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Influence of Oral Administration of Peppermint Tea on Biochemical and Histological Markers of the Liver of Wistar Rats

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Abstract
Reports have linked the consumption of peppermint tea to improve liver cell functioning and the consumption of peppermint tea has been on the increase. The aim of this experiment was to assess the influence of the oral administration of peppermint tea on the liver of Wistar rats using biochemical and histological findings. 20 male Wistar albino rats were grouped into 4, consisting of 5 rats in each group. They were given 10mg/kg b.wt, 30mg/kg b.wt and 50mg/kg b.wt concentration of peppermint tea in tap water for 4 weeks. The first group was on normal diet and received tap water instead of tea. Their blood samples were analyzed for alanine transaminase, aspartate transaminase, alkaline phosphatase, total protein, gamma-glutamyl transpeptidase,albumin, glucose and lactate dehydrogenase. The liver tissues were also processed for histological examination. The liver tissues were essentially normal and similar to the control tissues. The biochemical parameters studied were also normal and similar to the results obtained from the control animals. The consumption of peppermint tea days has no effect on the liver biomarkers and the histology of the liver of Wistar rats which confirmed the safety in the consumption of the studied.
Keywords: Peppermint, Tea, Wistar rats, Liver

1. Introduction

The use of plant extracts by traditional medical practitioners for the treatment of liver disorders has been on for centuries (Schuppan *et al*., 1999). Peppermint *(Mentha piperita)* herb teas have been shown to have good commercial values due to their many therapeutic functions. Reports have shown that the aqueous extracts of *Mentha piperita* have been widely used as folkloric medicine in the management of stress, anti-spasmodic, anti-nociceptive (Yousef *et a*l., 2015), antioxidant, antimicrobial, antiviral, anticarcinogenic, antitumorigenic, antiallergic (Singh *et al*., 2011). Peppermint tea polyphenol prevents oxygen free radical-induced hepatocyte lethality (Hasegawa *et al*., 1995; Cai *et al*., 2002). Among all tea polyphenols, the common phenolic compounds have been essentially flavonoids (rutin, quercetin and luteolin) and phenolic acids (Pereira *et al*., 2009). The phenolic acids exist predominantly as hydroxycinnamic acids (caffeic acid, *p*-coumaric acid, and rosmarinic acid) and hydroxybenzoic acid (glycyrrhetinic acid, ellagic acid and gallic acid) which protect against oxidative damage diseases (coronary heart disease, stroke and cancer) and also essential for the growth and reproduction of plants (Hollman, 2001). The protective effects of tea extracts or tea polyphenol against liver fibrosis and liver cirrhosis in rats have been reported (Xiao *et al*., 2002; Li *et al.,* 2004), and confirmed (Bun *et al.,* 2006) when a study on the hepatotoxicity of high concentration of the tea on Wistar rats was found to be safe.

The aim of this work was to compare biochemical findings of liver function tests with the histological observations and establish the relationship between consumption of peppermint tea and inflammatory reactions in the liver of Wistar rats.

2. Materials and Methods

Preparation of Tea Extracts

Peppermint tea was bought from the Trado-medical Centre, Ibadan, Oyo state, Nigeria. The tea extracts were prepared using hot water infusion. 15g of each tea sample was infused in 1.2Litres of hot water, the mixtures were then be filtered using No. 1 Whatman filter paper and the filtrate kept prior analysis.

Experimental animals

There were 4 groups of 5 albino rats each; the experiment was carried out according to the methods of (Li *et al.,* 2004) with slight modifications. Adult male albino rats weighing 150-170g were used according to the standard guidelines of the Care and Use of Experimental Animal Resources. The rats were allowed to acclimatize for a week before the experiment.

Oral Acute Toxicity Study

There were 4 groups of 5 albino rats each; the experiment was carried out according to the methods of (Li *et al.,* 2004) with slight modifications. Tap water, 10mg, 30mg and 50mg of the tea infusion was given to the rats in the groups respectively. The animals given tap water served as controls. The tea was administered orally and all the rats were placed under observation for 24 hours for possible deaths of the rats.

