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RENAL AND HEPATIC IMPACTS OF *CURCUMA LONGA* AND *MENTHA PIPERITA* IN ALBINO RATS

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ABSTRACT

The liver and kidney are organs that are easily affected by toxic substances, as result of their various roles in processing, biotransformation and excretion of substances. Herbs and many plant materials are among the numerous substances that can have direct impact on the liver and kidney. *Curcuma longa* and *Mentha piperita* are spices commonly used for culinary, pharmacological and medicinal purposes in Nigeria. This study was done to determine, the effects of the individual plants as well as their combination on the kidney and liver functions of albino rats. Forty-two albino rats of mixed gender, excluding pregnant animals were used for the experiment which lasted for 6 weeks, during which, animals were fed with commercial rat pellets and plant samples (in different percentages). The animals were divided into seven groups. Group A (Normal Control group), were fed with commercial rat pellets. The experimental groups comprised of Group B (2.5% turmeric), Group C (10% turmeric), Group D (2.5% mint leaves), Group E (10% mint leaves), Group F (2.5%, 1:1 of both plants) and Group G (10% 1:1 of both plants). After 6 weeks of feeding, animals were sacrificed, serum was collected and assayed for kidney and liver functions. Standard methods and procedures were used to check for urea, creatinine and total protein for the kidneys. ALT, AST and Albumin levels were used to check for liver function and histopathological examinations were done for both organs. The results showed a significant non-concentration dependent increase in urea level in the experimental groups ($p<0.05$). There was also non-significant increase in creatinine level in most of the experimental groups, especially the *Curcuma longa* (groups B and C). The liver function tests showed, a significant reduction in ALT activity ($p<0.05$) in most of the experimental groups except the 10% *mentha piperita* leaves group (group E). There was a significant dose dependent increase in AST activity ($p<0.05$) in the experimental groups compared to the control. There was a non-significant ($p>0.05$) lower albumin concentration in the experimental groups compared to the control. Histopathological evaluation showed normal kidney tissues in all groups, but revealed abnormal liver tissues in groups C, E and G animals. Therefore, it can be inferred that both plants may have the potential to cause deleterious effects on the kidneys and especially the liver, when taken at high concentrations over prolonged period of time.

Keywords: Renal, Hepatic, *Curcuma Longa*, *Mentha Piperita,* Curcumin, Steatosis.

I. INRODUCTION

Kidneys are particularly susceptible to toxic substances, because of their large surface area, high blood flow, high metabolic activity and possible active reabsorption and concentrations of toxins. As such, diverse forms of kidney injuries have been associated with toxic substances. The identities of toxic substances contained in African herbal medicines are largely unknown, and the toxicology and pathogenesis of these herbal preparations are unknown too (Luyckx, 2012). Botanical toxins are encountered both in common edible plants and medicinal herbs. The use of herbal preparations accounts for nearly 35% of all cases of Acute Kidney Injury (AKI) in Africa (Okunola, *et al*., 2012). The liver on the other hand, also plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents and herbal agents, when taken, in overdoses and sometimes even within therapeutic ranges, may injure the organ. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs, herbs and other substances. A group of enzymes located in the endoplasmic reticulum, known as cytochrome P-450, is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is not a single enzyme, but rather consists of a closely related family of 50 isoforms; six of them metabolize 90% of drugs. There is a tremendous diversity of individual P-450 gene products, and this heterogeneity allows the

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liver to perform oxidation on a vast array of chemicals (including most drugs and herbs) in phase1 (Chalasani *et al.,* 2014*)*. Herbal products most common adverse reaction is hepatotoxicity. Turmeric (*curcuma longa*) is a flowering plant, of ginger family, Zingiberaceae, the rhizomes are used in cooking (Imtiaz and Sabia, 2016). The plant is a perennial, rhizomatous, herbaceous plant. Turmeric is native to India and Southeast Asia (Daily *et al.,* 2016). The rhizomes are used fresh, boiled in water or dried after which they are ground into a deep orange-yellow powder commonly used as a coloring and flavoring agent in many Asian cuisines, especially for curries, as well as for dyeing, a characteristic, imparted by the principal turmeric constituent "curcumin" (Nelson *et al,* 2017). It is used in many products, dairy products, ice cream, yoghurt, orange juice, biscuits, popcorn, cereals and sauces. It is the principal ingredient in curry powders (Imitiaz and Sabia, 2016). Mint or *mentha* belongs to the Lamiaceae family, which contains around 15 to 20 plant species, including peppermint and spearmint. It is a popular herb that people use fresh or dried in many dishes and infusions. Peppermint (*Mentha Piperita*) also known as mentha balsamea wild (Keifer *et al*., 2007) is a hybrid mint, a cross between water mint and spear mint (Rita and Animesh, 2011). Both plants are very common in Nigeria, where they are used as a spice and for various pharmacological and medicinal applications.

