The Correlation of Histopathological Findings with Ultrastructural Changes in Hepatocytes after Yangonin “Ya”-Intoxicated Rats Alone and In Combination with EtOH: Sub-Acute & Sub-Chronic Study

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Abstract. Yangonin “Ya” has been used for centuries as a herbal supplement, for its mood-altering properties. It has been used as a recreation agent, for relaxation, as well as for pain relief. However, hepatotoxicity is a widespread problem associated with medicines in general. Most herbal supplements are metabolized by the liver, and thus the liver represents the target organ. At present, Yangonin toxicity appears to be “idiosyncratic”. Therefore, a study was designed in order to investigate the organelle-based changes in hepatocytes, after treatment with Yangonin alone and in combination with EtOH. Thirty rats were divided into five groups comprising of six animals each. The groups comprised of the control groups {(NCx) & (PCx)}, Yangonin (Ya) group, ethanol (EtOH) group, and the combination of (Ya) and ethanol (EtOH+Ya) group. The experiment was conducted over a period of 14 weeks, as a sub-chronic study. At the end of the 14th week, mitochondria, peroxisome, rough and smooth endoplasmic reticulum, and nuclei of hepatocytes, were evaluated using a scoring system. The results were compared with histopathological findings, as well. Treatment with Ya significantly induced hepatotoxic scores as compared to the control groups. Organelle injury scores increased significantly with Ya treatment, while rats that received “EtOH+Ya” showed the severest lesions of liver scores such as, severe hepatocellular degeneration, necrosis, and hypertrophy. Ultrastructural and histopathological scores in both groups were in very strong correlation ($r = 0.928$ for EtOH, $r = 0.921$ for Ya alone and $r = 0.903$ for Ya plus EtOH group). In conclusion, ethanol enhanced the sedative and hypnotic activity of Ya, and markedly increased toxicity. Findings based on TEM examination of organelles, supported the histological results as well as tissue lesions/injuries in hepatocytes, a result of hepatotoxin-induced hepatopathy.

Keywords. Hepatopathy, Yangonin, alcohol, ultrastructure, sER, mitochondria.

1. Introduction

Drug-induced hepatotoxicity has been cited as the most common reason for the withdrawal of approved drugs from the market. In addition, 50% of acute liver failure cases in the United States, have been attributed to drug-induced hepatic injury. The actual incidence of drug-induced hepatic...
injury is difficult to estimate, as most cases are under-reported, or sourced from retrospective studies. Another problem associated with determining prevalence, is the lack of information or data pertaining to self-medication and use of herbal products that could interact adversely with prescription and non-prescription drugs [1, 2, 3]. Though the incidence of drug-induced hepatic injury appears to be quite low, data from the Centers for Disease Control and Prevention (CDC) in the U.S., has reported that, about 1600 new acute cases of liver failure are reported annually, of which 41% are due to paracetamol hepatotoxicity [4]. The cases of adverse drug reactions and fatalities related to drugs, has more than doubled, during the period between 1995 and 2005 [5]. A lot of the cases of drug-induced liver injury are idiosyncratic, which means that the drug reactions cannot be predicted based on the known pharmacological properties of the substance, and therefore are often missed during pre-clinical stages of development. Regardless of their etiology, drug-induced hepatotoxicity is of major concern to the pharmaceutical industry, during the development of drugs, not only due to the increased risk for patients undergoing clinical trials but also to patients, after the launch of a new drug into the market. It could also result in increased costs and losses for pharmaceutical industries, due to the recalling of a drug at the later stages of development, or after it has been launched [6]. Piper methysticum Forster, is an ancient crop originating from Oceania (Polynesia, Melanesia and Micronesia [7]. Piper methysticum is cultivated for its rootstock which has been traditionally used to produce a drink. Piper methysticum extracts (both fresh and dried rhizome and roots) were prepared by grinding it into an aqueous suspension with water or coconut milk, and then strained and directly consumed [8, 9]. The effects of Yangonin (is found in Piper methysticum root as the main component) are considered to be mild, and less drastically mind-altering than most common drugs like alcohol, and when consumed in moderate amounts, there are no aftereffects such as "hangovers". Piper methysticum drinks when consumed, gives a sense of well-being, which is associated with its anxiolytic, sedative, muscle relaxant, and diuretic properties, among others [8]. Commonly observed side effects related to Piper methysticum consumption include allergic reactions, red eyes, yellowing of the skin, gastrointestinal complaints and lethargy, which affects the individual's appetite and ability to sufficiently eat [8, 10, 11]. In addition, due to confounding factors that may have contributed to the toxicity, it is difficult to confirm whether there is a direct association between Piper methysticum and severe liver toxicity. Therefore, we hypothesize that, a synergistic interaction between alcohol and Yangonin contributes to marked increasing in cell toxicity, particularly hepatocytes. The main aim of this study was to determine whether Yangonin (with or without alcohol) may predispose oxidative stress and free radicals and then cytotoxicity, by affecting levels of availability of hepatic glutathione. Also, to search the organelle-based changes in hepatocytes after Yangonin treatment (alone and in combination with EtOH) in experimental rats. As well as, to determine the correlation of histopathological changes with ultrastructural findings.

