The effect of ethanolic extract and Caffeine extract of the yerba Meta plant (*ilex paraguariensis*) on the vital functions and tissues of the liver and kidneys in guinea pigs

Raja Aburwais [(1)], Emhemed Abukhattala [(2)], Fadi Ashini [(1), and] Monder Arafat [(1)]
Pharmaceutical Technology, Medical Technology Faculty, Misrata [1]
School of Life Sciences, Libyan Academy Misurata [2]
r.aburwais@mtc.edu.ly

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Abstract

The active compounds contained in medicinal plants are generally the ones to which the therapeutic effect of diseases is attributed. This study aims to use the caffeine alkaloid extracted from the plant and the ethanolic extract of the yerba mate plant to know its effect on blood contents and rates of fat and vital tissues using experimental animals of the guinea pig type males, which are divided into five groups equally. The fifth group is a control group, and the first and second groups were injected with caffeine at a concentration of 10 and 20 mg/kg, respectively, and the third group was injected with yerba mate at a concentration of 200 mg/kg, while the fourth group was injected with mate at a concentration of 100 mg/kg. At the end of the experiment, blood samples were taken to examine lipid levels and blood biometrics, and organs (kidneys, liver) were removed. Transfer it to the laboratory to conduct histological examinations. It was concluded from these tests that the extract of ethanol from the Yerba mate plant at a concentration of 200 mg/kg reduces the level of cholesterol and triglycerides in the blood. It is estimated with statistical significance (P = 0.0035). However, a concentration of 100 mg/kg of Yerba mate extract increases kidney function rates and liver enzymes and has no effect on liver tissue The kidney. The results showed that caffeine at concentrations of 20 mg/kg and 10 mg/kg had no clear effect. On biochemical blood tests. A concentration of 20 mg/kg of caffeine showed tissue damage to the liver, the accumulation of fat around its cells, and destruction in the urinary tubules in the kidney, and to a lesser degree in a concentration of 10 mg/kg. It was concluded that ethanolic yerba mate extract and caffeine have an effect on liver and kidney tissue and a clear effect on biochemical rates.

I. INTRODUCTION

Caffeine is a widely used alkaline stimulant found in more than 60 plants. It is the most common of the coffee or tea plants, and other sources include the koala, cocoa, and mate. It is known that caffeine is a diuretic and a mood modifier, which leads to a spike in dopamine in the brain. Serotonin and catechol amines are metabolites of caffeine and the risks of discontinuation of use Caffeine can cause low levels of serotonin, anxiety, irritability, an inability to focus, and depression. Excess caffeine can lead to caffeine poisoning, caffeine-induced anxiety disorder, or sleep disturbance, and studies indicate that caffeine intake is a dangerous factor and causes abortion (Bastos et *al.*, 2005).

A study conducted by Heck and Mejia (2007) showed that latte tea has a stimulant effect on the central nervous system as it contains a caffeine alkaloid, which is attributed to its stimulant effect and effectiveness in regulating heartbeat. This is according to a study that found that latte tea is used as an alternative to a stimulant hot drink such as coffee or tea that contains caffeine as the main compound that affects the nervous system. The study showed beneficial properties of mate tea from a therapeutic point of view, such as treating hypercholesterolemia and reducing lipoprotein

levels, which reduces the risk of cardiovascular disease (Mejia, D., and Heck, 2007). A study conducted in the United States on the health benefits of mate tea (2008) found that mate tea has an effective therapeutic effect as an antimicrobial, anti-oxidant, diabetes prevention, and improvement of the digestive and nervous systems (Burris, K., et al., 2012). A study by Borges-Piovezan, et al. (2015) showed that a hot drink is a plant Mate has an antioxidant effect in ethanol extract, as it contains antioxidant compounds such as alkatannins, myricetin, flavonoids, and phenols. 4,5 dicaffeoylquinic acid and 3,3 dicaffeoylquinic acid (Dallago et al., 2011). This study aimed at the mate plant extract and the caffeine alkaloid compound extracted at the level of lipid profile and obesity in rats. Mate tea enables the brain to break down carbohydrates and convert calories to energy through exercise, given the large number of calories burned, which promotes losing weight for individuals on a diet and decreasing high blood pressure. It keeps the body balanced in the face of the stress associated with vigorous exercise (Wendling et al., 2016). Understanding the varieties of active substances present in the plant by chemical methods and extracting the caffeine compound using the caffeine extracted from the mate plant on experimental animals.