II. MATERIALS AND METHODS

Preparation of plant materials

Turmeric rhizhomes *(Curcuma longa)* and Peppermint leaves *(Mentha piperita)* were both bought from Eke Awka Market, Anambra state. The plant materials were properly identified by a Taxonomist in the Botany Department of Nnamdi Azikiwe University, Awka. Tumeric was given a herbarium number NAUH-21 while pepper mint leaf was assigned a herbarium number NAUH-220B. Thereafter, the rhizhome and leaves of the plants were washed under running tap water, shade dried at room temperature and ground into fine powder differently. Three portions were obtained, Tumeric alone, Peppermint leaves alone and a mixture of both plants in the ratio 1:1.

Animal Studies

A comparative/Analytic Cross-sectional study was used. Forty Adult Wistar rats comprising twenty males and twenty Non-pregnant females were bought from Ken's Animal House, Awka (Affliated to UNN, Enugu). They were acclimatized, weighed and then divided into seven groups of six rats each. They were fed for six weeks, weighed every week, after which they were sacrificed for kidney and liver enzymes studies as well as histopathological evaluation of the tissues. The animals were handled according to the guidelines set by the Nnamdi Azikiwe University Animal ethics committee, which gave consent for the experiment.

1. **Group A (Normal Control group):** Rats were fed with commercial rat pellets. Each rat was fed 15g of commercial rat pellets daily, the quantity was increased or decreased, depending on their spillover.
2. **Group B (Experimental group)**: Rats were fed with commercial rat pellets mixed with 2.5% turmeric.
3. **Group C (Experimental group):** Rats were fed with commercial rat pellets mixed with 10% turmeric
4. **Group D (Experimental group):** Rats were fed with commercial rat pellets mixed with 2.5% mint leaves.
5. **Group E (Experimental group):** Rats were fed with commercial rat pellets mixed with 10% mint leaves.
6. **Group F (Experimental group):** Rats were fed with commercial rat pellets and a mixture of 2.5% (1:1) of *Curcuma longa)* and *(Mentha piperita).*
7. **Group G (Experimental group):** Rats were fed with commercial rat pellets and a mixture of 10% (1:1) of *Curcuma longa)* and *(Mentha piperita).*

Kidney and liver function tests

Urea (Eisenwiener, 1976) and creatinine were analysed using Randox test kits according to the manufacturer's instructions and procedures. Serum biochemical indices routinely estimated for liver functions include aspartate aminotransferase (AST) (Reitman and Frankel, 1957), alanine aminotransferase (ALT) (Reitman and Frankel, 1957) and total bilirubin (Jendrassik and Grof, 1938). The procedures used were according to the diagnostic kits' manufacturer's instruction. Total protein concentration in the serum was determined by the Biuret method of Gornall *et al.,* 1949.

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Histopathological examination of kidney and liver tissues

Liver and kidney tissue samples were sent to Ife's Laboratory, Regina Ceali Road, Awka, Anambra State, Nigeria (fully licensed), for histopathological examinations. The process of fixation using 10% neutral buffered formalin was used to preserve and maintain structures of tissues and cells, thereafter, tissue samples were sectioned and trimmed. Tissue samples were then embedded in harder medium such as paraffin wax and divided into sections again using the microtome knife, then stained using Hematoxylin and Eosin (H&E) stains. The stains were used to target specific chemical component of the liver and kidney tissues. Hematoxylin, stains cell nuclei blue while Eosin, stains cytoplasm and other tissues pink. Light microscopy was finally used to view and analyse the prepared samples and a camera mounted on the microscope was used to capture and record the findings (Mcmanus and *Mowry* 1984).

