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# Proximate Composition and Role of *Telfairia Occidentalis* Pod on Monosodium Glutamate-Induced Hepatoxicity in Wistar Rats

**[1]Egbuonu, A.C.C., [1]Alaebo, P.O., [1]Njoku, C.J., [1]Njoku, G.C., [1]Ugwu, P. and [1]Akasike, C.I.**

[1]Department of Biochemistry, College of Natural Sciences,
Michael Okpara University of Agriculture, Umudike, Nigeria
Corresponding Author's email: tonycemalukegbuonu@yaho.com; egbuonu.anthony@mouau.edu.ng

**Abstract**
The enormous waste burden potential of unutilized pod of *Telfairia (T.) Occidentalis*, TOP, could be controlled by using it in diets, and as drugs that could mitigate potential hepatotoxic effect of monosodium glutamate, MSG; a common food flavouring. This study was on the proximate properties of TOP flour, TOPF, and role of the ethanol extract, TOPE on MSG-induced hepatotoxicity in rats. Twenty adult male Wistar rats (120 - 175 g) randomly allotted to five groups of 4 rats each in groups A, B, C, D and E respectively received normal saline (1 ml), MSG (8000 mg/kg), TOPE (200 mg/kg), MSG (8000 mg/kg) + TOPE (200 mg/kg) and MSG (8000 mg/kg) + TOPE (400 mg/kg) *via* oral gavage and daily for 14 days. The proximate properties (%) of TOPF were total carbohydrate (67.51±0.06) > ash (9.31±0.02) > crude fibre (7.88±0.02) > moisture (7.69±0.01 > crude protein (6.21±0.02) > fat (1.40 ±0.02), suggesting its prospective dietary potential. Significant ($P < 0.05$) alterations in the hepatic histology, hepatic functional parameters and associated diagnostic ratios in MSG-treated rats were incomparable to other rat groups. TOPE (200 mg/kg) elicited effect comparable to control and caused significant ($P < 0.05$) dose dependent reduction in the MSG effect. This demonstrated TOPE-related hepato-protection, and consistent protective role against MSG-induced hepatotoxicity, in the rats. Studies to harness the apparent dietary and hepatoprotective potentials of TOP in the rats are warranted and recommended.

***Keywords: Histology, T. occidentalis, Monosodium glutamate Hepatic functions, Diagnostic ratios***

## Introduction

The liver plays an important role in the body including glycogen storage, plasma protein synthesis and detoxification aimed at ensuring the proper production and storage of required metabolites (Imo *et al*., 2021) hence may be susceptible to injury particularly in situation of toxicity. Bioindicators of hepatic function and the associated diagnostic ratios are among the important determinants of animal health status (Jimoh *et al*., 2015). Hepatic functions indicators (alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; bilirubin, BIL and albumin, ALB, and the associated diagnostic ratios (AST:ALT, ALB:ALP, ALT:AST, BIL:ALB, ALB:BIL) are useful biomarkers in the evaluation of cirrhosis (Yang *et al*., 2020) and hepatic disease-associated ailments including hypertension (Rahman *et al*., 2020) and Covid-19 (Martinez and Franco, 2021). The diagnostic ratio, AST:ALT could predict the extent and type (extra or intra) of liver damage (Hall and Cash, 2022) while ALB:ALP could predict early stage of hepatocellular carcinoma (Feng *et al*., 2021). Liver histology predicts extent of agent-related effect in experimental animal model (Jubaidi *et al*., 2019). MSG is a sodium derivative of the acidic amino acid, glutamate, which is popular for its flavour enhancing effect but with potential adversity on the liver in animal models ((Airaodion *et al*., 2019; Kianifard *et al*., 2019). Utilizing plant-based wastes in diets and drugs warrants detailed study to preclude possible toxicity. Ongoing studies seek natural products to counter MSG-related adverse effect (Ibrahim *et al*. 2020). Recently, several natural products (Hajihasani *et al*., 2020) including *T. occidentalis* leaf extract (Owoeye *et al*., 2018) mitigated adverse effects caused by MSG in animal study models.