II. MATERIALS AND METHODS

2.1 Animals:

Experimental animals of the guinea pig (Qandi) type of male sex were used and obtained from an animal breeding center inside the city of Misurata and taken care of in a special laboratory in the Faculty of Medical Technology Misurata at room temperature for 8 days under all standard conditions; the average age is three months. Diet components for experimental animals

Guinea pig (Qandi) (feed 400 grams)

Vegetables contain 50 grams of fiber.

Water, 100 ml.

Group 1 was injected with caffeine 10 mg/kg, Group 2 was injected with caffeine 20 mg/kg, Group 3 was injected with ethanolic extract 200 mg/kg, Group 4 was injected with ethanolic extract 100 mg/kg, and Group 5 was injected control group.

2.2. 1 Preparing mate extract:

A quantity of dried herbs of mate from the legume stores in Misurata city was collected, and the active substances of the plant were detected. A concentration of 2% of mate plant extract was prepared in 96% ethyl alcohol on a cold basis, and then 20 g of mate plant was placed in a 200-ml conical flask on the shaker for 24 hours. The extract was filtered using filter paper and dried at room temperature. Thus, 2 gm of mate extract was taken, dissolved in 100 ml of sterile water, and injected with experimental animals (Shimoda *et al.*, 2006; Zheng et al., 2004).

2.2.2 Caffeine was extracted from Meta extract, as described by Jarrar, A., et al. (1999).

2.3 Phytochemical screening:

Phytochemical screening was conducted according to Raaman (2006). The alkaloid tests involved adding drops of the Wagner reagent to the extract and waiting for a few minutes. Additionally, in another test tube, 2ml of the extract was mixed with Mayer reagent, and the results were observed after a waiting period.

For the phenol test, 2 ml of the extract was combined with 2 ml of lead acetate reagent.

To perform the terpene test, a 35% concentration of ammonia was applied to the sprayed filter paper.

The saponins test required mixing 2.5 ml of the extract with 10 ml of distilled water, followed by stirring for a minute and heating.

For the flavonoids test, 5 ml of the extract was mixed with pieces of magnesium flakes and a drop of hydrochloric acid was added.

2.4 Blood tests: Biochemistry tests, Glucose, Enzyme tests, Ions tests, and body fat levels

2.5 Histology protocol

After the autopsy, a specimen of liver, kidneys, and tissues were taken, and the samples were fixed in a 10% formalin solution for 24 hours, then washed with water to remove traces of formalin from the tissue and dehydrated by placing it in a series of ascending alcohols (50%, 70%, 90%, 100%, and 100%). Therefore, the specimens were placed in xylene for cleaning, and the sections were stained with hematoxylin and eosin after the microtome was 5 m thick. A microscope examined the slides, and a microscope equipped with a digital camera took some pictures.

III. RESULTS

3.1 Results of detection of active substances:

The result was positive by Alkaloid test, as a brown precipitate appeared with the Wagner reagent and a white precipitate with the Mayer reagent. The result was positive by the Phenol test, as a bluish-green precipitate appeared with lead acetate reagent and a yellow-green precipitate with potassium hydroxide reagent. The result was positive for the Flavonoids test, as a red color appeared with the detector. The result was positive for the Saponins test, as honey-colored foam appeared with the detector. The result was positive for the Terpene test, as a yellow color appears with the detector in Tab. 1. These substances are what determine the therapeutic effectiveness of the plant, and it was proven in a study conducted by the World Health Organization that chemicals are responsible for the therapeutic effect.

Test	Terpenes	Saponins	Flavonoids	Phenols	Alkaloids
result	+	+	+	+	+

Tab 1: phyotochemistry screen of Meta extract

3.2 Blood tests

3.2.1 Liver enzymes:

3.2.1.1 Alkaline phosphatase (ALKP):

The groups that administered match extract doses (100 ml/kg and 200 ml/kg, respectively) showed the greatest levels of alkaline phosphatase, with both a P=0.0217 = 0.0217 significance level indicated. In comparison to a control group, the caffeine-injected groups had slightly increasing levels. As in Fig. 1.