2. Materials and Methods

2.1. Reagents

Yangonin is one of the six major kavalactones found in the kava plant. Commercially available Yangonin extract, a medium yellow powder was used (70% Yangonin HPLC Piper Methysticin Root extract) was obtained from Shaanxi Herb Sky Biotech Co., Ltd (Fiji) in one lot, which was received in batch (JT160314). The powdered extract was gavaged to each animal after being dissolved in distilled water (800 mg/kg. body weight/day). Below the chemical structure of “Yangonine” 4-Methoxy-6-[(E)-2-(4-methoxyphenyl) ethenyl] pyran-2-one (Figure 1).
2.2. Animals and Exposure

The study was conducted in the Pathology Lab (Vet. Med/UPM). The protocol of the study was approved by Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, University Putra Malaysia (UPM) with UPM/IACUC/AUP-R082/2015, the reference number for notice of approval. Thirty, nine-weeks-old Sprague-Dawley rats, weighing about 200±25gm were supplied with LAR Unit. They were housed in animal cages under standard conditions with a period of 12 h light/dark at 22 to 28°C and 70 to 80% relative humidity in the animal house, UPM. The animals were allowed to acclimatize for seven days before the start of the experiments. The animals were dosed with a standard rat chow pellet and allowed to drink water ad libitum.

2.3. Experimental Design

Animals were equally divided into five groups of approximately equal initial body weight, which are considered as negative control (NCx), positive control (PCx) and treated groups (EtOH, Ya and EtOH+Ya). The rats were examined for changes in body weight, feed and water intake, biochemical, liver parameters, macroscopically, microscopically histological changes and ultrastructural changes. At the end of the investigational period and under appropriate anesthesia by 87 mg ketamine/kg (b.w) mixed with 13 mg xylazine /kg (b.w), the animals were subjected to cardiac puncture to collect blood samples into sterile heparinized and non-heparinized test tubes for clinical chemistry study; the animals were then sacrificed by cervical dislocation, and finally, liver was dissected and weighted after being washed and blotted dry to somatic index determination. The absolute and the relative weights \[\left(\text{Liver W./body W.}\times 100\right)\] were recorded \[12\].

2.4. Histological examination

At sacrifice, whole necropsies were performed on all rats utilizing standardized methodology. Liver weight, size and color were determined from all animals. Tissues including macroscopic abnormalities were removed, fixed, and preserved in 10% neutral buffered formalin for microscopic evaluation. Four to five micrometers (µm) thick sections were cut and mounted onto glass slides, stained with hematoxylin and eosin (H & E), while for Transmission Electron Microscopy, small pieces of liver (1mm3 slices) were immediately fixed in 4% glutaraldehyde buffer. Ultra-thin sections were collected on copper grids, double-contrast staining was applied with uranyl acetate (100 mL methanol and 5 g uranyl acetate) and Reynold’s lead nitrate solution (1.76 g sodium citrate, 1.33 g lead nitrate, 50 mL distilled water and 8 mL 1N NaOH). Thin sections were examined under a transmission electron microscope (Hitachi H7100 TEM, Japan) \[13\]. Twenty-Five cells from each specimen were examined. Mitochondria, peroxisome, nuclei and smooth endoplasmic reticulum (sER) of hepatocytes were evaluated by using a previously described scoring system (Table 1). Twenty-Five nuclei, 60 mitochondria and 20 sER were examined for each animal.

