

Original Article

An experimental study on the impact of the Sudanese liquor (Aragi) in the etiology of liver damage

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Abstract

Background: Alcoholism is considered as one of the life threatening problems due to its enormous effects on liver.

Aims: To identify the biochemical and histological changes of Aragi on the liver of Wistar albino rats.

Materials and Methods: It was conducted in fifteen adult male and female Wistar albino rats, five of which were slaughtered at day (zero) for histological investigation. The other ten rats were divided equally between the control and tested groups. The control was given water while the tested group was given Aragi for sixty days. The levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were measured at days (zero), (30) and (60) for both groups. Liver samples were investigated for histological changes at day (60) for both groups.

Results and discussion: The results revealed a statistically significant increase in the levels of ALP at day (30) ($p < 0.01$), while GOT, GPT showed a significant increase at day (60) ($p < 0.01$), as compared to the control. Histological findings of the livers of Aragi drinker rats, at day (60) revealed different grades of multinuclear cell infiltration, fibrosis, focal necrosis, fatty degeneration, as compared to the control and day (zero) groups, which showed no histological alteration. It was observed that three females of the control group gave birth to new offsprings during the sixty days of the experiment while none of the Aragi drinker females did.

Conclusion: Aragi consumption plays a major role in liver damage which may lead to death, as well as impairment of fertility.

Key words: Aragi, enzymes, histology, liver, rats.

Introduction

Alcoholism is the most widely used term to describe patients with alcohol problems⁽¹⁾. Alcohol (ethanol) is the number one favorite mood altering drug in the United States⁽²⁾, it is not only a psychoactive drug but is also considered as part of the basic food supply in many societies. After ingestion most of the alcohol is metabolized in the liver by hepatic alcohol

dehydrogenase enzyme. A large intake of alcohol has enormous effects on nutritional status thereafter absorption, destruction and elimination begin, the kidneys and lungs excrete about one-tenth of the total alcohol ingested unchanged, the remaining alcohol undergoes oxidation. The oxidation of alcohol produces energy that results in temporary reduction of fear and conflict thereby strengthening drinking-

desire⁽³⁾. The major effect of acute alcoholism is associated with the central nervous system⁽⁴⁾.

Alcohol has been a constant presence in African social life for centuries as it has been in most parts of the world⁽⁵⁾, alcohol social consequences affect individuals other than the drinkers, such as traffic casualties, or incidence of violence in the family⁽⁶⁾.

Aragi is the native alcoholic drink commonly used in the Sudan; it has been the drink of choice to most people who take alcohol, due to its affordable price and availability. Both the amount of the drink consumed and the number of people taking it are assumed to have risen. This implies an increase in the risk of the emergence of toxicity due to Aragi intake⁽⁷⁾. Although Aragi consumption is widely spreading in Sudan, research in the adverse effects of Aragi is scarce, sparse and scanty. This study which aimed to identify the biochemical and histological changes of Aragi on the liver of Wistar albino rats was run to participate with other relevant researches in filling this gap.

Materials and methods

Preparation of traditional Aragi: The alcoholic drink used for this study is Aragi which is a traditional native Sudanese liquor prepared from dates fermented by yeast, which converts the sugar found in dates into alcohol. A small amount of yeast was added to boiled water and then poured into washed dates in a clean pot. The pot was firmly covered and left for 3 days, after which the fermented Aragi was then distilled and collected in a clean bottle, and then it was diluted to 50%.

Study subjects: This research was conducted in adult male and female Wistar albino rats weighing (109-142 grams). The animals were divided to two groups. The test group was given Aragi throughout the experiment, while the control group was given water instead. The choice of the study animals was based on

selection of adult, healthy rats and exclusion of young, diseased rats. All animals received humane care according to the guidelines outlined by the Committee for the Purpose of Control and Supervision on Experiments on Animals⁽⁸⁾.