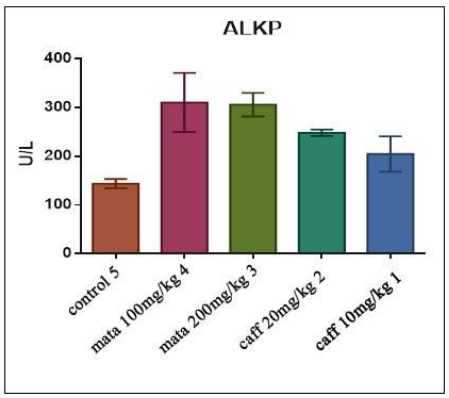

Figure 1 shows the level of alkaline phosphatase in the serum of experimental animals.

3.2.1.2 Alanine aminotransferase (ALT)

These results show a significant increase in the level of aminotransferase enzyme for group 4, which takes a dose of 100 mg/kg of ethanolic extract of mate with a

statistical significance of P ≤ 0.0001. In addition, it shows that group 1, which injects a dose of 10 mg/kg of caffeine, has a slight decrease with a statistical significance of P = 0.7441 in the aminotransferase enzyme compared to the control group. As in fig 2.

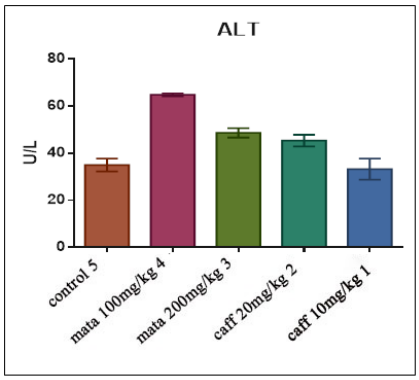

Figure 2 shows the level of aminotransferase enzyme in the serum of experimental animals.

3.2.1.3 Aspartate transaminase (AST)

The results showed a sharp increase in the level of aspartate transporter for the group that took a dose of 100 mg/kg of ethanolic extract of mate (P = 0.7785) compared to the control group and also noted the closeness of the level for the two groups that were injected with caffeine (Fig. 3).

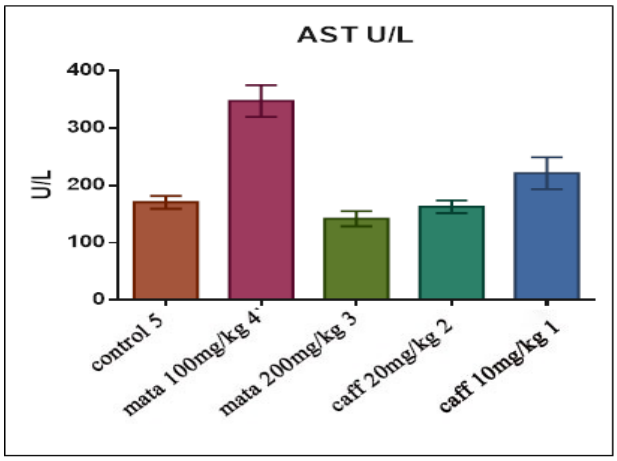

Figure (3) shows the level of aspartate transporter enzyme in the serum of experimental animals

3.2.2 Kidney function rates

3.2.2.1 Creatinine

The results showed a severe increase in the level of aspartate transporter for the group that took a dose of 100 ml/kg of ethanolic extract of yerba mate, estimated with a statistical significance P = 0.0017, while a slight decrease for the group that took a dose of 200 ml / kg, estimated with a statistical significance, P = 0.1544 from the ethanolic extract of yerba mate Compared with the control group, and also the observation of the level of convergence of the two groups that are injected with caffeine As fig:4.