2.5. Statistical analysis for histopathologic scores

The data was presented as mean ± SEM. In order to determine significant differences between groups, the Mann-Whitney U test was used for histopathologic scores, and for ultrastructure scores the Student’s t-test was employed. The Pearson correlation procedure was used to evaluate the correlation of histopathological and ultrastructural scores. Values of P < 0.05, were considered statistically significant.
Table 1. Criteria for histopathological scoring system (light and transmission electron microscope).

| Severity of Hepatopathy | Light microscopy (H&E) | Description |
|-------------------------|------------------------|-------------|
| Minimal                 | Grade 1                | 0% of organ affected (normal histological appearance of hepatocytes). |
| Mild                    | Grade 2                | 1–39% of organ affected (congestion with slight sinusoidal space and central vein dilatation). |
| Moderate                | Grade 3                | 40–79% of organ affected (cystic and fatty degeneration, hepatocellular hypertrophy and necrosis), and dilatation with congestion of sinusoids and central vein. |
| Marked (severe)         | Grade 4                | 80%–100% of organ affected. Greater severity of changes noted in grade 3 with Kupffer cells hyperplasia and hypertrophy and possible pathological evidence of liver damage/fibrosis and cirrhosis. |

Transmission Electron Microscopy/TEM

| Ultrastructure changes | Description |
|------------------------|-------------|
| Nucleus affected       | Normal |
|                        | Irregular chromatin distribution (margination, clumping) |
|                        | Increased and aggregation of heterochromatin with fragmented irregular envelope |
|                        | Degenerated and necrotic nucleus |
| sER and peroxisome proliferation | Normal |
|                        | Slight dilatation |
|                        | High proliferation and dilatation |
|                        | Presence of large degenerated area and highly vacuolization of sER and highly proliferation of peroxisome |
| Cytoplasmic vacuoles and lipid droplets accumulation | Normal |
|                        | Slight vacuolation of cytoplasm |
|                        | Moderate number of lipid droplets with cytoplasmic vacuoles |
|                        | Severe accumulation of lipid droplets in cytoplasm |

3. Results

Throughout the course of the sub-acute experimental study, mortality was not observed in either of the “Ya” alone groups, Ya in combination with EtOH group, nor any of the other treatment groups. The most notable clinical abnormalities were observed in rats that were administered Ya, mainly those given Ya in combination with EtOH. The abnormalities were observed from the first week of the trial and increased gradually with time; this persisted for the duration of the experimental study. Clinical findings included, abnormal breathing, ataxia, dehydration due to loss of appetite, indigestion, lethargy, and loss of coordination. On the other hand, the withdrawal of 800mg/kg per day of Ya ingestion (alone and in combination with alcohol), after the third week induced a detectable change in behavioral patterns such as, increased sleeping time which subsequently lead to a decrease in the amount of feed intake and water consumption. While, at the end of the experimental period, most of clinical signs were as arching of the back, twitching as well as seizures, cutaneous lesions, characterized by flaky, dry skin with at times yellowish discoloration, which caused peripheral scaly
eruptions followed by hair loss (alopecia) beneath the lower jaw, known as Kani Kani lesions (Figure 2).

![Figure 2](image_url)

**Figure 2.** Photographs demonstrating the clinical observation throughout the sub-chronic trial. Ya group: abnormal red, congested eyes of rats, ruffled coat with alopecia area (dermopathy lesion/ Kani Kani lesion) beneath the lower jaw and under two eyes. Et+Ya group: severe eyes and lids congestion with more severe Kani Kani lesion approximately for whole head especially under eyes with severe weight loss, hunched posture, ruffled coat, head tilt.

3.1. **Macroscopic investigation**

Gross examination of the liver revealed various gross pathological changes among animals in the Ya and EtOH+Ya groups. Multiple necrotic foci around 2-3 mm in diameter were seen in the liver of rats belonging to both the Ya and Ya+EtOH groups, at 14 weeks. Significant lesions were observed in the enlarged oily heavy liver of the Ya-intoxicated group, and similar gross changes in the liver were also seen in the EtOH+Ya supplemented group, but much more severe. Also, the liver of rats from the EtOH+Ya group, were mottled with a yellowish or pale appearance, sometimes darkened color, with hepatic granuloma, as compared to the NCx group. The liver of EtOH intoxicated group, were mostly pale, oily and enlarged.
Figure 3. Photograph illustrating the gross morphological changes of liver. At necropsy, control (Cx) showing a normal structure, color, size and weight for all rats. Moderate to severe fatty changes is observed in liver of rats from EtOH group and Ya group with necrotic dark area demarcated from unaffected region and rounded edges showing in Ya group. Et+Ya group showing congested, severe dark red colored liver, necrotic black area with rounded edges and increase size and weight compare to Cx group.