Group 1: Control; group without treatment; normal diet and 0% of the tea samples
Group 2: aqueous extract of Peppermint Tea; 10mg/kg b.wt
Group 3: aqueous extract of Peppermint Tea; 30mg/kg b.wt

Group 4: aqueous extract of Peppermint tea; 50mg/kg b.wt

Oral Sub-Chronic Toxicity Study

None of the animals in the mortality study died. Therefore, administration of the infusions continued for a further 4 weeks before intoxication. At the end of four weeks, the rats were weighed and blood samples were collected through cardiac puncture under chloroform anaesthesia into EDTA bottles for liver function tests. The animals were subsequently sacrificed by cervical dislocation and liver tissues were taken and immediately fixed in 10% formaldehyde for histological examination.

Biochemical and Histological analysis

The blood samples were centrifuged, plasma aspirated and analyzed for alanine transaminase, aspartate transaminase, alkaline phosphatase, total protein, gamma-glutamyl transpeptidase,albumin, glucose and lactate dehydrogenase according to standard biochemical methods using kits bought from Randox Laboratory distributor. The animals were subsequently sacrificed by cervical dislocation and liver tissues were taken and immediately fixed in 10% formaldehyde for histological examination.

3. Results

The result of the effect of *Mentha piperita* at different concentrations of the tea infusions on liver enzyme markers; gamma glutamyl transferase (ɤGT), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), total bilirubin (BIL), alkaline transferase (ALT), and aspartate transferase (AST), lactate dehydrogenase (LDH) and total protein (T. Prot.) is presented in Tables 1 and 2. The result showed that the various concentrations of *Mentha piperita* did not have any damaging effects on the liver as the values obtained from the biochemical investigation falls between the normal ranges of levels stated for the blood and below control values.

Table 1: Effects of the Peppermint tea extracts doses (at different concentration) after 4 weeks on serum biochemical markers of the albino rats in different groups compared to control

SAMPLES	GGT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)
CONTROL	5.05±0.58^{a}	56.22±4.14^{a}	36.00±1.67^{a}	28.70 ±1.10^{a}
P1	27.58±20.27^{a}	70.22±1.38^{a}	46.33±1.33^{a}	28.20±0.70^{a,b}
P3	25.05±10.42^{a}	69.06±6.90^{b}	35.50±1.83^{a}	25.70±1.10^{a}
P5	15.05±1.74^{a}	56.18±4.14^{a}	34.83±1.80^{a}	21.50±1.20^{b}
Normal	**5-48**	**45-115**	**8-48**	**7-55**

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE. P1 represents peppermint extracts at 10mg/kg.bwt; P3 represents peppermint extracts at 30mg/kg.bwt; P5 represents peppermint extracts at 50mg/kg.bwt. N=5; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatise; AST: Aspartate transferase ; ALT: Alanine transferase

Table 2: Effects of the teas extracts doses (at different concentration) after 4 weeks on serum biochemical markers of the albino rats in different groups compared to control

SAMPLES	LDH (U/L)	T.PROT (g/l)	ALB (g/dl)	TBIL(mg/dl)	GLU (mg/dl))
CONTROL	231.43±16.03^{a}	60.19 ± 0.42^{a}	37.57±0.54^{a}	0.59± 0.08^{a}	105.11± 2.58^{a}
P1	248.56±59.84^{a}	64.63± 0.25^{a}	37.87±0.12^{a}	1.16± 0.53^{a}	112.44± 1.87^{a}
P3	215.73±18.57^{b}	69.20 ± 1.52^{a}	38.47±2.57^{a}	0.91± 0.03^{a}	95.44± 2.13^{a}
P5	208.41±24.76^{a}	75.49± 0.74^{b}	44.70±4.60^{a}	0.44± 0.06^{a}	93.76± 0.26^{a}
Normal	**122-320**	**64-83**	**35-50**	**0.1-1.2**	**75 - 115**

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE. P1 represents peppermint extracts at 10mg/kg.bwt; P3 represents peppermint extracts at 30mg/kg.bwt; P5 represents peppermint extracts at 50mg/kg.bwt. N=5; LDH: Lactate dehydrogenase; T. PROT: Total protein; ALB: albumin; BIL: Total Bilirubin; GLU: Glucose.