However, TOP has not been used in this regard. *T. occidenalis* (fluted Pumpkin) which belongs to the genus *Cucurbita* and the family *Cucurbitaceae* is a common leafy and seed vegetable that is widely consumed by many people in Nigeria and the world over (Omimakinde *et al*., 2018; Akwukwaegbu *et al*., 2016). The fruit pod of *T. occidenalis*, TOP, is grossly underutilized (Nyong *et al*., 2021) as it is wholly discarded after removing the seed which places it as a major agro wastes source. There is little information on

the proximate properties of the pod and its potential to mitigate MSG effect. The TOP may have dietary value and may interact with MSG to exert unknown influence on the liver histology, functional and associated diagnostic ratios in animals. This study aimed to ascertain the crude dietary value of TOPF and also the role of ethanol extract of *T. occidemtalis* pod, TOPE, on the functional integrity of the liver in MSG-compromised rats. Crude dietary value was assessed through proximate contents analysis which is an important novel food discovery step to assess the nutritional profile and keeping quality of a new food source (Ahmed, *et al*., 2022). The influence TOPE on the liver function was assessed through the liver histology, liver function markers, including ALT, AST, ALP, ALB, BIL and associated diagnostic ratios (AST:ALT, ALB:ALP, ALT:AST, BIL:ALB, ALB:BIL) which were among the routine liver function bioindicators (Imo *et al*., 2021; Kalas *et al*., 2021).

## Material and Methods

### *Sample collection, preparation and extraction*

Ajinomoto, a brand of monosodium glutamate marketed by West African Seasoning Company Limited, was bought from Ubani industrial market in Umuahia, Nigeria. Matured fruits of *T. occidentalis* were collected in the month of April, 2016 from Ndioro district of Ikwuano, Abia state, Nigeria. The taxonomic identity of the plant was confirmed by Professor Hillary Edeoga of Plant Science and Biotechnology, Michael Okpara university of Agriculture Umudike, Nigeria. The pod was cut open with a knife to remove the seeds, washed under running tap water to remove the surface dirt and sliced into smaller sizes before sun-drying for 14 days. The dried sample was ground into powdery form using Arthur Thomas Laboratory Mill (Crypto model, USA) and kept till used to determine the proximate properties and to obtain the ethanol extract. To obtain the ethanol extract, two hundred grams (200 g) of the *T. occidentalis* pod flour, TOPF, was placed in a conical flask and extracted with 1200 ml of ethanol with constant stirring for 24 hours to facilitate the extraction process using a mechanical shaker (maintained at room temperature and at 200 rpm). Thereafter the solid particles were filtered using a Whatman No. 1 (125 mm) filter paper. The extraction process was repeated three times to achieve complete extraction. The filtrate was left to evaporate at room temperature and concentrated by heating to dryness in a water bath maintained at 60 °C to obtain a dried extract (referred to as *T. occidentalis* pod extract, TOPE*)* that was refrigerated until used.

### *Ethical adherence and Experimental Animals and Design*

The study adhered strictly to the ethical guidelines on animal use as stipulated by the National Research Council, NRC, USA (2011). Twenty adult male albino rats (120-175 g) obtained from the animal house unit of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria were used. All animals were allowed free access to food and water and were housed in aluminum cages maintained under standard laboratory conditions with light and dark cycles of 12 h each and room temperature of 25 ºC. Acclimatized adult male albino rats (120 - 175 g) were randomly allotted to five groups of 4 rats each. Group A rats received normal saline (1 ml). Group B rats received MSG (8000 mg/kg). Group C rats received TOPE (200 mg/kg) while groups D and E rats respectively received (in addition to MSG 8000 mg/kg) 200, 400 mg/kg of TOPE. Experimental administration was orally by gavage and daily for 14 days. At the end of 14 days experimental period, the animals were sacrificed by cervical dislocation, and blood samples were collected by cardiac puncture into plain bottles (to obtain clotted blood). The blood thus collected was allowed to clot after standing for 10 minutes at ambient temperature. Thereafter, the respective serum was separated by centrifuging the coagulated blood samples at 3000 × g for 15 minutes and used for the determination of ALT, AST, ALP, BIL and ALB levels. The liver was collected and preserved with 10 % formalin for histological assessment.

