Dietary effect of cocoa pod husk meal on organ histology and protein concentrations in brain regions of rabbits

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Abstract

Cocoa pod husks contain anti-nutrients that could cause digestive and nervous disorders in farm animals. The pod husks could pollute the environment if not properly handled; however, they can be treated and processed for livestock feeding. Therefore, this study evaluated the effect of dietary cocoa pod husk meal (CPHM) on the histology of internal organs and protein concentrations in brain regions of rabbits. Sixty rabbits (mixed breeds) with mean body weight (606.42 ± 1.30 g/rabbit) were used. Twelve *iso*-caloric (2500.12 Kcal/kg ME) and *iso*-nitrogenous (16.05% CP) diets were formulated. The dietary treatments contained sundried, fermented and hot –water treated CPHM each at 0, 12.5, 25 and 37.5% levels, respectively with the 0% serving as control. The rabbits were randomly distributed to the diets using a completely randomized design (CRD). The animals were raised to maturity before being sacrificed for histologic examination of kidneys, liver and testes. Heads of the slaughtered rabbits were severed, brain evacuated and differentiated into separate regions for protein concentration determinations. Results showed atrophies on the kidneys, liver and testes as the CPHM inclusion level increases with severe histological distortions occurring in rabbits fed sundried CPHM at 37.5%. The protein concentrations in the brain regions revealed significant ($p<0.05$) adverse effect on the amygdala, cerebral cortex and medulla oblongata. The study concluded that different forms of CPHM could be included in diets meant for rabbits at levels ≤ 25 %; higher levels will cause serious adverse effects on internal organs and brain function due to the residual theobromine.

Keywords: Brain, cocoa, digestion, husk, organ, rabbit

Introduction

The geometrical rate of population growth in Africa which was projected in 2004 to be 871 million people has been affected adversely by dwindling food resources (Gueye, 2007). Extant reports show that one billion people in the world are living in a state of malnutrition; of which 700 – 800 million are in Africa and approximately 70 % of these are rural poor farm families (FAO, 2012). In Africa, the twin problems of malnutrition and poverty have posed serious threat to human livelihoods. According to Allan (1983), more than 500 million people in the

developing countries are facing chronic malnutrition and abject poverty due to unsustainable income, inflation and unemployment. Animal production which accounts for 40 % of the total gross value of global agricultural productivity is likely to increase as the demand for livestock products is growing rapidly with increase in human population and urbanization (FAO, 2003). The geometrical population growth in the world depicts more demand for meat and associated animal protein, which presently is in a deficit (Attah *et al.*, 2011). This dearth of animal protein is mainly due to poor animal productive performance, especially in developing countries.

With the rapid exhaustion of limited animal products, increasing population and better life styles, it is now imperative to explore other aspects of livestock production to bridge the gap of inadequate protein intake from animal origin. According to FAO (2012), with effect from year 2000, the projected world requirement for animal protein would increase tremendously due to population explosion. Traditional ruminants and monogastrics are the main sources of domestic meat. However, rabbits (*pseudo-ruminants*) and other micro livestock species are emerging in many developing countries like Nigeria as potential sources of animal protein. The inadequacy of animal protein coupled with stiff competition between man and animals for agricultural produce call for enhancing the diversification and productivity of livestock and this has culminated in resurgence of interest in rabbit production (Oyadeyi *et al.* 2013; Ozung, 2021; Ozung *et al.,* 2021). The renewed interest in rabbit production in Nigeria has necessitated research into alternative feed resources that are readily available, under - utilized agro by - products or agro - industrial wastes like cocoa pod husks to substitute or supplement the highly demanded conventional cereals (Agunbiade *et al.*, 2001; Ozung, 2016). These wastes have adversely affected the environment by way of polluting and distorting the beauty of the natural ecosystem; hence the need to convert wastes to wealth via utilizing these husks in rabbit nutrition.

Rabbit production has been intensified owing to the fact that the animals are very prolific with short gestation length of 30 ± 2days and quick attainment of maturity (Oyadeyi *et al.*, 2013). Rabbit meat is healthy as it is low in cholesterol (50 g/100 g); fat (4 g/100 g); energy (124 Kcal/100 g) but high in protein (22 g/100 g) (Aduku and Olukosi, 1990). According to Ozung (2016), rabbit meat is rich in crude protein (21.79 – 22.39 %) and energy range between 100.92 and 115.86 Kcal/100 g. The researcher further reported that rabbit meat is rich in minerals such as calcium (0.12 – 1.90 mg/kg), manganese (0.01 – 0.07 mg/kg), magnesium (0.09 – 0.48 mg/kg), potassium (10.16 – 14.81 mg/kg) and phosphorus (0.01 – 11.44 mg/kg). The meat has a complete amino acid profile compared to plant protein sources; it has good flavour, rich in nutrients as well as easily digestible (Cheeke, 1986).

Literature reports (Ijaiya *et al.*, 2005; Ozung *et al.,*2011) abound on the use of agro - industrial by – products and farm waste materials in livestock nutrition. The prices of most of these by – products have escalated as a result of high demand; thereby necessitating the quest for cheaper alternatives common in the tropics, for the purpose of promoting optimum performance characteristics and reduction in cost of production and to make rabbit farming profit oriented to the teaming unemployed youth and rural poor farmers in Nigeria (Tuluen and Patrick, 2021). Most diets for rabbits consist of ingredients from plant sources (NRC, 1977; Raharjo *et al.,*1986), composite mixture of

table scraps (Shueir, 1985) and agro by – products (Ijaiya *et al.*, 2005; Ozung *et al.,*2011). One of the promising agro by - products that can be utilized in rabbit diet formulations is the cocoa pod husk. Cocoa pod husks constitute 75 percent of the entire cocoa fruit on fresh weight basis (Fagbenro, 1992). Adomako (1991) reported that cocoa beans account for less than 2.55 % of the whole fruit. Cocoa pod husks contain 6 - 7 % crude protein, 9 - 10 % total ash, 1 – 8 % crude fat (ether extract) and 23 – 33 % crude fibre (Donkor *et al.*, 1991). Furthermore, Sobamiwa and Longe (1994) reported that the metabolizable energy of cocoa pod husk is moderate and ranges from 2000 – 2100 kcal/kg; which is comparable to that of palm kernel cake, soybean meal, rice bran and brewers dried grain (Anon, 1989). The processed cocoa pod husks have been reported to have low theobromine content (Donkor *et.*, 1991). The crude fibre is easily digestible (Opeke, 1997). Cocoa pod husk is digestible by all classes of livestock, especially ruminants and rabbits (Ozung, 2016). However, the high crude fibre content (21.49 – 34.82 %) hinders its effective utilization by monogastrics (Abiola and Tewe, 1991). This constraint calls for the treating and processing of the cocoa pod husks by various methods (fermentation, hot – water treatment, urea, enzyme, fungal treatment and microbial detheobromination), so as to promote digestibility and biodegradability in animals.

