily calorie limits for alcohol consumers. Elevated levels of triglycerides, low-density lipoprotein and non-high-density lipoprotein suggest potential cardiovascular risks associated with beer consumption. The study prompts questions about the impact of specific clarifying agents on beer nutrition, underscoring the necessity for further research and potential adjustments in brewing practices. Recommendations include mindful beer consumption, exploring alternative clarifying agents and establishing guidelines for clarifier use in beer production to safeguard consumer health. Overall, this research contributes to the awareness of the nutritional implications of beer consumption, advocating for a considerate approach to brewing practices and consumer choices.

SIGNIFICANCE STATEMENT

This study discovered that animals fed beers clarified with isinglass, Irish moss, chill guard and gelatin all showed a significant decrease in high-density lipoprotein that can be beneficial for understanding amplified de novo lipogenesis which may lead to cellular damage. This study will help the researchers uncover the critical areas of alcohol-induced heart-related diseases that many researchers were not able to explore and show empirical evidence. Thus, a new theory on considerations for choosing beer clarifiers may be arrived at.

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