### *Determination of proximate properties, serum indicators of liver functions and associated diagnostic ratios*

The proximate contents (moisture, ash, crude fibre, fat, protein and carbohydrate) were respectively determined by methods as referenced and described earlier (Egbuonu and Osuji, 2016; Egbuonu *et al*., 2016). The serum indicators level of liver function (ALT, AST, ALP and total bilirubin) were respectively determined with Randox commercial Kits based on methods as referenced earlier (Egbuonu *et al*., 2018a). Serum albumin level was determined by the method based on Bromocresol Green (BCG) reaction as reported earlier (Egbuonu *et al*., 2018b). Corresponding diagnostic ratios including AST:ALT, ALB:ALP, ALT:AST, BIL:ALB, ALB:BIL, were calculated from the determined parameters.

### *Histological preparation and examination of the liver*

The liver was prepared and examined for histological alterations as reported recently (Egbuonu *et al*., 2022). The prepared slides were examined with a Motic™ compound light microscope using × 4, × 10 and × 40 objective lenses. The photomicrographs of selected images were captuered using a Motic™ 9.0 megapixels microscope camera at × 100 and × 400 magnifications.

### *Statistical analysis*

The descriptive statistics and test for significance in mean of the data were by one-way analysis of variance (ANOVA) with the statistical package for social sciences (SPSS) version 16. The Duncan's multiple range tests were used to identify the means that differ significantly at $P < 0.05$. Group results were expressed as mean ± standard error of mean, SEM, (n = 4).

## Results and Discussion

The proximate properties (%) as shown in Table 1 revealed total carbohydrate (67.51±0.06) as the highest proximate content in TOPE followed in decreasing order by ash (9.31±0.02), crude fibre (7.88±0.02), moisture (7.69±0.01, crude protein (6.21±0.02) and fat

(1.40 ±0.02). MSG significantly increased ($P < 0.05$) ALT, AST, ALP and BIL but decreased ($P < 0.05$) ALB levels in the rats compared to others. TOPE significantly decreased ($P < 0.05$) ALT, and ALP levels compared to control, MSG and others but decreased ($P < 0.05$) AST, BIL and ALB levels compared only to MSG (Table 2).

Results of the effect on the calculated diagnostic ratios showed that MSG significantly increased ($P < 0.05$) BIL:ALB ratio but decreased ($P < 0.05$) ALB:ALP and ALB:BIL ratios of the rats compared to control and others. However, MSG significantly increased ($P < 0.05$) AST;ALT but decreased ($P < 0.05$) ALT:AST ratios of the rats compared to control and the extract plus MSG co-treated rat groups. TOPE significantly decreased ($P < 0.05$) these ratios (except AST:ALT ratio) compared to control, MSG and other groups. Concomitant exposure to MSG and TOPE resulted to decreased ($P < 0.05$) AST:ALT and BIL:ALB ratios but increased ($P < 0.05$) ALB:ALP and BIL:ALB ratios compared to MSG (Table 3).

The liver sections of rats in MSG group showed evidence of periportal infiltration of mononuclear inflammatory leucocytes and multifocal areas of hepatocellular necrosis with infiltration of mononuclear leucocytes (White arrow) in the portal area (P). The liver sections of rats in TOPE and MSG + TOPE co-treated groups showed histoarchitecture comparable to that of the rats in the control that showed the normal hepatic histo-architecture with normal hepatic lobules, normal hepatocytes arranged in cords around the central veins (CV), cords radiating towards the portal area (P) which showed normal hepatic vein (HV) and bile duct (B) (Figure 1).

Proximate analysis is an important step in novel food discovery as the components indicate the nutritional profile and keeping quality of the new food source (Ahmed *et al*., 2022). Herein, the proximate properties (%) of TOPF as shown in Table 1 indicated preponderance of carbohydrate and other food components, suggesting prospective dietary and keeping quality potentials of TOPF. The high carbohydrate content of TOPF compares with the earlier reports for fluted pumpkin pod (Omimakinde *et al.,* 2018; Nyong *et al*., 2021), the rich carbohydrate source, cassava (Nilusha *et al*., 2021) and higher than that reported by Akpabio and Ikpe for *Aneilema aequinoctiale* leaves (2013). The preponderance of carbohydrate in TOPF suggests that TOPF could be utilized in composite flour formulations (Rachman *et al*., 2020). Moisture content, the determinant of shelf life of food is low in TOPF which will support its long keeping life compared to commercial wheat flour (12.49 %) and the recommended moisture content (13 %) for edible cassava (Nilusha *et al*., 2021). The ash content which indicates the inorganic minerals and the least fat content in the TOPF, respectively are comparably higher and lower than those in *Aneilema aequinoctiale* leaves (Akpabio and Ikpe, 2013) suggesting that TOPF is a rich dietary source for minerals with prospective utility value in low fat diet formulations, and unlikelihood to form starch- lipid complex that hampers food swelling and solubility (Nilusha *et al*., 2021). Protein content of TOPF is higher than that recorded for cassava (Nilusha *et al*., 2021) but lower than 17.05 % reported for *Aneilema aequinoctiale* leaves (Akpabio and Ikpe, 2013). The crude fibre is higher than 1.34 % reported for *Aneilema aequinoctiale* leaves (Akpabio and Ikpe, 2013). Barring water content which was favourably lower, the other proximate contents in TOPF were higher than those reported for jackfruit, pineapple and banana fresh fruits (Saputri *et al*., 2022). Therefore, TOPF is a prospective rich source of nutrients mix with good keeping quality and possible utility in low fat-diet formulations.