Fermented and unfermented cocoa pod husk meal have been used in determining the growth rate, feed efficiency and meat quality of rabbits as well as the physiological responses of rabbits (Ozung, 2016). There is paucity of physiological findings on rabbits fed cocoa by –products in Nigeria, hence; this study evaluated the effect of differently treated dietary cocoa pod husk meal on the histology of internal organs and protein concentrations in brain regions of rabbits.

Materials and methods

Location of the study

This study was carried out from 1st March – 30th September, 2021 (duration of 7 months) at the Rabbitry Unit of the Teaching and Research Farm, University of Calabar, Calabar, Cross River State, Nigeria. According to the GeoNames geographical database (2021); Calabar is located at 4.9517^0 latitude and 8.322^0 longitude (in decimal degrees) with an average elevation/altitude of 42 metres. While, Akpan *et al.* (2006) reported that Calabar is located at latitude 3^0N of the equator and longitude 7^0E of the Greenwich meridian, with a land mass of 233.2 sq. miles (604 km^2). The annual rainfall ranges from 3000 – 3500 mm (average of 1,830 mm) per annum and the average daily temperature is 25^0 C/77^0 F which increases to 30^0C (86^0F) in August. The relative humidity is between 70 and 80 %, while the wind speed/direction is 8.10 km/h west and the cloud is broken at 1000 ft with little cumulonimbus at 2200 ft.

Collection and processing of cocoa pod husk meal (CPHM)

Freshly broken composite cocoa pod husks were obtained from the fermentation units of the Cocoa Research Institute of Nigeria (CRIN) sub - station at Ajassor, Ikom LGA of Cross River State. The pods were collected during the main production season in West Africa (March – September) (Opeke, 1997). The broken pods were washed and sun - dried to constant weight, bulked and milled with hammer mill to produce cocoa pod husk meal (CPHM). The resultant meal was divided into three (3) portions: The sundried CPHM (SCPHM), Fermented CPHM (FCPHM) and Hot water - treated CPHM (HCPHM), respectively. Cocoa pod husk meal for the fermented treatment was thoroughly mixed with 60 % water, relative to its weight as

ascertained by Bello *et al.* (2012) and bagged in an air tight polythene bag. This was allowed to stay for three (3) days under room temperature, thereafter, it was opened and shade dried to constant weight; before being packed, bagged and stored in a cool dry place until it was used for diet formulation. The final portion of CPHM was treated with hot water that was boiled to 100^0 C for 15 minutes (Odunsi *et al.*, 1999; Olubamiwa *et al.,* 2006; Adeyina *et al.*, 2010) which was later drained, shade dried and stored in air-tight containers for later use in feed formulation.

Experimental diets

Twelve (12) *iso- nitrogenous* (16.05 % CP) and *iso –caloric* (2500.12 Kcal/kg ME) diets were formulated in line with the nutrient needs of rabbits as recommended by Aduku and Olukosi (1990). Each processed form of CPHM was included at 0, 12.5, 25 and 37.5 % levels for T_1, T_2, T_3, and T_4 (Sundried CPHM), T_5, T_6, T_7, T_8 (Fermented CPHM) and T_9, T_{10}, T_{11}, T_{12} (Hot – water treated CPHM), respectively in the experimental diets. Diet without CPHM (0 percent) served as control in the experiment. The choice of these levels was based on earlier reports on the use of much lower levels of CPHM for poultry, pigs and rabbits without adverse and significant effects on growth performance characteristics (Teguia *et al.*, 2004; Olubamiwa *et al.*, 2006; Adeyina *et al.*, 2010). Feedstuff purchase/choice and procurement of cocoa pod husks as well as methods of processing CPHM and ration formulation gave primary consideration to least cost and maximum biological returns (Ogunwole *et al.*, 2010).

Experimental rabbits, management and ethical approval

Sixty (60) weaned mixed breed rabbits between 5 and 6 weeks old of both sexes (24 bucks and 36 does), (average initial body weight of 606.42±1.30 g) were used in this study. The rabbits were purchased from a reputable rabbitry (Domino farms, Use - Offot) in Uyo, Akwa Ibom State. They were managed based on standard experimental procedures. On arrival at the rabbitry facility, the animals were provided with anti – stress vitalyte at 0.50 g per 75 litres of chlorine - free water. Concrete drinking troughs and fabricated feeding troughs (empty beverage cans nailed to the wooden board) were provided in each cage.

The rabbits adjusted for two weeks before the actual commencement of the feeding trial and within this period; they were placed on commercial pelleted grower mash and screened against ecto and endo parasites via subcutaneous injection of Ivermectin (Kepromec) at the recommended level (0.20 ml per rabbit). Thereafter, the animals were subjected to 21 weeks feeding and at maturity (5 months), they were sacrificed for morphometric evaluations.

Housing and equipment

The experimental animals were housed individually in double tier wooden hutches (with wire mesh floor) measuring 65 × 65 × 65 cm (L × H× W) and raised 25 cm from the ground and placed in a standard rabbitry with half walls to allow for cross ventilation.

Experimental design

Animals were randomly distributed to the test diets in a simple Completely Randomized Design (CRD) experiment. They were twelve (12) dietary treatments with five (5) rabbits (2 bucks and 3 does) per treatment. The rabbits were assigned to the various treatments after equalizing for body weight and sex.

Evacuation of brain regions and determination of total protein concentrations

The rabbit heads were obtained and cut - off at the *occipito – atlantal* joint and placed in refrigerated containers for coding.

The heads were thereafter frozen at – 20^0 C for 14 days before there were dissected (Bitto *et al.*, 2000). During the process, each frozen head was placed in a dorsoventral position on ice – cold porcelain tile for cutting (Egbunike, 1981) before the brain was dug out. The brain was further freed of meninges, weighed and dissected out into different regions (Egbunike, 1981) based on the brain atlas of rabbits. Samples of each brain region (cerebral cortex, cerebellum, amygdala, hypothalamus, pons, mesencephalon, hippocampus, medulla oblongata) were homogenized separately in 1 % (w/v) 0.1 M ice - cold phosphate buffer with 0.10 % Triton X – 100. The total protein concentrations in brain regions were thereafter evaluated by the Biuret method as outlined in the Boehringer Mannheim Diagnostica Manual (1979) as already reported by Bitto *et. al.* (2000) and Gbore and Egbunike (2012).

Histopathological examination

Gross histopathological examination of the internal organs (kidneys, liver) and gonads (testes from rabbit bucks) in each dietary treatment was determined at the Diagnostic Laboratory of the University of Calabar Teaching Hospital (UCTH) after they were carefully removed and fixed with 10 % formalin solution. The gross histopathological examination was carried out using standard laboratory methods (Drury and Wallington, 1976).

Statistical analysis

Data obtained in this study were subjected to one – way Analysis of Variance (ANOVA) using General Linear Model (SAS,1999) for a completely randomized design (CRD). Significant means were compared using the Least Significance Difference (LSD) method (Steel and Torrie, 1980).