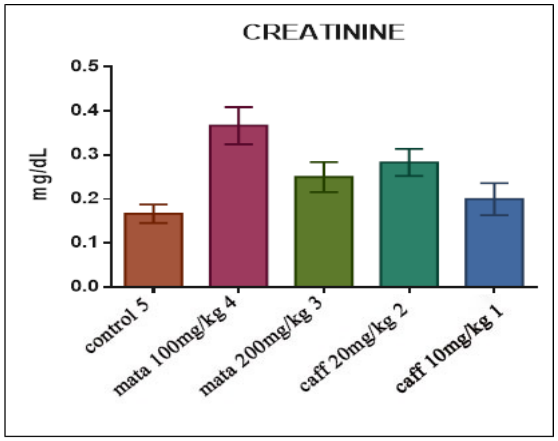

Figure (4) Creatinine in the serum of experimental animals

3.2.2.2 Urea

The results showed an increase in the level of urea for the group that takes a dose of ethanolic extract of Mate with an amount of 100 ml / kg and it is estimated with statistical significance P value = 0.0167, while the rest of the other groups are close to the control group estimated by statistical significance P = 0.0001 as fig:5.

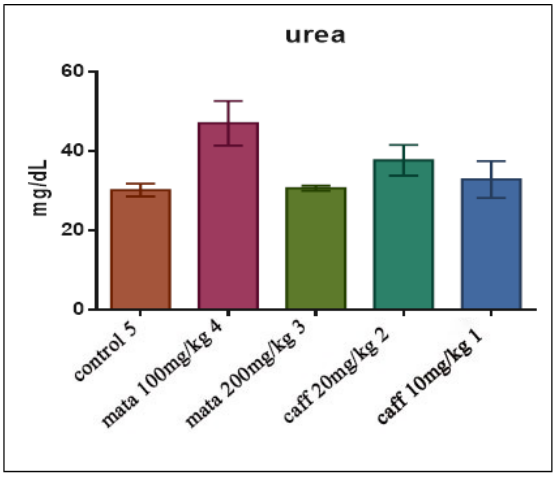

Figure (5) The level of urea in the serum of experimental animals.

3.2.3 Body fat levels

3.2.3.1 Cholesterol

The results of low cholesterol were estimated with statistical significance for the group that exposed a dose of ethanolic extract of lutein in the amount of 200 mg/kg, P≤0.0035, while a decrease was estimated with statistical significance for the group that took a dose of extract in the amount of 100 mg/kg P ≤ 0.0419, as observed in fig:6.

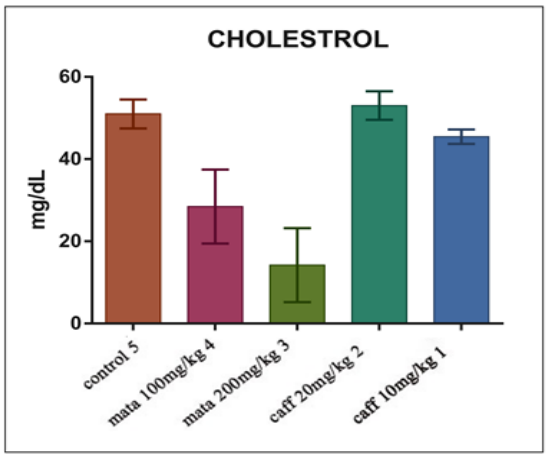

Figure (6) the level of cholesterol in the serum of experimental animals

3.2.3.2 Triglyceride

The findings demonstrated a reduction in triglycerides within the cohort subjected to a 200 mg/kg dosage of an ethanolic extract of Mate. The statistical analysis indicated a significant distinction (P = 0.7346) when compared to the control group (Fig. 7).

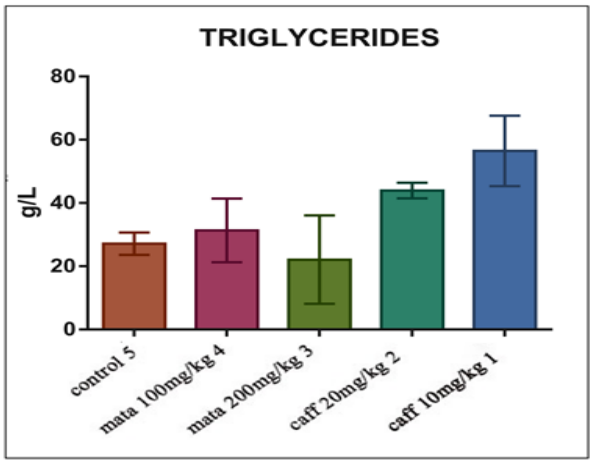

Figure (7) Triglyceride level in the serum of experimental animals

3.3 Examination of the tissues of the liver and kidneys in the treated animal

3.3.1 Histology of the Kidney:

The kidney tissue characteristics for the treatment group, groups given yerba mate at a concentration (100 mg/kg, 200 mg/kg) indicated by Figures (10, 11), and groups administered caffeine at a dose (10 mg/kg) represented by Figure 8. All histological alterations, which appear to have histological features in the renal tubules, The findings of the renal tissue of those treated with a dose of caffeine of 20 mg/kg as shown in figure (9), on the other hand, revealed Figure (09) demonstrates the effect of deposits in the tubule lumen and tubular cell rupture compared with the control group result as shown in Figure (12).