Statistical analysis

Statistical comparison between different experimental groups was done by one-way analysis of variance (ANOVA). Differences between values were considered statistically significant at the $p < 0.05$ level. All values are expressed as mean ± SD.

III. RESULTS

The kidney function tests are shown in table 1. The results showed that there was significant non-concentration dependent increase in urea level in the experimental groups (B-G) compared to the control ($p<0.05$). This increase was higher in the mint leaf groups and the groups that were fed with the mixture. There were also non-significant increases in creatinine level in most of the experimental groups especially the turmeric and mixture groups compared to the control ($p>0.05$). There was a reduction in creatinine level in the mint leaves groups, though it increased with concentration increase. Most of the groups had non-significantly lower total protein (TP) levels ($p>0.05$) than the control except the 10% mixture group.

Table 1: Effect of dietary supplementation of turmeric and pepper mint leaves in single and mixed forms on kidney function biomarkers of wistar rats.

Groups	Urea (mg/dl))	Creatinine (mg/dl)	Total protein (g/dl)
A (Normal control)	24.88±2.46	0.87±0.36	72.25±9.47
B (2.5% Turmeric)	56.71±5.60	0.91±0.21	59.87±1.85
C (10.0% Turmeric)	31.96±5.05	1.33±0.40	57.71±0.75
D (2.5% Mint leaves)	98.12±9.61	0.65±0.13	54.30±2.18
E (10.0% Mint leaves)	69.46±14.33	0.92±0.14	64.14±8.34
F (2.5% mixture)	123.40±18.75	0.97±0.48	60.93±3.37
G (10.0% mixture)	121.64±13.47	0.89±0.21	75.71±5.64

The liver function tests are shown in table 2. The results showed that there was a significant lower ALT activity ($p<0.05$) in most of the experimental groups (B-G) except the 10% mint leaf group compared to the control. There was a significant rise in AST activity ($p<0.05$) in the experimental groups (B-G) compared to the control. The AST activity increased with increase in concentration of both samples. There was a non-significant decrease in albumin levels ($p>0.05$) in the experimental groups, except the 2.5% turmeric group (group B) which had a little higher albumin level compared to the control.

Table 2: Showing the effect of dietary supplementation of turmeric and pepper mint leaves in single and mixed forms on the average liver function parameters of wistar rats.

Groups	ALT (U/L)	AST (U/L)	Albumin (g/dl)
A (Normal control)	40.10±8.87	79.00±13.16	5.15±0.72
B (2.5% Turmeric)	11.20±2.54	126.30±9.20	5.29±0.30
C (10.0% Turmeric)	25.90±5.28	129.80±12.80	3.87±0.99
D (2.5% Mint leaves)	24.15±2.44	94.13±3.72	3.56±0.50

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E (10.0% Mint leaves)	42.25±2.97	128.50±8.98	4.69±0.59
F (2.5% mixture)	19.33±6.93	101.13±10.97	4.78±0.55
G (10.0% mixture)	27.90±4.04	99.67±2.52	4.82±0.19

Histopathological examination results

The results showed that all kidney tissues examined in all groups, appeared normal, Group A animals had normal kidney and liver tissues, but the liver tissues of groups C, E and G showed various degrees of steatosis and presence of fats, which are quite abnormal features. The animals that took 10.0% turmeric (group C) and 10.0% mixture of both plants (Group G) had severe macrovesicular steatosis.