The experimental model used was as follows:

$Y_{ij} = \mu + T_i + E_{ij}$

Where:

Y_{ij}: Observed value

μ: Overall mean value

T_i: Random effect of the i^{th} processing method of CPHM

E_{ij}: Random residual error

Results

Weight of brain and total protein concentrations in brain regions

Results showing the weight of brain and total protein concentrations of rabbits fed diets containing differently processed forms of CPHM are presented in Table 1. The weight of rabbit brain obtained in this study showed significant effect ($p < 0.05$) of diets. The values obtained were 10.14, 13.98, 8.11 and 11.74 g in the sundried CPHM; 8.36, 10.12, 14.05 and 6.79 g in the fermented CPHM as well as 7.60, 7.83, 7.59 and 6.41 g in the hot – water treated CPHM in diets containing 0, 12.50, 25.00 and 37.50 percent inclusion levels, respectively. The protein concentrations recorded in the Amygdala, Cerebral cortex and Medulla oblongata showed significant differences across dietary treatments. The range of protein concentrations in the Pons was 0.29 – 0.96 g/100ml in the sundried CPHM; 0.32 – 0.73 g/100ml in the fermented CPHM and 0.22 – 0.65 g/100ml in the hot- water treated CPHM. Cerebellum recorded 0.48 – 1.54 g/100ml in the sundried CPHM; 0.38 – 1.07 g/100ml in the fermented CPHM and 0.33 – 0.86 g/100ml in the hot – water treated CPHM. Hypothalamus recorded 0.24 – 0.64 g/100ml; 0.26 – 0.70 g/100ml; 0.36 – 0.80 g/100ml for the sundried, fermented and hot – water treated CPHM), respectively. Mesencephalon recorded 0.44 – 0.96 g/100ml; 0.16 – 0.64 g/100ml; 0.26 – 0.65 g/100ml in the sundried, fermented and hot –water treated CPHM, respectively and Hippocampus recorded 0.34 – 0.66 g/100ml; 0.17 – 0.78 g/100ml; 0.35 – 1.11 g/100ml in the sundried, fermented and hot – water treated CPHM,

respectively and were statistically similar without a particular trend, across dietary treatments except for the pons and cerebellum in the sundried and fermented CPHM groups, respectively. The results showed higher concentrations of total protein in the fermented and hot – water treated CPHM groups and lower values in the sundried CPHM group.

Histopathological lesions of major organs

The histopathological lesions of the kidney, testis and liver of rabbits are summarized in Table 2, with the respective photomicrographs presented in Plates 1a – 3l. Results show normal tissue architecture in the organs fed the control diets, with progressive tissue disintegrations, damages, vacoulations and atrophies as the levels of cocoa pod husk meal increased across the dietary treatments. The sundried cocoa pod husk meal group showed gross histological atrophies in all the organs, followed by the hot – water treated group and least in the fermented cocoa pod husk meal group.

Discussion

The weight of brain of rabbits fed experimental diets revealed a fluctuating trend but with significant ($p< 0.05$) differences between treatments. The protein concentrations in the amygdala, cerebral cortex and medulla oblongata were significantly ($p< 0.05$) affected by treatments. There were similarities in the concentrations of protein in pons, cerebellum, hypothalamus, mesencephalon and hippocampus; implying that cocoa pod husk meal may not have adverse effects in protein concentration of these brain regions. Total protein concentrations in the 37.5 % level of CPHM inclusion were significantly increased in amygdala for the hot – water treated CPHM group compared to values in the sundried and fermented groups. A major effect of diet on the protein concentration was recorded in the amygdala and medulla oblongata where the control diet in the fermented CPHM group was superior to other diets in the sundried and hot – water treated CPHM groups, respectively. The highest protein concentrations in the amygdala (hot – water treated CPHM), cerebral cortex (fermented CPHM), medulla oblongata, pons, cerebellum and mesencephalon (sundried CPHM) and hippocampus (hot – water treated CPHM) were obtained in the highest inclusion level of CPHM (37.5 %) compared to values in the control diet for the different treatment methods. This implies that there was the absence of interference of residual theobromine with neural mechanisms involved in protein synthesis in the brain regions of rabbits. Therefore, an important brain region like the cerebellum which serves as a major coordinator of the other processes of the brain including neurosecretions (EPSA, 2008) will perform optimally well with CPHM based diets. This finding has however, contradicted the findings of Bitto (2008) who reported significantly lower protein concentrations and interference of residual papain in the functioning of the cerebellum of rabbits fed unripe pawpaw peel based - diets. The protein concentrations obtained in this study for the cerebral cortex, cerebellum and amygdala were comparable with the findings of Bitto (2008), but protein concentrations in medulla oblongata, pons, hypothalamus, mesencephalon and hippocampus are fairly higher than the values earlier reported by Bitto (2008). These differences could be attributed to dietary effect as residual theobromine in this study has a rather antagonistic effect to papain used in the earlier study.

The outcome of histopathological evaluation of some organs (testes, kidney and liver) of rabbits sundried, fermented and hot – water treated cocoa pod husk meal – based diets and the associated photomicrographs, revealed some histopathological damages to these organs

by the dietary treatments compared with the control in each treated form of CPHM. The photomicrographs of the testes appeared normal in the control, but there were obvious testicular changes with induced alteration in spermatogenesis accompanied by noticeable lumen filled with degenerated spermatids as the level of CPHM inclusion increased across dietary treatments. The effect was most pronounced in the sundried CPHM group, followed by the hot – water treated CPHM and least in the fermented CHPM group. These findings have confirmed testicular degeneration and abnormal spermatozoa due to residual theobromine toxicity. This observation further agrees with the findings of EFSA (2008) that theobromine affects gonads in rodents. The histopathological examination outcome of the kidney revealed visible enlargement of the capsular loop with vacoulations and extensive degeneration of tubular epithelial tissues especially at the highest inclusion level (37.5%) of the CPHM compared with the control (which showed normal kidney architecture) and lower inclusion levels (with little atrophy of the glomeruli), irrespective of the treatment method. This study has further confirmed that residual theobromine from different forms of CPHM at high inclusion level has toxic effect on the kidneys of rabbits. This is in line with the report given by EFSA (2008) that rodents exposed to theobromine from any source will suffer from kidney toxicity and depressed weight gain. It was further observed that rabbits fed the 37.5 % sundried, fermented and hot – water treated CPHM inclusion level showed gross liver hepatic degeneration, swollen hepatocytes, obliterated sinusoids and general necrosis which were diffused as compared to the mild effects at the lower inclusion levels. The most consistent histopathological finding in the liver was a high incidence of cellular infiltration by mononuclear cells and degeneration due to theobromine toxicity. Akande *et al.* (2011) had earlier reported lesions like severe fatty changes in the liver, widely distributed internal petechial haemorrhages or ecchymoses and catarrhal enteritis. This finding has also revealed that theobromine from CPHM has adverse effects on the liver of rabbits. It agrees with the finding of EFSA (2008) that rabbits, dogs, dairy cows and horses exposed to theobromine will suffer liver damage, while pigs will show poor growth and enteritis. It is therefore obvious that experimental diets containing anti – nutritional factors like theobromine from cocoa products or other toxic compounds will induce histopathological damages to major organs like testes, kidney and liver in animals like rabbits. This observation corroborates the findings of Ewuola *et al.* (2003) who fed *Fusarum verticulloids* culture feed material to rabbits.

