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AMELIORATIVE EFFECT OF TALINUM TRIANGULARE AGAINST OXIDATIVE STRESS INDUCED BY SMOKE FISH

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ABSTRACT: Smoking of fish entails exposing fish to high temperatures and smoke from burning wood or other substances. This can result in the development of pro-oxidant chemicals in the body, such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). DNA, proteins, and lipids can be damaged by PAHs and HCAs, which can lead to oxidative stress and inflammation. *Talinum triangulare* play a significant role in maintaining and enhancing human health and quality of life. They are utilized by humans as essential ingredients in food, cosmetics, colors, and medications. The aim of the work is to study the effect of *Talinum triangulare* on smoke fish induced oxidative stress on Wistar rats. Twenty-eight healthy male Wistar rats weighing 190-210g, was grouped into 4 group, with 7 rats per group. First group chow pellet with distill water, group 2 exposed to smoke fish daily for 28 days, group 3 and 4 were exposed to smoke fish and administered 100 mg/kg and 200 mg/kg of *Talinum triangulare* extract respectively. The results revealed that smoke fish caused oxidative stress leading to oxidative damages in both liver and kidney tissues, thereby resulting in high accumulation of ALT, AST, Creatinine kinase, uric aicd and urea in the blood stream, it was ameliorated and reduced by 100 mg/kg bw and 200 mg/kg bw of *T. triangulare.* With 200 mg/kg bw showing more effectiveness in combating oxidative stress relating diseases which act as a strong antioxidant.

Keywords: Kidney, Liver, Oxidative Stress, Smoke Fish, *Talinum Triangulare*

1.0 Introduction

Fish is one of the most popular foods in the world which is known to produce substantial amount of protein. It is preserved to stop its degradation, deterioration and increase its nutritional properties by deep-frying, grilling, broiling, roasting, boiling, baking, smoking, stir-frying, and braising. Most fish smoking (roasting) is one of the most common methods adopted for fish preservation. It is believed it's improving its sensory properties such as flavour and taste.

Incomplete wood combustion during smoking can produce considerable amounts of polycyclic aromatic hydrocarbons (PAHs) which can penetrate through the surface of products (Jira *et al.,* 2006; Yusuf *et al.,* 2015).

It is known that in mammalian cells, polycyclic aromatic hydrocarbons (PAHs) undergo metabolic activation to diol, and epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (Lightfoot *et al.,* 2000; Yusuf *et al.,* 2015)

Talinum triangulare (Water leaf) is an edible leafy vegetable that belongs to the family Portulaceae, it has been known for strong medicinal potential and nutritional importance, previous researches shows that talinum triangulare has high alkaloid, tannins, alkaloids, saponins, phenol and flavonoids which justified that is a good antioxidant essential for preventing /treating various diseases and oxidative stress related diseases (Bioltif and Edward, 2020). The leaves of the plant are used to speed up the elimination of faeces from the body; treat gastrointestinal disorder, feed livestock; dropsy, swelling, oedema and reduce cardiovascular diseases (Ameh and Eze, 2010). Aja *et al.,* (2010) shows this vegetable has high level of carotenoid (biologic antioxidant), antioxidant is known to protect cell against damaging reaction oxygen species, because imbalance between antioxidant and reactive oxygen species results in oxidative stress leading to cellular damage causing inflammation, ischemic injury and neurodegenerative diseases. Presence of carotenoids may help provide protection against these aforementioned diseases and enhancing the immune system (Aja *et al.,* 2010).

The aim of this study is to assess the preventive impact of *Talinum triangulare* extracts (TTE) against oxidative stress in the rats exposed to smoked fish.

2.1 Materials and Methods

All reagents were of analytical grade supplied from Sigma Aldrich Company, Louis USA and from RANDOX Laboratory Limited, UK. Twenty-eight healthy male Wistar rats weighing 190-210g and aged 8-10 weeks were obtained from the Animal Care Unit of Redeemer University Ede's Faculty of Veterinary Medicine. The animals were quarantined for 7 days and randomized by body weight into the test and the control groups

2.2 Preparation of *T.triangulare* extracts

The leaves of plant material were washed with distilled water, allowed to drain, and air-dried for three weeks at room temperature in the Biochemistry laboratory. Using a blender, the air-dried samples were reduced to a fine powder. Cold extraction was performed by soaking 100g of powdered Talinum triangulare in 1000ml of 50% ethanol (50:50, ethanol: water, v/v) for 24 hours with continuous agitation in a separating funnel before running off the extracts into a beaker. It was filtered and the filtrate was evaporated using a rotary evaporator to get *T.trangulare* extracts.