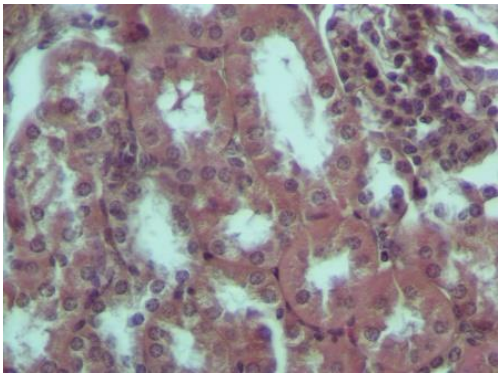

Figure 8: Group administered caffeine at a dose (10 mg/kg)

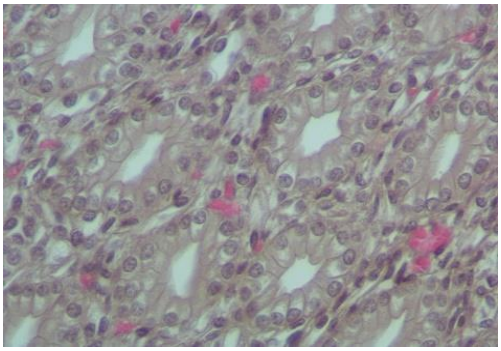

Figure 9: Group administered caffeine at a dose of 20 mg/kg

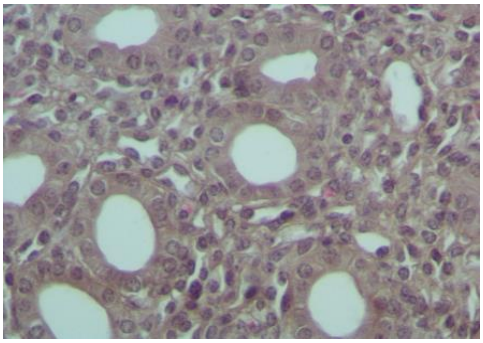

Figure 10: Group administered ethanolic yerba mate extract at a dose of 200 mg/kg.

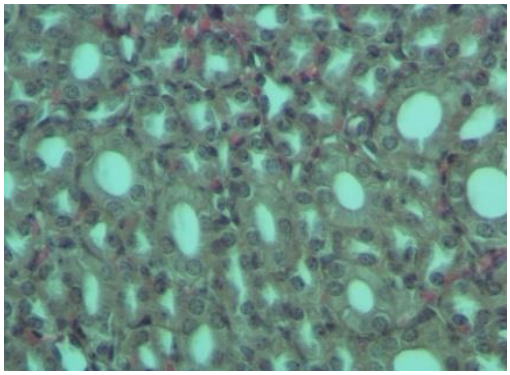

Figure 11: Group administered ethanolic yerba mate extract at a dose of 100 mg/kg.