Conclusion

Within the experimental conditions of this study, it is therefore concluded that sundried, fermented and hot – water treated cocoa pod husk meal could be included in diets meant for rabbits at levels ≤ 25 %. The fermented CPHM was most superior, followed by the hot-water treated and lastly the sundried treated CPHM. At levels not beyond 25 %, the residual theobromine and other associated anti-nutrients in cocoa husks will promote optimum protein concentrations in the brain regions and ensure histological integrity of internal organs.

Based on the findings of this study, the fermented cocoa pod husk meal (CPHM) based - diet showed superiority in the parameters considered; it is therefore recommended that fermented cocoa pod husk meal should be included in diets meant for rabbits at a level not exceeding 25%.

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Table 1: Weight of brain and total protein concentrations in brain regions of rabbits fed cocoa pod husk meal - based diets

Parameter	SCPHM				FCPHM				HCPHM				
(g/100ml)	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	
	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	SEM
Wt. of brain (g)	10.14^{c}	13.98^{a}	8.11^{d}	11.74^{b}	8.36^{d}	10.12^{c}	14.05^{a}	6.79^{e}	7.60^{d}	7.83^{d}	7.59^{d}	6.41^{e}	0.76
Amygdala	0.55^{d}	0.99^{c}	0.53^{d}	0.77^{c}	1.31^{a}	0.40^{e}	0.68^{c}	0.66^{c}	0.80^{c}	0.52^{d}	0.52^{d}	1.15^{b}	0.08
Cerebral cortex	0.34^{d}	1.01^{a}	0.48^{c}	0.73^{b}	1.01^{a}	0.42^{c}	0.53^{c}	0.94^{a}	0.66^{c}	0.74^{b}	0.64^{c}	0.65^{c}	0.06
Medulla oblongata	0.26^{e}	0.49^{d}	0.65^{c}	0.98^{b}	1.27^{a}	0.51^{cd}	0.66^{c}	0.65^{c}	1.03^{b}	0.83^{b}	0.54^{cd}	0.47^{d}	0.08
Pons	0.29	0.56	0.69	0.96	0.44	0.32	0.50	0.73	0.22	0.62	0.54	0.65	0.06
Cerebellum	0.69	0.48	0.55	1.54	0.38	0.53	0.66	1.07	0.86	0.83	0.59	0.33	0.10
Hypothalamus	0.37	0.24	0.32	0.64	0.32	0.26	0.55	0.70	0.80	0.47	0.36	0.76	0.06
Mesencephalon	0.54	0.50	0.44	0.96	0.20	0.16	0.64	0.52	0.65	0.61	0.26	0.56	0.06
Hippocampus	0.43	0.40	0.34	0.66	0.17	0.36	0.78	0.55	0.74	0.55	0.35	1.11	0.07

SCPHM: Sundried Cocoa Pod Husk Meal
FCPHM: Fermented Cocoa Pod Husk Meal
HCPHM: Hot -water treated Cocoa Pod Husk Meal
S.E. M: Standard Error of Mean

Table 2: Histopathological lesions of some organs in rabbits fed cocoa pod husk meal (CPHM) based diets

	Organs		
Dietary Treatment	Testis	Kidney	Liver
0 % Sundried CPHM	Normal appearance of the general tissue structure of Sertoli cells and Spermatogonic cells and Spermatozoa at different stages of development. Plate 1 (a)	Normal appearance of the general tissue structure of the kidney. Plate 2 (a)	Normal tissue structure with no noticeable atrophy of hepatocytes Plate 3(a)
12.50 % Sundried CPHM	No obvious testicular changes in structure, though with intense staining Plate 1(b)	The cyto – structure of the Kidney, exhibiting normal appearance of the glomeruli with no obvious vacuolization and necrosis. Plate 2(b)	The hepatocytes appear normal with no distortion in the tissue structure. Plate 3(b)
25.00 % Sundried CPHM	Spermatozoa at different stages, with no testicular damage of the general tissue structure Plate 1(c)	The cyto – structure of the Kidney with little atrophy and hypertrophy of the glomeruli and some blood spots. Plate 2 (c)	Dilation of sinusoids and prominent central vein. Plate 3 (c)
37.50 %t Sundried CPHM	Induced alteration in spermatogenesis with noticeable lumen filled with degenerated spermatids. Plate 1(d)	Noticeable vacuolization in renal tubules Plate 2(d)	Damaged and enlarged central vein, swollen hepatocytes and sinusoids. Plate 3(d)
0 % Fermented CPHM	Normal appearance with good numerical population of motile germ cells. Plate 1(e)	Normal appearance of the general tissue structure of the kidney with no obvious tubular loss. Plate 2(e)	Normal tissue structure with no atrophy of hepatocytes. Plate 3(e)
12.50 % Fermented CPHM	No noticeable testicular distortion and spermatid number. Plate 1(f)	Noticeable degeneration of the glomeruli. Plate 2(f)	Venous damage with expanded central vein/ sinusoids. Plate 3(f)
25.00 % Fermented CPHM	No noticeable reduction in the volume of mature spermatozoa with no	Atrophy of the glomeruli Plate 2(g)	Blue stained areas of the collapsed portal tract.

	alteration in testicular morphology. Plate 1(g)		Plate 3(g)
37.50% Fermented CPHM	Degenerated spermatids and tubules with alteration in testicular morphology Plate 1(h)	Visible enlargement of the capsular loop with vacoulations Plate 2(h)	Chronic congestion of the blood, representing accumulation of erythrocytes in centrilobular region (nutmeg liver). Plate 3(h)
0 % Hot – water treated CPHM	Normal appearance of testicular structure and spermatids Plate 1(i)	Normal appearance of the general tissue structure of the kidney with no obvious tubular loss. Plate 2 (i)	Normal tissue structure with no noticeable atrophy of hepatocytes. Plate 3(i)
12.50 % Hot – water treated CPHM	Induced alterations in spermatogenesis with noticeable differences in lumen of various tubules. Plate 1(j)	Cloudy swellings and degeneration of tubular epithelia with atrophy of the glomeruli. Plate 2(j)	Extensive venous damage with expanded central vein and dilated sinusoids. Plate 3 (j)
25.00 % Hot – water treated CPHM	Affected tubules filled with degenerated spermatids Plate 1(k)	Congested blood vessels with atrophy of the glomeruli and enlargement of the capsular loop Plate 2 (k)	Passive congestion of the liver with accumulation of neutrophils around the portal veins. Plate 3 (k)
37.50 % Hot – water treated CPHM	Affected tubules filled with degenerated spermatids and noticeable reduction in the population of sperm cells in seminiferous tubules. Plate 1(l)	Extensive vacuolization in renal tubules Plate 2 (l)	Destroyed hepatocytes, congested blood vessels and erythrocytes infiltrated from portal area. Plate 3(l)