All the tissue sections obtained from the liver of experimental Wistar rats fed with the peppermint tea extracts from 10 mg/kg b.wt to 50 mg/kg b.wt concentration were not different from tissues from the control animals. All the sections were essentially normal without any inflammatory lesion (Fig 1)

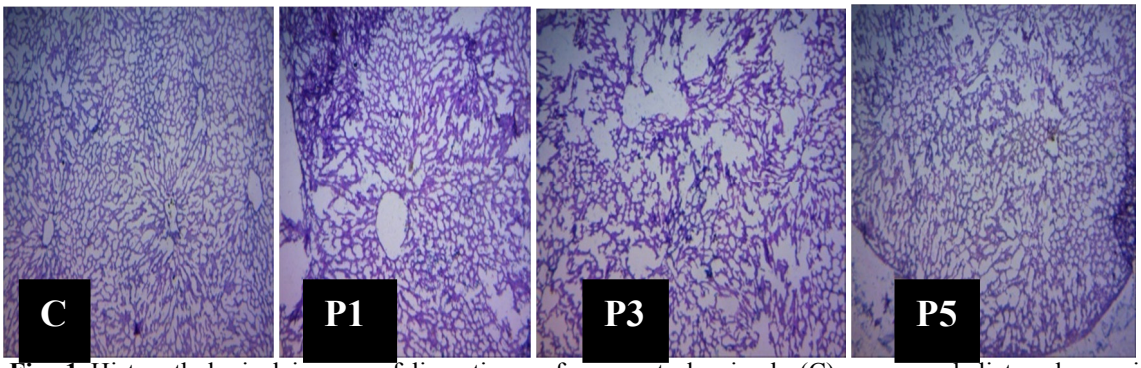

Fig. 1 Histopathological images of liver tissues from control animals (C) on normal diet and experimental animals treated with peppermint tea extract doses 10, 30 and 50 mg/kg b.wt (P1, P3 and P5).

4. Discussion

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion (Mengel *et al.,* 2005). A large number of plants and formulations have been claimed to have hepatoprotective activity. Compared with other herbs, because of wonderful pharmacological function, peppermint was most widely used in formulations, applied in therapy of many diseases, especially liver diseases. Tea is the most consumed beverage in the world and there are numerous reports of the health benefits of tea. Few of the beneficial effects of tea are its anti-inflammatory property (Heping *et al.,* 2007), and its anticancer property (McKay and Blumberg, 2002). Peppermint tea extract has been used to decongest nasal airflow (Eccles *et al*, 1990; Schafer *et al*, 1986), reduced postoperative nausea (Orani *et al*, 1991; Tate, 1997), acts as local anaesthetic (Galeotti *et al*, 2001) and possesses anti-inflammatory effect (Atta and Alkofahi, 1998). Peppermint tea rosmarinic acid (Peterson and Simmonds, 2003) has been described to have antibacterial, antiviral, and antioxidative, and anti-inflammatory activities (Szabo *et al.,* 1999).