Study area: This study was carried out at the Biomedical Research Laboratory (2)-Ahfad University for Women. Biochemical analysis was conducted at the National Health Laboratory. Histological examination was performed at Faculty of Veterinary Medicine – University of Khartoum.

Diet of rats: The diet given to the rats consisted of a mixture of (meat + flour + milk + salt + oil).

Study procedure: This experimental case control study was conducted in a total number of 15 rats, 5 of which were slaughtered at day (0) for histological investigation of the liver. The other 10 rats were divided equally between the control and the tested groups.

Both groups were kept under the same environmental conditions and were provided with the same amount of water and food. Rats were kept in their new location for 10 days before starting the experiment (as a period of adaptation). Since day (0) up to day (60) the test group was given a calculated dose of Aragi (equivalent to one cup taken by a human being) and a small amount of Aragi was added to their drinking water (so as to ensure presence of alcohol in their blood). The control group was given water instead of Aragi. Blood samples (from both groups) were collected at days 0, 30 and 60 for biochemical analysis. Furthermore at day (60) all rats were slaughtered for histological investigation of the liver and the results were compared with that of day (0) and the control.

Biochemical analysis: Blood samples were collected from the retro-orbital plexus of rats using heparinized capillary tubes as described by Khanna et al⁽⁹⁾. This

procedure was performed after anaesthetizing the rats through inhalation of chloroform soaked in a piece of cotton. Blood samples were collected into heparinized sample containers. The levels of GOT, GPT and ALP liver enzymes were determined by colorimetric method using Plasmatec kits ⁽¹⁰⁾.

Histopathological examination: The specimens were collected immediately after slaughtering and fixed in 10% formaldehyde and were investigated according to the method described by Bancroft and Gumble ⁽¹¹⁾, and then light microscopic examinations were carried out at magnifications of 4x, 10x and 40x. Histopathological findings, of portal tract lesions, focal necrosis, fibrosis and fatty degeneration, were expressed qualitatively with + sign(s) (+: slight, ++: moderate and +++: severe) according to the dissemination of pathologies. Other findings were reported as present or absent as described by Ustundag et al ⁽¹²⁾.

Statistical analysis: Mean values in plasma parameters were compared using the student's paired T-test to detect the difference. Chi square was applied to estimate the correlation between plasma parameters and time.

Results

According to visual observation the following findings were noticed: Compared to the control, the Aragi drinkers showed more food consumption and different behaviors. The three females of the control group gave birth to new offsprings during the sixty days of the experiment while none of the Aragi drinker females did.

Biochemical analysis

There was a significant elevation in the blood level of GOT hepatic enzyme at day (60) ($P < 0.01$), after administration of Aragi to the test group, as compared to the control (Table 1).

Table1: Effect of Aragi on the level of GOT enzyme

	Day 0	Day 30	Day 60
Control group	35.2 ± 2.7	166.9 ± 15.9	324 ± 67.8
Aragi drinkers	34.6 ± 4.7	192.0 ± 20.8	521.6 ± 16.6*

(Data were expressed in mean ± standard error of mean)

* = ($P < 0.01$) (at 99% confidence)

Similar to the effect on GOT there was a significant elevation in the blood level of GPT hepatic enzyme at day (60) ($P < 0.01$), after administration of Aragi to the test group, as compared to the control (Table 2).

Table 2: Effect of Aragi on the level of GPT enzyme

	Day 0	Day 30	Day 60
Control group	27.2 ± 1.98	173.8 ± 12.0	333.9 ± 68.7
Aragi drinkers	31.0 ± 3.01	198.4 ± 18.7	528.6 ± 14.1*

(Data were expressed in mean ± standard error of mean)

* = ($P < 0.01$) (at 99% confidence)

Regarding ALP hepatic enzyme there was a significant increase at day (30) ($P < 0.01$), as compared to the control (Table 3).