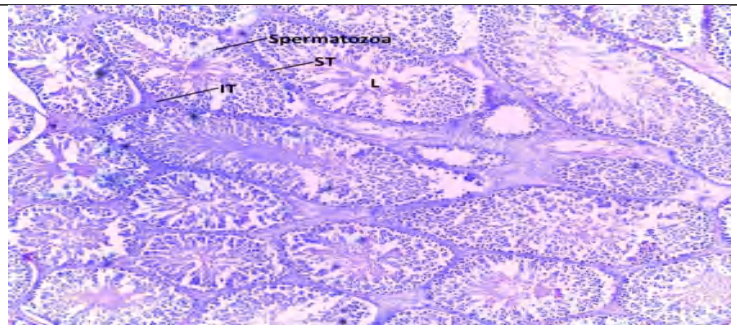
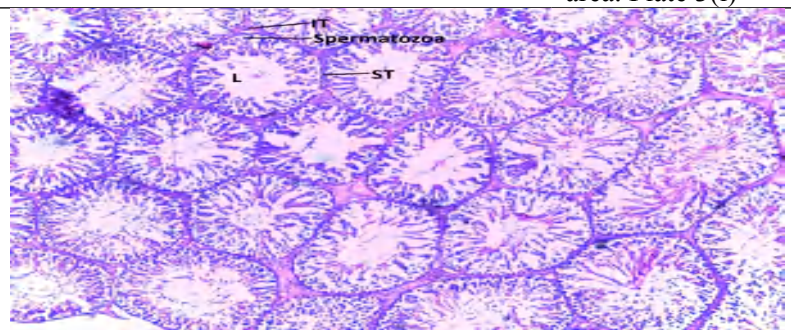
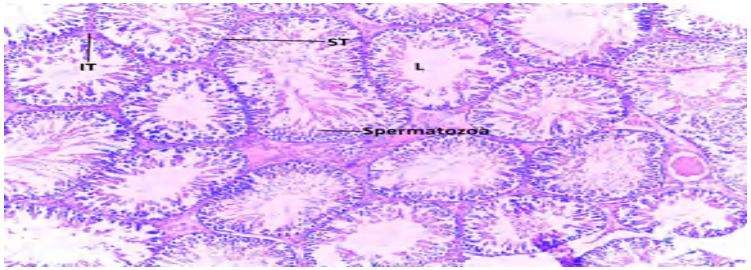


Plate 1(a): Percent CPHM Plate 1(b):



Testis in 0 Sundried (×400) Testis in 0

Percent fermented CPHM (×400)



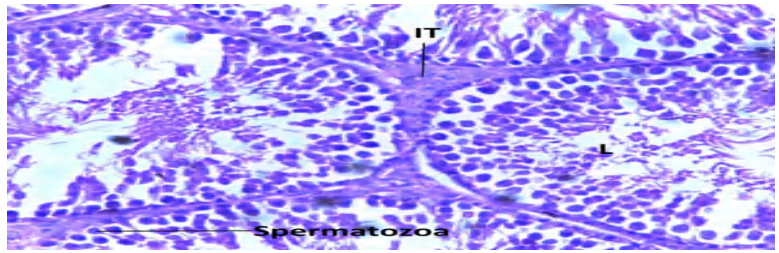
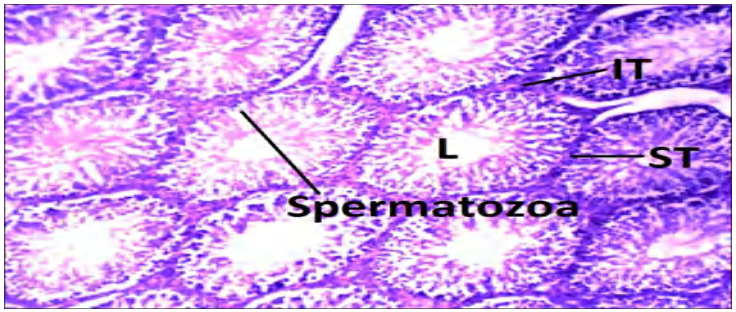


Plate 1(c): Testis in 0 Percent hot –water treated CPHM (×400)

Plate1(d): Testis in 12.50 Percent Sundried CPHM (×400)



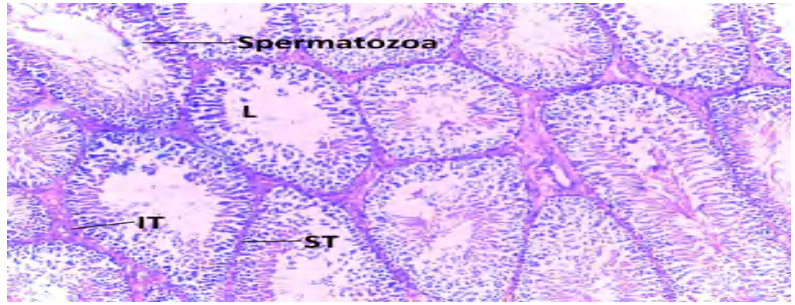
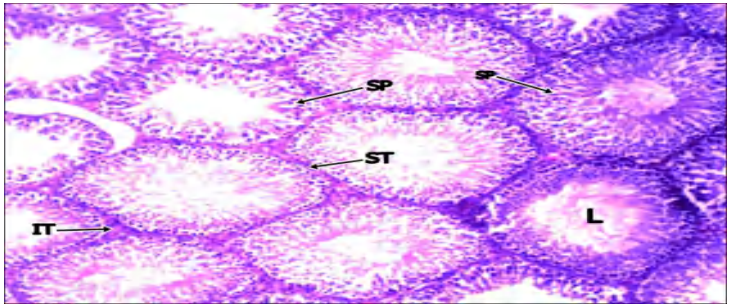


Plate 1(e): Testis in 12.50 Percent fermented CPHM (×400)

Plate 1(f): Testis in 12.50 Percent hot –water treated CPHM (×400)



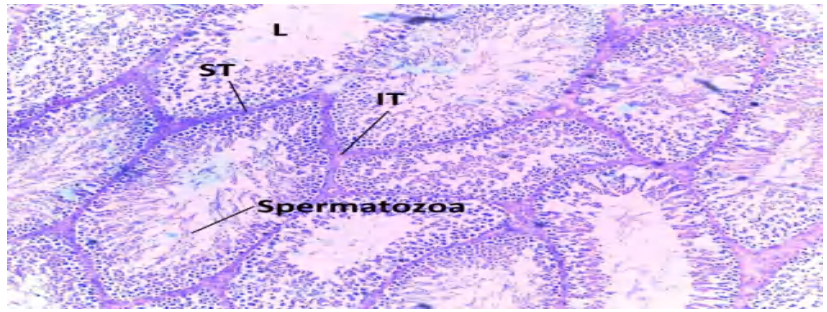


Plate 1(g): Testis in 25.00 Percent Sundried CPHM (×400)