The common liver function bioindicators include ALT, AST, ALP, BIL and ALB as assessed herein and significant alteration in these bioindicators indicated underlying health issues associated with hepatic dysfunction (Choi *et al*., 2020; Kalas *et al*., 2021) or collapse in hepatic response involved in the host defense mechanisms (Kalas, *et al*., 2021). This may explain why liver function texts are almost always assessed to preclude possible toxic influence of a prospective new food source. In this study, the proximate profile of TOPF suggested its nutritional potential. The present study further explored the influence of TOPE on MSG-related hepatotoxicity in rats to establish its possible dietary hepato-protective potential. Results revealed that the significant ($P < 0.05$) alterations in the hepatic functional parameters, the associated diagnostic ratios and hepatic histology in MSG-treated rats were incomparable to the control and the other rat groups. This demonstrated and collaborated the adversity of MSG on the functional and histological integrities of the rats' liver in line with recent study reports (Airaodion *et al*., 2019; Kianifard *et al*., 2019), and in association with simultaneous elevation in ALT and AST levels observed herein in the MSG rats' group (Abdel-Ghaffar and Abdelghaffar, 2022; Egbuonu *et al*., 2022). Increased activity of ALP as in MSG rats' group indicated hepatocellular dysfunction or bone disease which may result from increased *de novo* synthesis by liver cells but diminished excretion and bile flow as in obstructed biliary system (Feng *et al*., 2021; Kalas *et al*., 2021). This could be supported by the concomitant elevation of ALP and BIL levels as in MSG-treated rats herein which indicated a cholestatic pattern of liver damage (Kalas *et al*., 2021). The excretion and conjugation of BIL are closely linked to the conjugating and excreting functions of the liver. BIL level generally increased due to an excretion defect in the liver, notably cholestasis (Kalas *et al*., 2021) which may be a pointer to a novel mechanism of MSG-related liver damage warranting follow-up. Decreased ($P < 0.05$) ALB level in MSG rats' group reported in this study indicated inflammation of the rats' liver and consequent compromised functional capacity of the rats' liver to synthesize protein. This is plausible as ALB synthesis occurs in the liver while the level diminishes in consequence to systemic inflammation hence it is a marker of liver inflammation

and impaired functional capacity of the liver related to protein synthesis (Kalas *et al*., 2021; Feng *et al*., 2021). This suggests that aside protein excretion defect, protein synthesis defect owing to liver inflammation may be another mechanism by which MSG exerts toxic response on the liver.