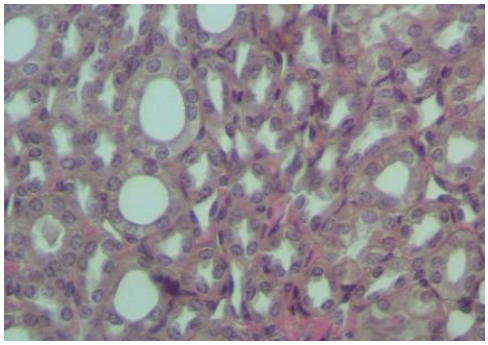

Figure 12: Histological anatomy of the kidney of the Control group

3.3.2 Histology of the liver:

The examination observed that increasing rats used in the study had a tissue change in the livers as compared to the controls. The morphology of the liver of the group given caffeine at a dose of 20 mg/kg (Fig. 14) revealed the presence of necrosis of cells and fat accumulation, leading to tissue laceration and necrosis of cells. Figure 13 shows an increase in fat in the tissue and the appearance of a white area in the cytoplasm, as well as minor necrosis of certain hepatocytes, at a dose of 10 mg/kg. While the liver tissue of the groups fed 100 mg/kg and 200 mg/kg, respectively, shows that liver cells seem to be largely normal and there are no changes in the histological structure when compared with control, The figure (15, 16) shows the liver tissue of the groups fed 100 mg/kg and 200 mg/kg, respectively, where the liver cells appear to be largely normal and there are no changes in the histological structure (Fig. 17).

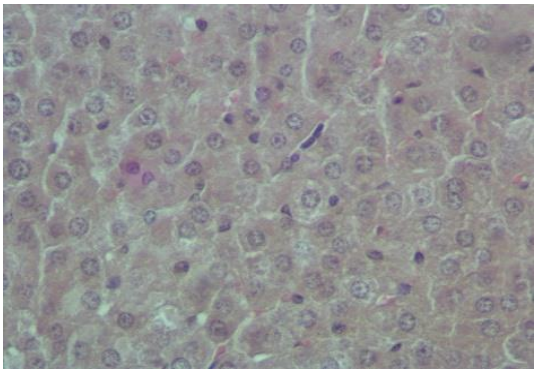

Figure13: Group administered caffeine at a dose of 10 mg/kg)

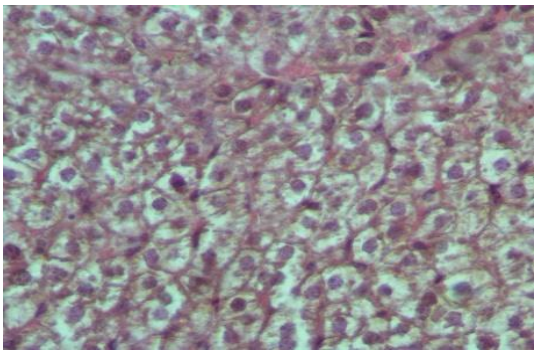

Figure14: Group administered caffeine at a dose of 20 mg/kg)

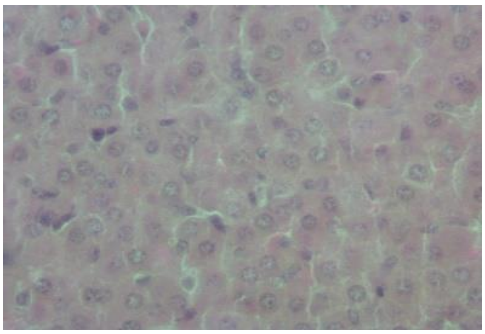

Figure15: Group administered ethanolic yerba mate extract at a dose of 100 mg/kg)

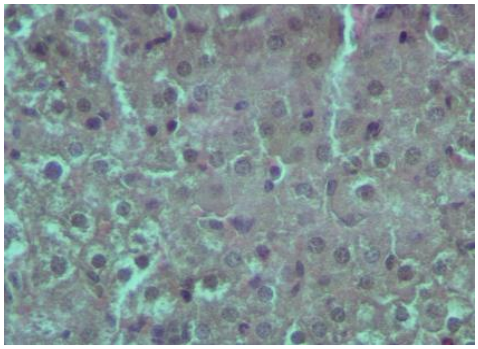

Figure16: Group administered ethanolic yerba mate extract at a dose of 200 mg/kg)

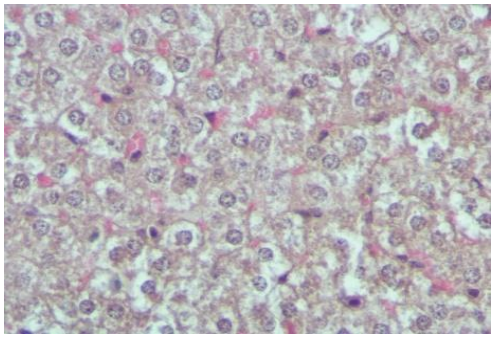

Figure 17: Histological anatomy of the Liver of the Control group

VI. Discussion

Caffeine may have a preventive effect against Alzheimer's and Parkinson's, although the evidence is unclear. It may help patients avoid cirrhosis of the liver. Whether consumed a few days before reaching a high altitude, caffeine might help reduce the severity of acute mountain sickness. Caffeine consumption has been associated with a lower incidence of type 2 diabetes in one meta-analysis. Caffeine use on a regular basis lowers the risk of developing Parkinson's disease and delays the disease's progression.