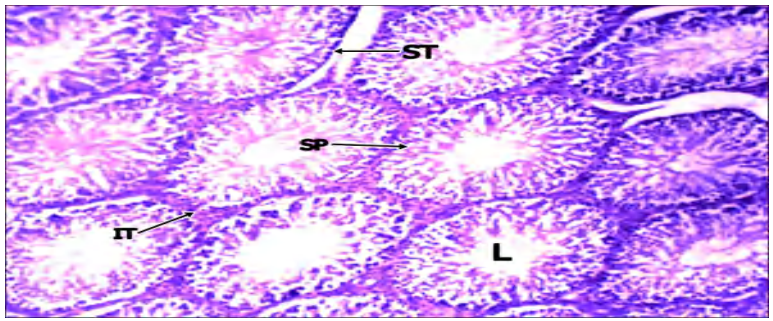


Plate 1(h): Testis in 25.00 Percent fermented CPHM (×400)

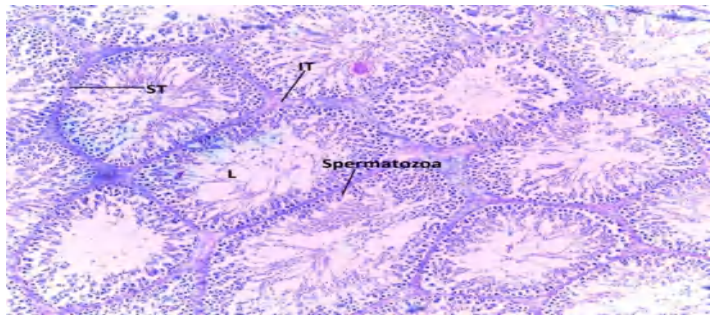
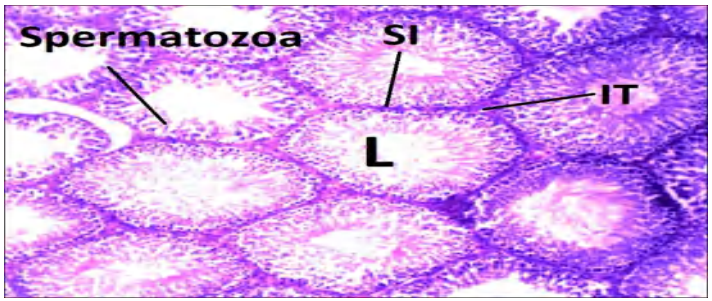


Plate 1(i): Testis in 25.00 Percent hot – water treated CPHM (×400)

Plate 1(j): Testis in 37.50 Percent Sundried CPHM (×400)



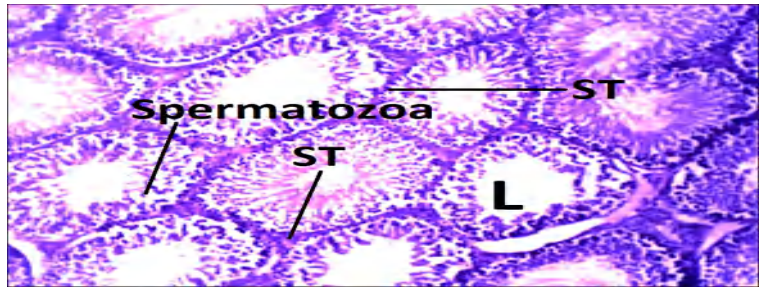
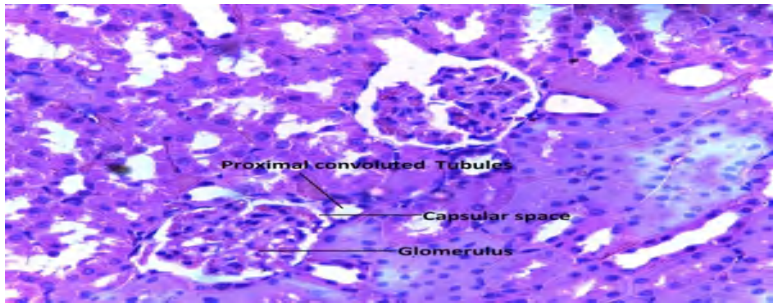


Plate 1(k): Testis in 37.50 Percent fermented CPHM (×400)

Plate 1(l): Testis in 37.50 Percent hot –water treated CPHM (×400)



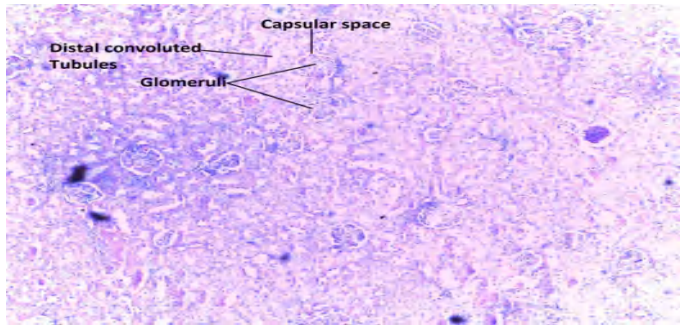
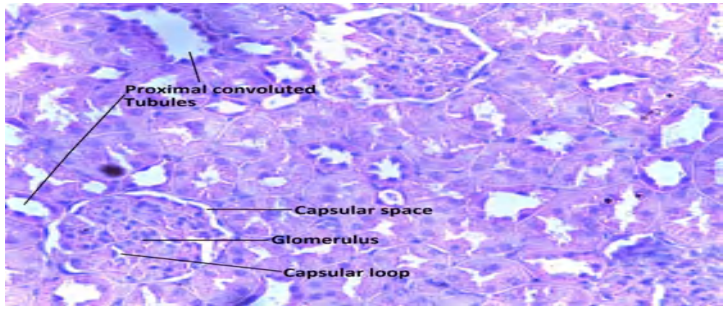


Plate 2 (a): Kidney in 0 Percent hot – water treated CPHM (×400) Plate 2(b): Kidney in 12.50 Percent Sundried CPHM (×400)



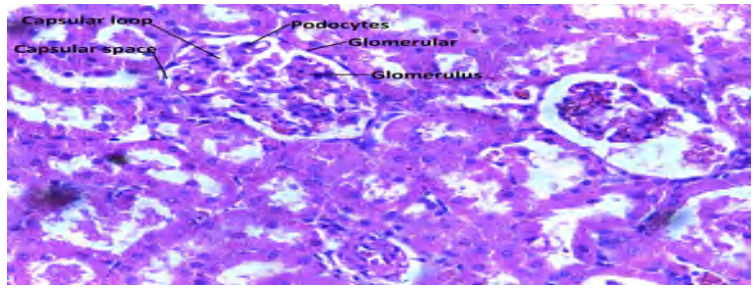


Plate 2 (c): Kidney in 12.50 Percent fermented CPHM (×400) Plate 2(d): Kidney in 12.50 Percent hot – water treated CPHM (×400)