Normal or absolute values of liver enzymes may not rule out or may be poor indicator of liver diseases (Antipass *et al*., 2020). Both cases of high and low albumin levels indicated hepatic dysfunction (Choi *et al*., 2020). Thus, associated diagnostic ratios are used to augment the common liver function results (Castera, 2018). Concomitant elevation in the levels of ALT, AST, ALT:AST ratio and ALP as in the MSG rats' group indicated a spiked pathological risk (Kohsari *et al*., 2021). Herein, result of the associated diagnostic ratios supported the absolute value result of the liver function test. Recent study showed that the AST:ALT ratio accurately predicted liver damage (Antipass *et al*., 2020; Anand and Singh, 2019; Castera, 2018) while ALB:ALP ratio reflected liver inflammation reactions (Feng *et al*., 2021). TOPE (200 mg/kg) elicited effect comparable to control and caused significant ($P < 0.05$) dose dependent reduction in the discussed MSG effects. This demonstrated TOPE-related benefit on these hepatic function bioindicators and capacity to consistently mitigate attendant alteration of these bioindicators caused by MSG in the rats. Liver histology outcome complements agent-related effect on the liver function tests in experimental animal model (Kianifard *et al*., 2019; Jubaidi *et al*., 2019). Herein, as shown in Figure 1, the liver sections of rats in MSG group showed evidence of periportal infiltration of mononuclear inflammatory leucocytes and other indicators of liver damage whereas the liver sections of rats in TOPE and MSG + TOPE co-treated groups showed histoarchitecture comparable to that of the rats in the control that showed the normal hepatic histo-architecture. These confirmed that TOPE exhibited beneficial influence on the rats' liver and protected the rats' against MSG induced hepatotoxicity. The present study agreed with that of Ogbonnaya and Uadia (2014) which concluded that *Telfairia occidentalis* pod extract elicited dose-dependent protective effect on liver function parameters in rats. Thus, the present study results support the hypothesis implicating defect in the functional capacity of the liver to synthesize and excrete protein as a probable mechanism of liver damage following MSG intoxication in rats'.

**Conclusion**

The proximate properties result indicated the prospective dietary potential of TOP while the animal study result demonstrated TOPE-related hepato-protection, and consistent protective role against MSG-induced hepatotoxicity, in the rats. Studies to harness the apparent dietary and hepatoprotective potentials of TOP in the rats are warranted and recommended.

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**Table 1: Proximate properties composition (%) of TOPE**

| Proximate properties | Fat | Protein | Ash | Moisture | Fibre | Total Carbohydrate |
|---|---|---|---|---|---|---|
| **Composition (%)** | 1.40 ±0.02 | 6.21±0.02 | 9.31±0.02 | 7.69±0.01 | 7.88±0.02 | 67.51±0.06 |

***Results are mean ± standard error of mean, SEM (n = triplicate determinations)***

**Table 2: Role of *T. occidentalis* pod extract, TOPE, on hepatic bioindicators in MSG-compromised rats**

| Groups | ALT (IU/L) | AST (IU/L) | ALP (IU/L) | BIL (mg/dl) | ALB (g/dl) |
|---|---|---|---|---|---|
| Control | 74.48±5.13$^{b}$ | 11.67±1.31$^{a}$ | 34.85±0.92$^{b}$ | 0.33±0.03$^{a}$ | 6.85±0.44$^{e}$ |
| MSG (8000 mg/kg) | 97.75±2.10$^{e}$ | 20.50±0.41$^{e}$ | 50.75±1.25$^{e}$ | 0.53±0.05$^{d}$ | 3.32±0.15$^{a}$ |
| TOPE (200mg/kg) | 69.25±0.95$^{a}$ | 15.80±0.82$^{d}$ | 34.00±1.15$^{a}$ | 0.45±0.06$^{c}$ | 3.86±0.16$^{d}$ |
| MSG (8000 mg/kg) + TOPE (200mg/kg) | 81.00±0.41$^{c}$ | 15.62±0.85$^{c}$ | 45.50±0.29$^{d}$ | 0.45±0.03$^{c}$ | 3.56±0.13$^{b}$ |
| MSG (8000 mg/kg) + TOPE (400mg/kg) | 84.50±0. 65$^{d}$ | 14.25±0.96$^{b}$ | 45.25±0.63$^{c}$ | 0.38±0.05$^{b}$ | 3.63±0.02$^{c}$ |

***Results represent mean± S.E.M of group serum results obtained (n = 4). Mean values in the same column having different letters of the alphabet, are statistically significant at P < 0.05. Control group (A), MSG group (B), TOPE (200 mg/kg) group (C), MSG + TOPE (200 mg/kg) group (D) and MSG + TOPE (400 mg/kg) group (E)***