The results revealed that the administration of peppermint tea infusions at two dosage levels 30 and 50 mg/kg b.wt to rats for 4 weeks significantly ($p<0.05$) decreases serum levels AST and ALT when compared to the control rats (except licorice extract at dose 50 mg/kg b.wt with elevated value). These results agreed with that of Gupta and Misra 2006, who reported that the administration of tea extract reduced the elevated serum levels of ALT and AST induced by paracetamol. This reduction could be attributed to the protective effect of some phytochemicals present in peppermint tea extract and the maintenance of the functional integrity of hepatic cells. Clinical benefits of peppermint have been reported that they are hepatoprotective, antihepatotoxic, an have been used successfully in clinical trials to treat viral hepatitis (Xu-ying *et al.,* 2009; Armanini *et al.,* 2002; Tripathi *et al*, 2000; Lucida and Wallace, 1998). Biochemical analysis of serum ALP enzyme showed a significant ($p<0.05$) decrease in serum ALP level of rats orally given peppermint tea (P3) in a dose of 30 mg/kg b.wt when compared to the control rats. On the other hand, there was no significant change in serum ALP enzyme in rats given peppermint tea (P1 and P5) in doses of 10 and 50 mg/kg b.wt when compared to the control group as it has been shown by Tayeb *et al.* (2010), who investigated on Chamomile tea infusion.

Regarding another biomarkers of liver toxicity, the present results showed that serum levels of ALB and T.PROT were significantly ($p<0.05$) increased in rats after administration of peppermint tea extract at two dosage levels 30 and 50 mg/kg b.wt, when compared to the control group. Similar results were demonstrated by Tayeb *et al.* (2010) who reported significant increases in serum ALB and TPROT after administration to Chamomile tea infusion to the rats. This is because albumin is synthesized by the liver and often transports or binds to drugs that may negatively influence total protein and albumin metabolism (Tayeb *et al., 2*010).

Lactate dehydrogenase (LDH) is intracellular enzyme that recognized as a potential marker to assess liver toxicity (Agrahari *et al.,* 2007). This study showed no significant change in serum LDH levels in rats orally given; peppermint tea (P1 and P5) at dose 10 and 50 mg/kg b.wt when compared with the control group. The reduction in serum LDH could be attributed to the protective effect of peppermint tea and maintenance of the functional integrity of hepatic cells (Gupta and Misra, 2006). Concerning serum TBIL analysis, the results showed that there was a no significant ($p<0.05$) difference in serum TBIL in rats induced with peppermint tea infusion at all tested doses. It was concluded by (Nkozi *et al.,* 2005) that TBIL can be used as a measure of binding, conjugation, and excretory capacity of hepatocytes. Of greater interest is the fact that serum GGT, a more specific and sensitive diagnostic marker for liver problems, presented no statistical significant difference in the peppermint tea at all doses tested. Elevated serum GGT activity can be found in diseases of the liver and is similar to alkaline phosphatase (ALP) in detecting disease of the biliary tract (Betro *et al.,* 1997).

In this study, rats supplemented with peppermint tea at high levels dosage 30 and 50 mg/kg b.wt

produced lower serum concentrations of glucose. This in contrary with what happen after administration of chamomile tea extracts (Sailesh and Padmanabha, 2014), where a reduced serum concentration of glucose in Wistar albino rats was obserced when compared with control group. This result is an indication that the teas extract especially peppermint tea infusion may influence the insulin-sensitive cell receptors or binding activity (Xie *et al*., 2011). It is well-known that insulin prevents gluconeogenesis, thereby decreasing the serum contents of glucose (Xie *et al*., 2011).

The histological observations of liver sections from the experimental animals fed with *Mentha piperita*teas at doses 10, 30 and 50 mg/kg b.wt, generally showed a normal structure with hexagonal lobules, central veins and peripheral triads embedded in connective tissue. The studied tea infusions have no adverse effect on the liver

The normal histological features in the liver of rats treated with antioxidant-containing tea extracts supplements as shown by the representative images of the peppermint tea treated groups suggest that antioxidants can actively stabilized liver tissues against oxidative damage that could caused by free radical generation by organophosphate toxicity (Prakasami *et al.*, 2001; Hsu *et al.*, 2001). Hence, the studied tea extract can arrest the harmful effect on liver cells through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals and stimulate the regeneration of damaged tissues and cells as did green tea (El Daly, 2011).

5. Conclusion

We conclude that oral administration of peppermint tea at dosage 10, 30 and 50 mg/kg b.wt for 4 weeks has no effect on the liver biomarkers and the histology of the liver of Wistar rats which confirmed the safety in the consumption of the studied teas.

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