3.2. Light Microscopic evaluations

Through H&E microscopic examination, the liver of the control (Cx) group, appeared a normal architecture of hepatic tissue while, Ya-intoxicated rats revealed different histopathological changes, which observed primarily (initial 3 weeks) in the centrilobular zone and the two zones were significantly less affected. Later, at week 14th most of hepatic lesions were seen in the outer/periportal zone. Angiectasis, vacuolization and dilatation of hepatic sinusoids with hyperplastic of the endothelial layer of the central vein. Clear hepatocellular necrosis and hypertrophic (3-4 times larger than normal hepatocytes), other hepatocytes showed cystic and fatty degeneration (macrovesicular and microvesicular steatosis). The lesions observed in the sections of Ya-intoxicated rats, were also seen in the EtOH+Ya group, but with more severity and more diffuse, especially in the periportal zone and the hypertrophic cells appeared to be six times larger than normal hepatocytes.
Figure 3: Photomicrograph of liver from Control groups showing normal limit, represented by normally arranged hepatocytes around the central vein together with normal sinusoidal capillaries. Fatty change (steatosis) was observed in diffuse manner all over the hepatocytes (black arrow) with severe dilatation and congestion of central vein and sinusoidal space, moderate centrilobular necrosis, hypertrophic of some Kupffer cells, with slight periductal fibrosis surrounding the bile ducts (blue arrow) in Ya treated group. Et+Ya group showing clear centrilobular necrosis (red arrow) and centrilobular cystic degeneration (black arrow), hepatocellular hypertrophy in central zone (purple arrow), Kupffer cells hypertrophy and hyperplasia (shiny green arrow), dilation and multifocal angiectasis of sinusoids (yellow arrow), proliferation endothelial layer if central vein (light blue arrow) (H&E 40X).

Figure 4.
3.3. Electron Microscopic evaluations

TEM displayed numerous vacuoles and fat droplets in the cytoplasm of hypertrophic hepatocyte, severe degeneration and necrosis of hepatocytes organelles, an irregular nucleus with highly irregular chromatin distribution and dense electron membrane-bound granules in cytoplasmic affected cells of Ya and EtOH+Ya groups. Many of nuclei displayed an indistinct membrane, and dark staining revealed a dense amorphous mass of clumped nucleoplasm, and most of the nucleus of the hepatocellular necrotic cells were in pyknosis and sometimes in karyolysis. Large activated Kupffer cells with hypertrophic stellate cells in dilated blood sinusoids, were also seen. The higher magnifications revealed, many lysosomes in KCs, filled with electron-dense nanoparticles and extensive swollen mitochondria. Massive dilated/expanded sER with well-developed cisternae of rER and high proliferation of peroxisomes were also observed.

![Figure 5](image.png)

Figure 5. Transmission Electron micrographs from untreated (Cx) normal manifestation of hepatocyte with a euchromatic nucleus (N) as well as developed a nuclear membrane (yellow head arrow), and the cytoplasm contains normal mitochondria (m), an array of rough endoplasmic reticulum (rER) and smooth endoplasmic reticulum (sER), normal peroxisome (P). EtOH-treated rat showing hepatocytes...
with intracytoplasmic vacuolization (V), clumping of its organelles, extensive lipid droplets “steatosis liver” (f), swollen mitochondria (m), and large stellate “Ito” cells in the space of Disse, dilatated blood sinusoids (S) with hypertrophic Kupffer cells (KC) and degenerative hepatic nuclei (N) surrounded by a fragmented irregular nuclear envelope, while the hepatocytes of Ya-treated rats (alone and in combination with EtOH) showing intracytoplasmic vacuolization (V), swollen mitochondria (m), dilatated blood sinusoids (S) with severe hypertrophic Kupffer cells (KC), severe dilated smooth endoplasmic reticulum (sER) and highly proliferation of peroxisome (P), electro-dense bodies (mb) and debris of necrotic nuclei, a lot of lysosomes (Ly) and collagen fibers adjacent to Kupffer cells.