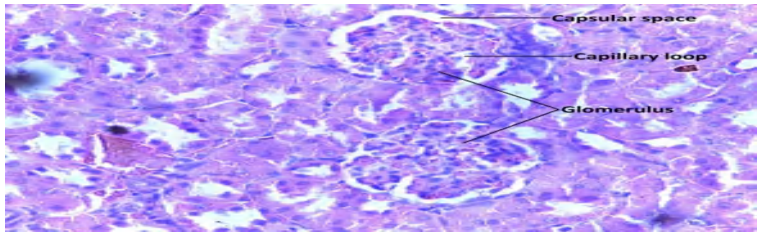


Plate 2 (e): Kidney in 25.00 Percent Sundried CPHM (×400)

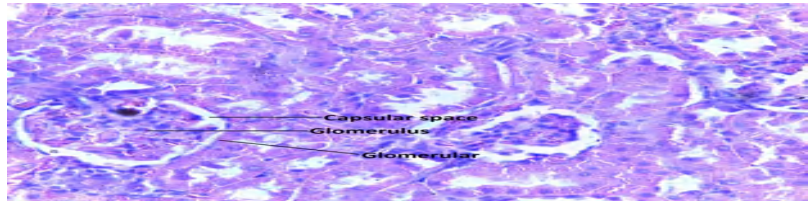


Plate 2 (f): Kidney in 25.00 Percent fermented CPHM (×400)

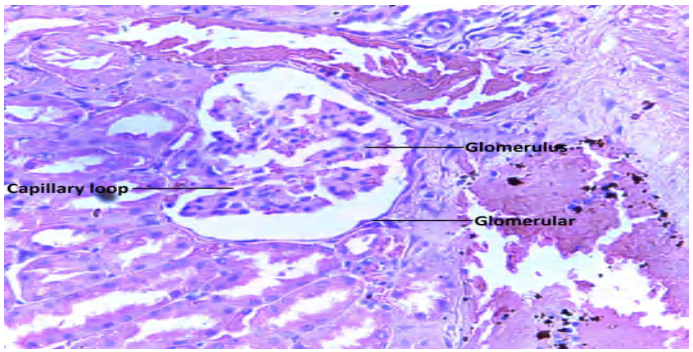


Plate 2 (g): Kidney in 25.00 Percent hot – water treated CPHM (×400)

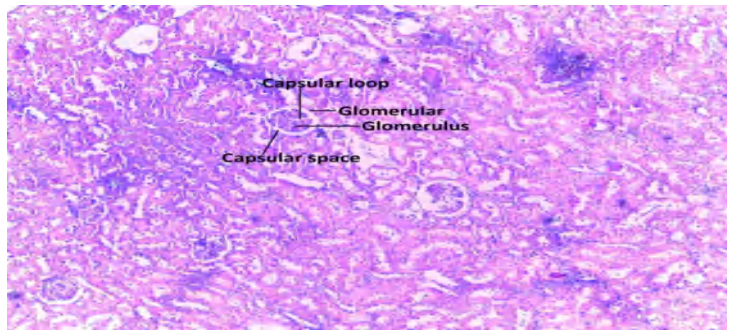


Plate 2(h): Kidney in 37.50 Percent Sundried CPHM (×400)

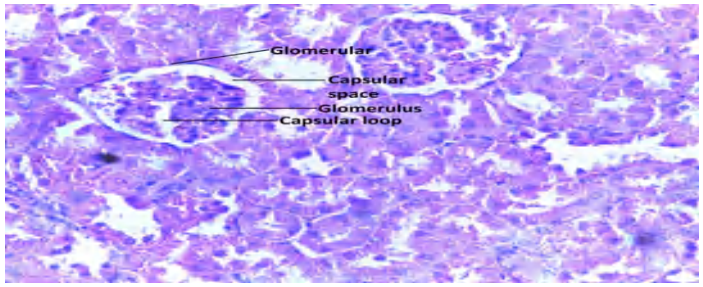


Plate 2(i): Kidney in 37.50 Percent fermented CPHM (×400)

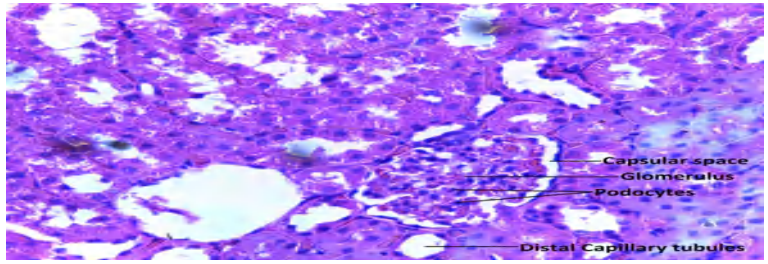


Plate 2 (j): Kidney in 37.50 Percent hot – water treated CPHM (×400)

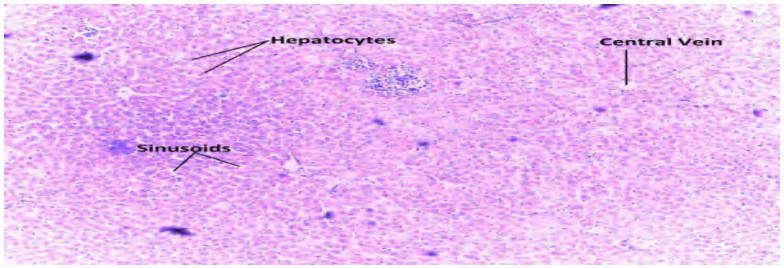


Plate 3(a): Liver in 0 Percent Sundried CPHM (×400)

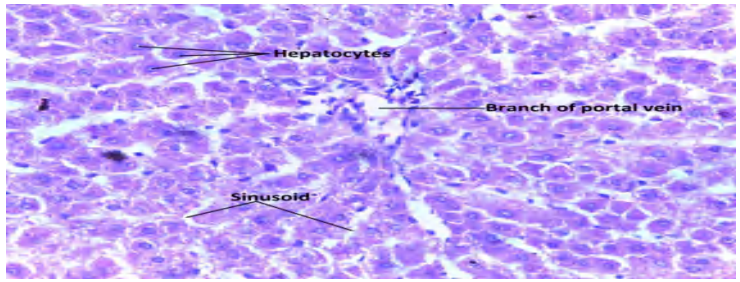


Plate 3(b): Liver in 0 Percent fermented CPHM (×400)

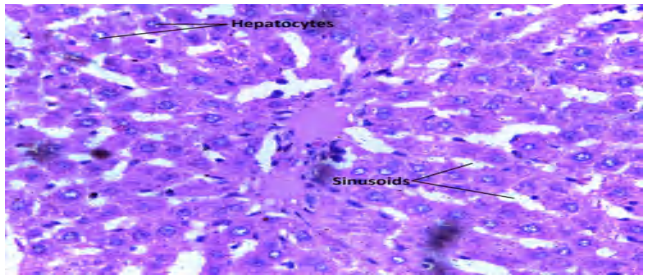


Plate 3(c): Liver in 0 Percent hot – water treated CPHM (×400)