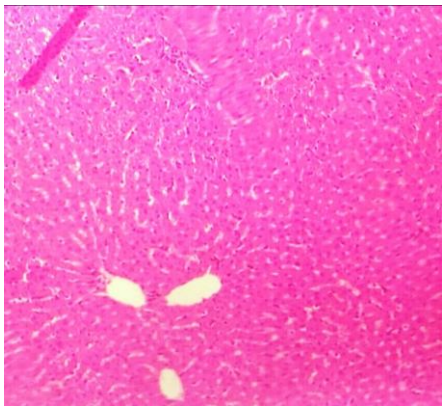

Fig 1: Liver

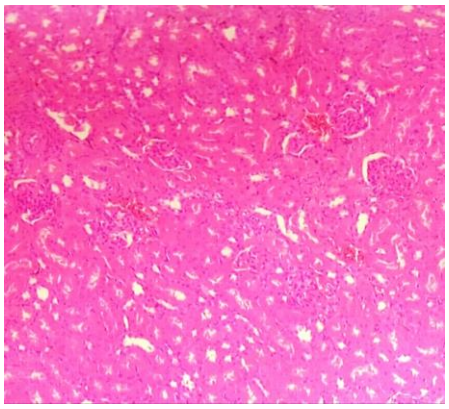

Fig 2: Kidney

GroupA (control): Histologic appearance of normal liver and kidney (H&E stain x 20 Magnification).

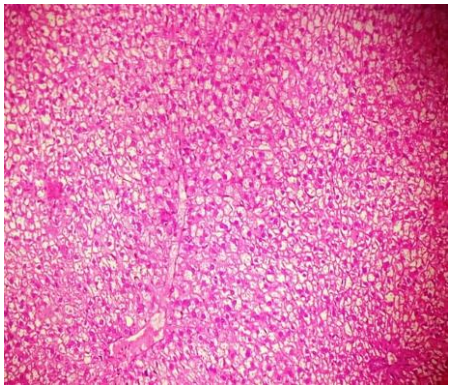

Fig 3: Liver

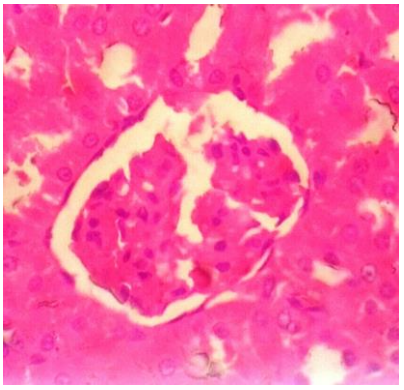

Fig 4: Kidney

Group C (10.0% Tumeric): X3 Normal**:** Histologic appearance of fatty liver disease, Liver tissue with a severe macrovesicular steatosis (H&E stain x 20 Magnification) and a normal kidney (H&E stain x 40 Magnification).

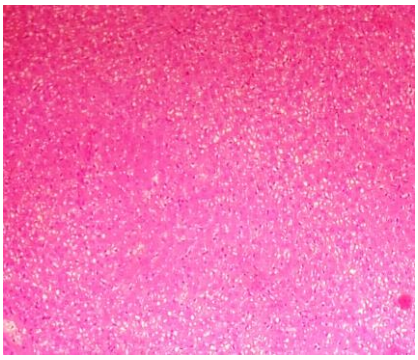

Fig 5: Liver

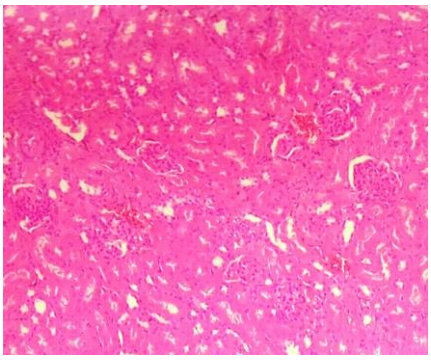

Fig 6: Kidney

Group E: (10.0% Pepper Mint) X3: Histologic appearance of fatty liver disease, Liver tissue with a mild macrovesicular steatosis (H&E stain x 20 Magnification) and normal kidney (H&E stain x 20 Magnification**)**.

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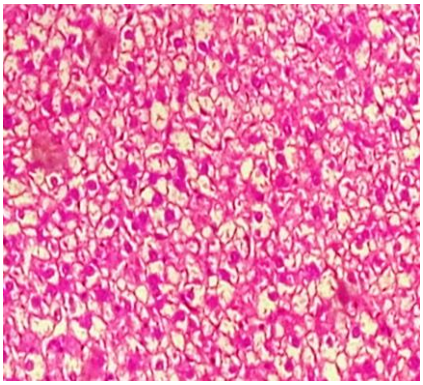