**Table 3: Role of *T. occidentalis* pod extract, TOPE, on some diagnostic ratios of hepatic bioindicators in MSG-compromised rats**

| Groups | AST:ALT Ratio | ALB:ALP Ratio | BIL:ALB Ratio | ALB:BIL Ratio | ALT:AST Ratio |
|---|---|---|---|---|---|
| Control | 0.15±0.06$^{a}$ | 0.19±0.06$^{e}$ | 0.04±0.08$^{a}$ | 20.75±0.05$^{e}$ | 6.38±0.02$^{e}$ |
| MSG (8000 mg/kg) | 0.20±0.0$^{d}$ | 0.06±0.05$^{a}$ | 0.15±0.09$^{e}$ | 6.26±0.06$^{a}$ | 4.76±0.0$^{b}$ |
| TOPE (200mg/kg) | 0.22±0.0$^{e}$ | 0.11±0.03$^{d}$ | 0.11±0.06$^{c}$ | 8.57±0.07$^{c}$ | 4.38±0.02$^{a}$ |
| MSG (8000 mg/kg) + TOPE (200mg/kg) | 0.19±0.02$^{c}$ | 0.07±0.08$^{b}$ | 0.12±0.06$^{d}$ | 7.91±0.01$^{b}$ | 5.18±0.05$^{c}$ |
| MSG (8000 mg/kg) + TOPE (400mg/kg) | 0.16±0.0$^{b}$ | 0.08±0.02$^{c}$ | 0.10±0.04$^{b}$ | 9.55±0.05$^{d}$ | 5.92±0.0$^{d}$ |

***Results represent mean± S.E.M of group serum results obtained (n = 4). Mean values in the same column having different letters of the alphabet, are statistically significant at P < 0.05. Control group (A), MSG group (B), TOPE (200 mg/kg) group (C), MSG + TOPE (200 mg/kg) group (D) and MSG + TOPE (400 mg/kg) group (E)***

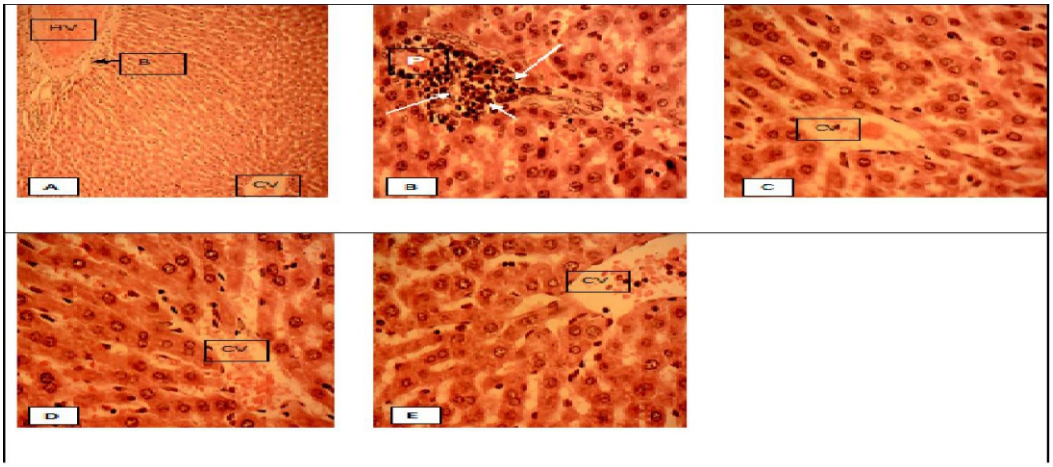


***Figure 1: Photomicrograph of the influence of TOPE on the liver histology in MSG-compromised rats (N/B: Control group (A), MSG group (B), TOPE (200 mg/kg) group (C), MSG + TOPE (200 mg/kg) group (D) and MSG + TOPE (400 mg/kg) group (E). Hematoxylin and Eosin (H & E) stained × 400).***

*Notes: (P = Portal area; HV = Hepatic vein, CV = Central vein, B = Bile duct)*

*A (Control): Sections of the liver collected from the rats in Group A showed the normal hepatic histo-architecture with normal hepatic lobules and normal hepatocytes arranged in cords around the central veins (CV). The cords radiate towards the portal area (P) which showed normal hepatic vein (HV) and bile duct (B). H & E x 400.*

*B: Showed evidence of periportal infiltration of mononuclear inflammatory leucocytes and multifocal areas of hepatocellular necrosis with infiltration of mononuclear leucocytes (White arrow) in the portal area (P). H & E x 400.*

*C, D and E: Showed similar hepatic histo-architecture as in the control with normal hepatic lobules, normal hepatocytes arranged in radiating cords around the central veins (CV). The hepatocytes which are arranged in cords radiate towards the portal areas which showed normal hepatic vein (HV) and bile duct (B). H & E x 400.*