There are several studies on the effects of yerba mate extract on different organs and functions in animals. One study found that yerba mate extract made from the leaves of the plant has protective effects against oxidative DNA damage in liver, kidney, and bladder cells in mice. (Gao Y, et al. 2014) Another study evaluated the protective effect of yerba mate extract against oxidative damage caused by ultraviolet radiation and found that it has

antioxidant properties and protection against DNA oxidation. (De Andrade F, et al. 2009) A study on adult rats found that yerba mate could improve liver metabolism and increase antioxidant defenses. However, none of these studies specifically addresses the effect of ethanolic extract and caffeine extract of the yerba mate plant on the vital functions and tissues of the liver and kidneys in guinea pigs (Pimentel GD, et al. 2012).

Caffeine's effect has become a large part of our lives; it changes appetites, stimulates the central nervous system, lowers exhaustion, and enhances concentration; nevertheless, if consumed in excess, people may feel fatigued and weak. While humans discuss the beneficial effects of caffeine, they usually believe that it stimulates the central nervous system, reduces exhaustion, and raises attentiveness. However, if one drinks more than 500-600 mg of caffeine per day, the reverse impact of caffeine can begin. Coffee is not a stimulant, as it commonly causes side effects for which individuals prefer to drink it, such as fatigue, headaches, and weakness. The most effective stimulant without negative effects is, at the end of the day, physical exercise.

As a regulator of lipid and glucose metabolism, the liver is the first gland to be affected by a continuous injury from high-calorie loading, which can lead to fatty liver. In relation to stages of disease progression, nonalcoholic liver cirrhosis displays a wide spectrum of indicators, ranging from steatosis alone, steatosis with inflammation, steatosis with hepatocellular injury, or steatosis with sinusoidal fibrosis. The researchers discovered that coffee drinkers have a 21% reduced risk of chronic liver disease compared to those who do not drink it, based on the medical history and coffee intake of half a million Britons.

Several people are more sensitive to the effects of caffeine than others are, and they can experience side effects after only 200-300 milligrams of caffeine consumed during the day. Furthermore, people who do not drink caffeine on a regular basis are more susceptible to various side effects. Body weight, age, smoking habits, tension, stress, and gender are among the characteristics that may affect sensitivity to caffeine. It was shown that the effect of caffeine on males is greater than on females.

According to a study conducted by (Farley, Joel, and Baylink, 1980), coffee has an effect on raising ALKP. Several studies have shown that drinking coffee regularly improves liver function, reduces fibrosis, and protects against liver cancer thanks to antioxidants, chlorogenic acid, and dipteran.

In a recent study, it was found that drinking coffee regularly reduces the incidence of liver cancer by 51%, and the more coffee is drunk, the greater the protection against liver cancer.

A team of American researchers studied the coffee drinking habits of more than 27,000 people, aged 20 or older, and took note of the main markers of liver function in blood samples, including the enzymes alanine (ALT), alanine (AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT). (Machado et al., 2008; Liu et al., 2014).

The researchers found that among the participants who drank three or more cups of either caffeinated or decaffeinated coffee in a group for a 24-hour period, their liver enzyme levels were lower than those of non-coffee drinkers, indicating that they promoted liver health. also agrees with a study conducted by Liu et al., in which ALT showed that caffeine raises the level of aminotransferase enzyme. A study conducted by Machado et al. found that the AST rate is affected by caffeine, and this study coincides with Al, Mosimann, and El's (2006) findings that the ethanolic extract of mate lowers cholesterol levels.

This study agrees with a 2018 study conducted by Oliveira et al. The level of triglycerides is reduced by the extract of ethanolic lactic acid (Oliveira et al., 2018). This agrees with another study conducted in 2017 by Tarnopolsky that the level of creatinine is affected by the extract of ethanolic mate (Anyanwu et al., 2017), whereas this study agrees with another study conducted by Helen that Urea level is affected by caffeine.

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