Fig 7: Liver

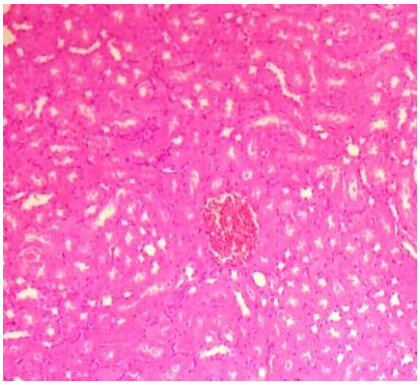

Fig 8: Kidney

Group G: (10.0% Tumeric + Mint) x1: Histologic appearance of fatty liver disease, Liver tissue with a severe macrovesicular steatosis (H&E stain x 40 Magnification) and a normal kidney (H&E stain x 20 Magnification).

IV. DISCUSSIONS

Kidney function assessment (serum urea, creatinine and total protein concentrations) was carried out on the control animals (group A), which were the animals fed with only commercial rat pellets and the experimental animals (groups B-G) which were the animals fed with commercial rat pellets and supplements of turmeric and pepper mint leaves in different percentages as shown in table 1.

Serum Urea and creatinine concentrations are reliable diagnostic tools used to detect renal injury or disease. Elevated concentration of both parameters beyond normal, usually indicate, impaired kidney function. Lower than normal level of serum total protein may indicate liver or kidney problem, especially when paired with elevated serum urea and creatinine concentrations.

Group B and C (animals that received 2.5% and 10% diet supplementation of turmeric alone) had higher serum urea (56.71±5.60 and 31.96±5.05 respectively, $p<0.05$) and higher serum creatinine (0.91±0.21 and 1.33±0.40 $p>0.05$ respectively) when compared to the control (group A) (table 1). They also had a non-significant lower total protein concentration than the control (group A) (table 1) $p>0.05$. This result differs from what was obtained by Abd-Elmegid *et al*., 2021, which reported that "curcumin", the active component of turmeric had no side effect on kidney function of rats, after 14 days feeding. Jogdand *et al*., 2017 also reported a nephroprotective effect of turmeric on rats fed with turmeric rhizome for 15 days. The wide discrepancy between this present study and those studies mentioned, might be because, Abd-Elmegid *et al*., 2021, used only "Curcumin", an isolated active component of turmeric, instead of crude turmeric rhizhome and for a shorter duratioin. The study done by Jogdand *et al*., 2017 was also for a short period of time (15 days). This present study was however, carried out on a longer duration of 6 weeks, which might have contributed to the increase in the level of kidney function biomarkers specifically, serum urea. The longer duration of this experiment might have increased the exposure time of the animals to the plant biochemical constituents. The isolation and direct use of bioactive component of turmeric in the form of "curcumin" might help to sieve out other phytochemical components of turmeric which might have negative impact on the kidneys when taken at high doses on prolonged period of time. Similar study done by Oluwafemi *et al*., 2021, reported however, the nephrotoxicity of turmeric rhizome, supporting the results obtained in this study. The use of whole turmeric rhizome without any form of processing in this study, for a long period of 6 weeks might have contributed to the elevated serum urea ($p<0.05$) and creatinine levels ($p>0.05$), as well as lower total protein concentration ($p>0.05$) observed in these groups (B and C), when compared to the control group (A). This suggests that, turmeric may be nephrotoxic especially at high dose. The myriad of phytochemicals found in turmeric (Anyaoku *et al.,*2023) with their various biochemical effects might have several impacts on the kidney function of the animals. The non-significant lower total protein level compared to the control might be attributed to reduced protein metabolism as a result of the presence of high antinutrient concentrations like tannins since it can inhibit absorption of proteins, leading to lower total protein level.