Table 2. Lesion scoring for liver tissue obtained at 14th weeks sub-chronic trial.

| Groups                        | NCx & PCx | EtOH | Ya | EtOH+Ya |
|-------------------------------|-----------|------|----|---------|
| Hepatocellular necrosis       | 0.00      | 0.86±0.04 | 1.78±0.14 | 2.18±0.10 |
| Hepatocellular hypertrophy    | 0.00      | 0.74±0.02 | 1.76±0.14 | 2.03±0.13 |
| Cystic and fatty degeneration | 0.00      | 1.67±0.07 | 1.26±0.06 | 1.72±0.12 |
| Total lesions score           | 0.00      | 3.27±0.29 | 4.80±0.17 | 5.93±0.14 |

| Groups                        | NCx & PCx | EtOH | Ya | EtOH+Ya |
|-------------------------------|-----------|------|----|---------|
| Nucleus affecting             | 0.00      | 0.42±0.03 | 0.63±0.02 | 0.93±0.02 |
| sER & Peroxisome Proliferation| 0.00      | 0.55±0.06 | 1.02±0.04 | 1.19±0.03 |
| Cytoplasmic vacuoles and lipid droplets accumulation | 0.00 | 0.83±0.04 | 1.03±0.05 | 1.24±0.03 |
| Total lesions score           | 0.00      | 1.80±0.12 | 2.68±0.13 | 3.36±0.1 |

The histopathologic lesions scores of light microscopes in EtOH, Ya and EtOH+Ya groups are a very strong correlation with Ultrastructural changes scores in that groups (r = 0.928 for EtOH Group, r = 0.921 for Ya group and r =0.903 for EtOH+Ya group) (Figure 5). While the table (3) represent the correlation between lesions under light and transmission electron microscope.

Table 3. The correlation between lesions of light and transmission electron microscope.

| TEM lesions                                         | Light microscope lesions (H&E) |
|-----------------------------------------------------|--------------------------------|
|                                                     | Hepatocellular necrosis | Hepatocellular hypertrophy | Cystic and fatty degeneration |
| Nucleus affecting                                   | .988**                  | .978**                   | .893*                        |
| sER & Peroxisome Proliferation                      | .998**                  | .995**                   | .873*                        |
| Cytoplasmic vacuoles and lipid droplets accumulation | .966**                  | .955**                   | .954**                       |