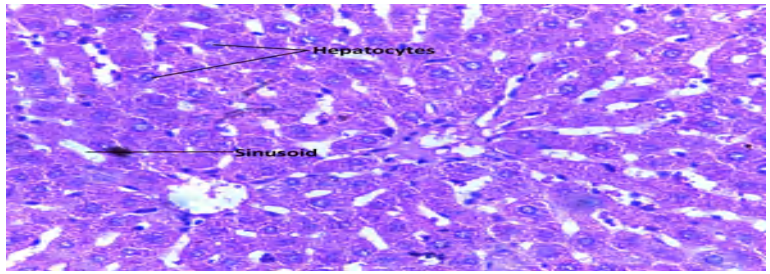


Plate 3(d): Liver in 12.50 Percent Sundried CPHM (×400)

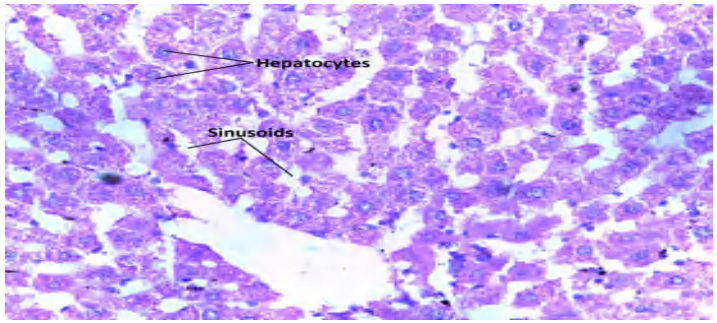


Plate 3(e): Liver in 12.50 Percent fermented CPHM (×400)

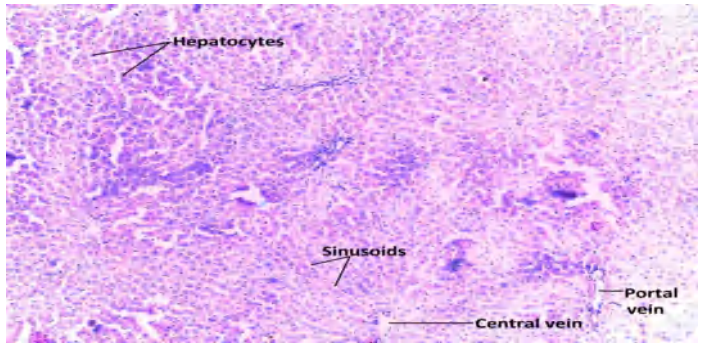


Plate 3 (f): Liver in 12.50 Percent hot – water treated CPHM

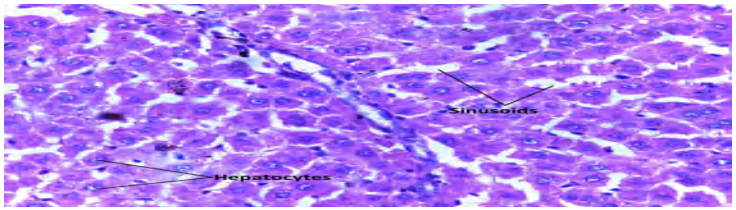


Plate 3 (g): Liver in 25.00 Percent Sundried CPHM (×400)

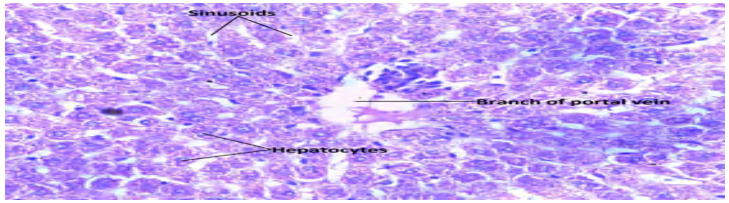


Plate 3(h): Liver in 25.00 Percent fermented CPHM (×400)

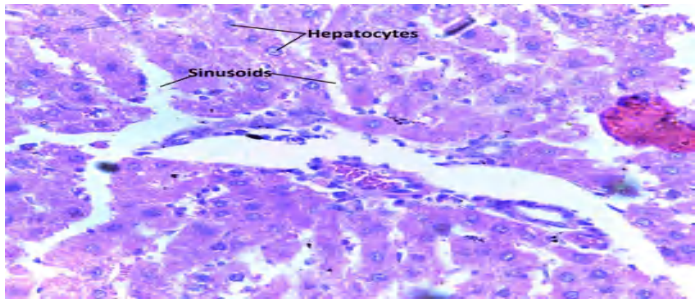


Plate 3(i): Liver in 25.00 Percent hot – water treated CPHM (×400)

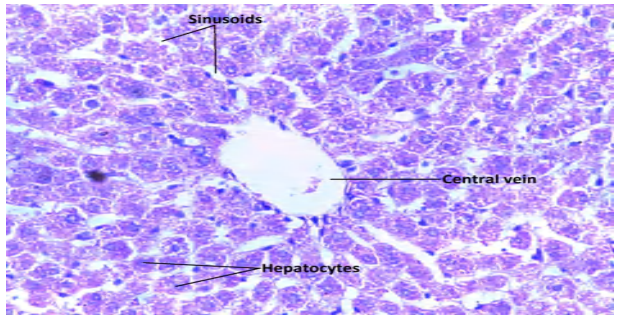


Plate 3(j): Liver in 37.50 Percent Sundried CPHM (×400)

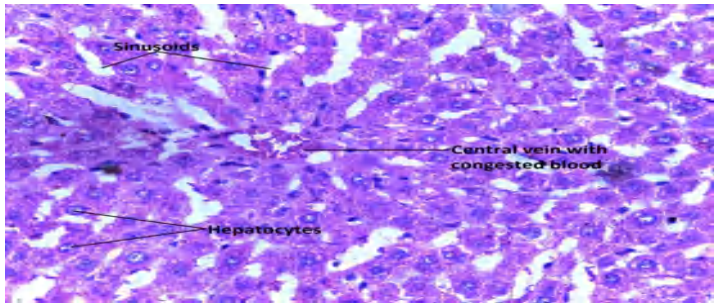


Plate 3(k): Liver in 37.50 Percent fermented CPHM (×400)

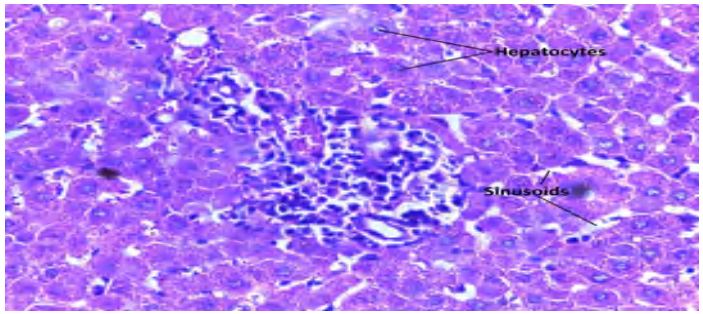


Plate 3(l): Liver in 37.50 Percent hot – water treated CPHM(×400)