Similarly, groups D and E (rats fed with 2.5 % and 10% pepper mint leaves supplements) also had higher serum urea concentrations when compared to the control group (group A) (table 1) $p<0.05$. Group D animals (2.5%

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pepper mint supplement) had lower creatinine level compared to the control group (table 1) $p>0.05$, but it increased non-significantly with rise in pepper mint concentration in group E animals (10% pepper mint supplement) compared to the control group (table 1) $p<0.05$. The total protein concentration of groups D and E were found to be lower than the control group (group A) (table 1) $p>0.05$. It can be deduced from this result that Pepper mint leaves, may also have negative effect on the kidneys, especially at a high dose since the groups that took 10% pepper mint leaves supplements (group E) had a significant higher urea concentration, a non-significant elevated creatinine level and a lower total protein level ($p>0.05$) when compared to the control animals (groups A). However, available data shows that ethanol extract of Pepper mint leaves has ameliorative effect on renal derangements associated with gentamicin (Ullah *et al*., 2014). This is not in agreement with the findings of this study. The presence of various phytochemicals in pepper mint leaves, notably "pulegone" (Dolzhenko *et al*., 2010) may be responsible for the negative effect of this plant on the kidneys, evidenced by the significant rise in serum urea concentration in these groups (D and E).

Groups F and G (animals that consumed a mixture of pepper mint leaves and turmeric at 2.5% and 10% diet supplementation), had the highest significant serum urea concentration (123.40±18.75 and 121.64±13.47, $p<0.05$ respectively), far above the normal control (group A) (table 1). Their serum creatnine levels were also elevated non-significantly (0.97±0.48 and 0.89±0.21, $p>0.05$ respectively) beyond the control group. The group fed with 2.5% mixture of both plants (group F) had lower total protein concentration when compared to the control group ($p>0.05$), while the group fed with 10% mixture of both plants (group G) had higher total protein concentration than the control group ($p>0.05$). The result from this study implies that pepper mint may have more serious negative effects on the kidneys than turmeric, judging from the observed rise in serum urea concentration in the animals that fed on 2.5% and 10% pepper mint leaves supplements (group D and E), which even rose farther when combined with turmeric (table 1). These groups of rats that took the mixture of both plants as supplement (groups F and G) had significantly, the highest elevated level of serum urea. They also had an increased creatinine level $p>0.05$, compared to the control in this experiment. This result, implies that a mixture of the two plants (pepper mint leaves and turmeric), taken at the same time for a long duration may have adverse effects on the kidneys as seen in this study (table 1) (groups F and G). This therefore suggests that these two plants (turmeric and pepper mint leaves) should not necessarily be taken concomitantly for a long period, as they may have strong negative effects on the kidneys. The presence of various phytochemicals in both plants might be responsible for their negative effects on the kidneys. Pepper mint for example is known to contain a toxic compound, "pulegone" and other compounds such as menthofuran (Dolzhenko *et al*., 2010) which may have negative effects on the kidneys. Tumeric is also known to increase the risk of nephrolithiasis because of its oxalate content.

This study also analysed the liver function of the animals fed with turmeric and pepper mint leaves in single and combined forms. Table 2, shows the result of the liver function assessment done on the animals after 6 weeks feeding.

Liver biomarkers ALT, AST and the serum Albumin were used to indicate liver's physiological state. The liver enzymes ALT and AST are released into the blood stream when the integrity of the hepatocytes are compromised. Levels of serum Albumin and proteins are also vital indicators of impaired or normal functions of the hepatocytes.

Groups (B and C), (animals that received 2.5% and 10% turmeric), had significant lower ALT levels when compared to the control (group A) (table 2) $P<0.05$, a significant higher AST levels compared to the control (group A) (table 2) $p<0.05$ and a non-significant lower albumin level in Group C compared to the control (table 2) ($p>0.05$). A lower Albumin level may indicate kidney, liver diseases, inflammations or infections. Since these groups (B and C) had a significant rise in AST levels above the control group at $p<0.05$, though with a significant lower ALT level. It can be deduced that turmeric rhizhome may be hepatotoxic at high concentration. Lower doses of turmeric may not however be toxic to the liver especially when used as a spice, judging from the observed lower ALT in groups B and C, especially group C (2.5% turmeric) (table 2). A high dose of turmeric taken for a longer period may be hepatotoxic since the serum AST was very high at 2.5% and 10% supplementation in this study (table 2) $p<0.05$. The study done by Oluwafemi *et al*., 2021, supports that Turmeric rhizhome powder at higher concentrations (above 10% of a whole recipe), may have deleterious

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effect on the liver. Another study carried out by Akudike *et al*., 2015, stated that Turmeric may help to reduce effects of metronidazole on the liver. In other words, turmeric may not be hepatotoxic especially at a low dose.