** Correlation is high significant at the level 0.01, *Correlation is significant at the 0.05.
4. Discussion

The current study investigated the histopathological findings and their correlation with ultrastructural results in hepatocytes, after EOH treatment in “Ya” induced experimental liver toxicity. As described previously in a few studies, *Piper methysticum* ‘awa’ feeding induced histopathological changes in the liver of experimental animals [12, 14]. Examination of the H & E-stained liver sections from Ya-gavaged rats under the light microscope, revealed different histological changes in hepatic architectures, such as clear cytoplasmic vacuolation and lipid droplets accumulation, other hepatocytes showed degeneration and necrosis with deep acidophilia and pyknotic nuclei; sometimes extensive karyorrhexis or fragmented nuclei were indicative of acute hepatocellular necrosis. Therefore, TEM examination revealed that, Ya induced alterations in chromatin distribution in the nuclei of hepatocytes. Furthermore, abundant heterochromatin was detected, disorganization of nuclear content as margination and clumping of chromatin, in Ya treated animals could be morphological evidence of injury in the nucleus. In addition, severely degenerated nuclei were detected, and most of the nuclear content was abnormal in appearance such as, fragmented irregular nuclear envelope and disturbed heterochromatine among the Ya group, which has been previously reported to induce DNA damage [14, 15]. These results support the well-known fact that, nucleoplasmic constituents represent the structural counterpart of transcription and processing of messenger and ribosomal RNAs and are therefore highly sensitive indicators of cellular activity [16]. Such a scoring system may be potentially useful for the researchers, in monitoring the changes in the ultrastructure of hepatocytes under pathogenic stress, or medications. The most significant ultrastructural changes with Ya treatment,
occurred in the mitochondria, sER and peroxisomes. The molecules in the electron transport chain, which play the central role in ATP synthesis, are found in the cristae mitochondria. It is also well known that mitochondria are a major source of endogenous production of ROS [17]. The major mitochondrial pathology observed in this study was, edema, the major ultrastructural sign of cellular injury, extensively invading the matrix resulting in swelling of the organelle and structural damage to cristae. Though edema was seen in both groups, it was much more pronounced in EtOH+Ya treated animals. In addition, there was marked disruption in cristae with EtOH+Ya treatment, and cristae integrity was highly affected. Swelling and a loss of regular cristae structure with a ruptured outer membrane, characteristics of deteriorated function of mitochondria [18, 19, 20], were observed among the rats of the EtOH+Ya group. In rats receiving “EtOH+Ya”, an increase in liver weight was consistent with an increase in the incidence of hepatocellular hypertrophy, as observed under the light microscope. Hepatocellular hypertrophy was recognized as, an irregular, diffuse increase in cell size associated with ground-glass cytoplasmic eosinophilia and cytoplasmic glycogen content and enlarged nuclei, primarily located in the centrilobular regions. Severely dilated ERs are indicative of severely damaged hepatocytes [21]. When the ER of rats treated with Ya were examined under TEM, serious damage was observed in the form of irregular lamellar organization, large dilatations and focal breaks in rER, vacuolisations, myelin figures and high proliferation in sER in various regions. However, more severe changes (focal and dilatations) were observed in rER and sER of EtOH+Ya treated animals. Besides the absence of a normal or intact appearance, that is known to be irreversible in the toxic state, as a result of the synergistic action of more than one toxic compound [22], sacs forming lamely-like shapes (disorganization) and vacuolization with high dilatation, contribute to the abnormal functioning of the ERs. The possible mechanisms that could explain the hepatocellular hypertrophy observed in Ya-intoxicated rats, suggest that the induction of drug-metabolizing enzymes by administration of Piper methysticum extract could cause the intrahepatic accumulation of proteins and lipids. It is a result of a slowdown in protein catabolism and particularly those intracellular proteins degraded in lysosomes by autophagy, after the intake of awa in combination with EtOH [12, 23, 24, 25, 26] Another possible mechanism suggests that, hepatomegaly occurs because the awa extract causes a significant induction and modulation of drug metabolizing enzymes particularly CYP isozymes [27, 28]. It is associated with a concomitant increase in peroxisome proliferation in hepatocytes and proliferation of the smooth endoplasmic reticulum (sER). Furthermore, the expansion and proliferation caused significant hepatomegaly, and these findings are in full agreement with previous results of [29] and [30] who reported that hepatocellular hypertrophy is indicative of enzyme induction, protein synthesis enhancement, and increased hepatocyte size could be a result of the proliferation of subcellular cytoplasmic organelles (typically sER and/or peroxisomes) or formed due to the accumulation of glycogen, lipid and water (hydropic degeneration). In addition, the results of the current study corroborated with the findings of previous studies, in respect to hypertrophic hepatocytes that could be caused by acetaldehyde, after the induction of CYP during alcohol consumption [31]. Through the process of binding the tubulin of microtubules, acetaldehyde blocks the secretion of proteins, which leads to increased levels of protein, lipid, water and electrolytes, causing hepatocytes to enlarge, a hallmark of alcoholic liver disease [32]. The excessive generation of free radicals following Ya supplementation, significantly increased MDA levels and was more pronounced in combination with EtOH, which adversely affected cellular membrane integrity and mitochondrial function, as demonstrated by the TEM results. This could be responsible for the enlargement of hepatocytes, via a decreased activity and/or levels of the Na-K ATPase pump (located in the plasma membrane), due to decreased levels of ATP following mitochondrial change, or through direct membrane damage caused by ROS, generated after Ya consumption, resulting in the influx of sodium (accompanied by water) causing cellular swelling/enlargement. These findings were in accordance with a previous study, which reported that, awa root extract induced mitochondrial dysfunction, and a 35% to 40% decrease in total cellular ATP after 3 to 4 hrs of treatment, respectively [33]. However, the highest increase in lesion scores particularly hepatomegaly, observed in the EtOH+Ya combination group, could be a result of a synergistic action or herbal-drug interaction, of ethanol with Piper
methysticum, which produced more severe adverse effects. To date, electron microscope findings in hepatocytes as a result of hepatotoxins, have not been defined systematically. Moreover, it was unclear whether the changes observed in various organelles were reversible or not. However, the present study was not only able to identify the specific changes observed in different organelles, but also provided a better assessment and measurement of ultrastructural injury. Furthermore, this study is the first of its kind, a preliminary investigation of the histopathological lesion scores under a light microscope, following treatment with Ya and in combination with EtOH.

5. Conclusion

In conclusion, this study elucidated changes in morphology of hepatocyte organelles, following the induction of a certain hepatotoxin. Ya was shown to change or alter the morphology of major organelles of hepatocytes and hastened the development of hepatopathy, particularly in combination with EtOH. The structural adverse changes observed in hepatocyte organelles in this study, are likely the cause of significant histological injuries. Since the transmission electron microscope is the highest magnification tool at present, modeling new ultrastructural scoring systems including more organelles and parameters can be useful in estimating the degree of injury and outcome of alternative treatment strategies in management of chronic liver diseases.

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