Table 3: Effect of Aragi on the level of ALP enzyme

	Day 0	Day 30	Day 60
Control group	115.7 ± 9.5	404.8 ± 100.1	179.2 ± 11.9
Aragi drinkers	100.4 ± 15.5	611.4 ± 15.2*	195.4 ± 19.6

(Data were expressed in mean ± standard error of mean)

* = ($P < 0.01$) (at 99% confidence)

Histopathological findings

The results of the histological findings for the livers of the different study groups were expressed in table (4).

Table 4: Histopathological findings of the study groups

Histological findings	Day 0 N=4	Control (day 60) N=4	Aragi drinkers (day 60) N=4
<i>In portal tract MNCI</i>			
Slight (+)	1	-	-
Medium (++)	-	-	2
Severe (+++)	-	-	2
<i>Fibrosis</i>			
Slight (+)	-	-	-
Medium (++)	-	-	1
Severe (+++)	-	-	3
<i>Focal necrosis</i>			
Slight (+)	-	-	-
Medium (++)	-	-	-
Severe (+++)	-	-	4
<i>Fatty degeneration</i>			
Slight (+)	-	-	-
Medium (++)	-	-	2
Severe (+++)	-	-	2

N=number of rats

MNCI=Multi Nuclear Cell Infiltration

It was noted that by the end of the second month one of Aragi drinker rats died, and one of the control group rats escaped, thus the net number of rats for each group became four instead of five.

Discussion

Most of health professionals agree that alcohol affect practically every organ in the human body. Alcohol consumption has been linked to more than 60 disease conditions. The World Health Organization estimated that there are about two billions people worldwide who consume alcoholic beverages and 76.3 million with diagnosable alcohol use disorders. Alcohol causes 1.8 million deaths (3.2% of total) and loss of 58.3 million (4% of total) of disability-adjusted life years⁽⁶⁾.

The selected biochemical parameters were liver enzymes represented by GOT, GPT and ALP. There was a statistically significant increase in the levels of ALP at day (30) ($p < 0.01$), while GOT, GPT showed a significant increase at day (60) ($p < 0.01$), as compared to the control.

A previous study reported that various biochemical parameters have assumed significance in alcohol use disorder⁽¹³⁾.

Another study revealed that plasma GOT values were significantly higher in the ethanol group compared to the control ($P < 0.01$)⁽¹²⁾.

Histological findings of the livers of Aragi drinker rats, at day (60) revealed different grades of multinuclear cell infiltration, fibrosis, focal necrosis, fatty degeneration, as compared to the control and day (zero) groups, which showed no histological alteration.

Purhit et al, mentioned that, in the initial stages of the alcoholic liver disease fat accumulation in hepatocytes lead to the development of fatty liver (steatosis), this may progress to hepatitis and fibrosis, which may lead to liver cirrhosis⁽¹⁴⁾.

Kown et al, reported that different grades of fatty liver developed in all the rats that received alcohol as part of their diets⁽¹⁵⁾. Fatty liver can be synthesized due to excess alcohol, and thus patients were susceptible to liver fibrosis⁽¹⁶⁾.

Chronic intoxication with ethanol is probably the most common cause of liver fibrosis⁽¹⁷⁾, which leads to hemodynamic and functional abnormalities that, when extensive, may be life-threatening. The formation of fibrosis and granulation tissue occupies the places of normal hepatocytes, which then leads to dysfunction of the liver⁽¹⁸⁾.

In conclusion this experimental case control study revealed severe malfunction of the hepatic activity, due to excessive Aragi intake, represented by marked elevation in the levels of GOT, GPT and ALP enzymes. Furthermore, there was a great cellular damage manifested by medium and severe multinuclear cell infiltration, severe fibrosis, medium and severe focal necrosis, medium and severe fatty degeneration. It was also noticed that female fertility was greatly affected and the body weights were not stable. Besides all these substantial findings, it was noticed that some of the drunken rats were so aggressive that they badly injured each other.

Further researches are recommended to investigate the effects of alcoholism on female's fertility.

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