Groups D (animals fed with 2.5% pepper mint leaves supplements) had a significant lower ALT level, higher AST Level ($p > 0.05$) and non-significant lower Albumin level compared to the control (group A) (table 2). When the concentration of Pepper mint leaves was in increased to 10% in Group E animals, their ALT and AST levels increased above the control group ($p > 0.05$). They also had lower Albumin level compared to the control animals (group A) (table 2) $p>0.05$. This implies that Peppermint leaves when taken at a high dose may also have deleterious effect on the liver especially over a long period of time, as seen in the increased level of liver enzymes and lower Albumin levels in group E (animals fed with 10% pepper mint supplements). Lower doses of peppermint leaves may not be hepatotoxic. The hepatotoxicity of Peppermint leaves therefore can be said to be dose dependent. The result from the study done by Ubani *et al*., 2022 and Khalil *et al*., 2015 are in agreement with the result of this study. According to Ubani *et al*., 2022, methanol leaf extract of Peppermint may not be hepatotoxic. Also, work done by Khalil *et al*., 2015 claims that pepper mint oil (*mentha piperita*) may rather possess hepatoprotective effect. The difference between these studies mentioned and the current study is the fact that the whole plant (*mentha piperita*) was used for the experiment in this study. There was no extraction using any agent, no processing was done either. The entire leaves were used in a raw form. The use of the whole plant may therefore have more impact on the liver by increasing the concentration of phytochemicals in the system. This may account for the increased liver enzymes seen in group E animals (animals fed 10% peppermint supplement) probably because of the presence of higher concentration of phytochemicals in the leaves than in the oil or any extract. The studies done by Ubani *et al*., 2022 and Khalil *et al*., 2015 used only extracts of the leaves which are likely to contain lower concentration of phytochemicals and may therefore not be hepatotoxic

Groups F and G, had a significant lower ALT, higher AST and non-significant lower Albumin levels, compared to the control group (table 2). From these results, it can be inferred, that these plants (turmeric and pepper mint leaves) are safer at low doses and when taken alone. The health benefits may be enjoyed at low concentrations as seen in their ALT reduction property. However, higher doses or a combination of these plants may be hepatotoxic, as evidenced in the significant increase in AST levels in all the treatment groups as well as lower albumin level ($p>0.05$) except in the group B animals. Both turmeric and pepper mint leaves contain various phytochemicals, some of which are common in both plants. Some are reported to be toxic, for example “pulegone” and “menthofuran” in peppermint. Therefore, taking a mixture of these plants at the same time may increase the concentration of some phytochemicals in the body, there by affecting the liver negatively.

The histopathological examination of the kidney and liver tissues showed that animals that consumed higher concentration of the plant samples (10%) had abnormal liver tissues, even though their kidneys appeared normal (groups C, E and G). Animals in groups C and G had severe macro vesicular steatosis (figs 3 and 7). Steatosis, is an abnormal accumulation of fat deposits in tissues especially the liver, which can be triggered by various factors including cell toxins (Schwingel *et al*., 2011) and malnutrition (Dalvi *et al*., 2018). The steatosis observed in the experimental animals in this study, may be linked to the phytochemical contents of the plants as well as the concentration consumed. These phytochemicals may have some negative effects on liver tissues especially at high concentration over a long period of time. Some phytochemicals such as tannin may even hinder absorption of nutrients in the GIT, causing malnutrition. Tumeric and pepper mint leaves contain various phytochemicals including tannins, oxalates, phytates, flavonoids etc (Anyaoku *et al*., 2023, Enemor *et al.,* 2020). Some of these phytochemicals are known antinutrients. Malnutrition is one major cause of steatosis. Overconsumption or abuse of these plants over a long time may be toxic to cells, causing steatosis by various mechanisms. This result also infers that a combination of these two plants especially at high doses may have potential deleterious effect on the liver tissues, hence should be avoided.

V. CONCLUSION

Tumeric and pepper mint leaves can be safely used as normal spices in food and drinks, however, taking very high concentrations of each plant or a mixture of both, over prolonged period of time, may have detrimental effects on the kidney and liver.

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