2.3 Experimental Design

Group 1	Fed with chow pellets and distilled water only for 4 weeks
Group 2	Fed smoked fish ad libitum daily for 4 weeks
Group 3	Fed smoked fish ad libitum + 100mg/kg *T.triangulare* extract daily for 4 weeks
Group 3	Fed smoked fish ad libitum + 200mg/kg *T.triangulare* extract daily for 4 weeks

2.4 Biochemical assay

Histopathological examination was carried out using the method of Avwioro, 2010,

Determination of kidney function markers such as creatinine kinase, Urea, uric acid, serum total protein was determined colorimetrically using commercially available Randox laboratory assay kits UK. In addition, Assay of Aspartate Aminotransferase (AST) activity, Alanine Aminotransferase (ALT) Activity, Alkaline phosphatase activities were determined using Randox laboratory kits, England. **L-** γ – glutamyltransferase activity **(**GGT) were carried out using fortress laboratory kits. Albumin activity was determined according to the procedure of Reinhold, (1953). Bilirubin activity was carried out according to the procedure of Jendrassik and Grof (1938). Misra and Fridovich's, (1972) method was used to determine SOD activity. Catalase activity was assayed following the method of Luck (1974). Moron *et al.* (1979) approach was used to determine reduced glutathione (GSH). Lipid peroxidation were determined by monitoring the degrees of lipid peroxides in the supernatant described by Niehaus and Samuelsson, (1968).

2.3 Statistical analysis

All statistical data were presented as mean ± SEM and studied with a test of one-way analysis of variance using Graph Pad Prism for Windows version 7.04 (Graphic Pad Software, San Diego, CA, USA). Significant differences between groups were calculated with the Tukey's Multiple Comparison Test at a significance of $p<0.05$

3.0 Results

The result of the effects of the leaf extract of *T. tringulare* on kidney biomarkers in smoked fish induced lipid peroxidation in albino rats are presented in Table 1

There were Significant increases in alanine amino phosphate, alanine amino transfarase, urea, creatinine kinase and uric acid in the regime of rats fed with smoked fish when compared to the control group (Table 1). However Significant decrease were recorded in alanine phosphate, alanine transfarase, urea, creatine kinase when compared to rats given smoked fish and 100mg/kg *Talinum triangulare*

Table 1: Effect of *Talinum triangulare* on Kidney biomarkers in smoked fish- induced lipid peroxidation in albino rats.

PARAMETERS	ALP	ALT	UREA	CK	URIC
Normal control	65.19±1.50^{b}	87.74±1.94^{a}	47.38±1.67^{b}	25.11±1.04^{b}	20.44±0.29^{a}
Smoked Fish	71.45±2.07^{a}	89.90±2.56^{a}	52.09±1.00^{a}	29.34±1.70^{a}	21.82±0.33^{a}
Smoked Fish+ 100mg/kg *Talinum triangulare*	61.55+1.87^{c}	82.37±2.40^{b}	45.33±2.1°C	23.31±1.17^{e}	16.70±1.47^{b}
SmokedFish+ 200mg/kg *Talinum triangulare*	54.29+1.17^{d}	74.58±1.93^{c}	41.70±1.52^{d}	18.61±1.01^{d}	13.17±0.38^{c}

All numerical data were expressed as mean± SEM(n=5) significant at p<0.05-0.001
CK= Creatine Kinase, ALT = Alanine Amino Transferase, ALP = Alkaline phosphatase,

3.2 The result of the effects of the leaf extract of *T. triangulare on* kidney antioxidant enzymes of rats fed with smoked fish are presented in Table 2.

There was significant increase in concentration of Superoxide dismutase group, catalase, glutathione dehydrogenase, glutathione reductase and total protein group (table 2) when compared to the control group. However, significant decrease in SOD, Catalase and GSH were recorded in compares with regime of rats that were fed with to smoked fish.

Table 2: Effect of *T. triangulare* on Kidney Antioxidant Enzymes in rats fed with smoked fish

Parameters	SOD	CAT	GPx	GSH	TP
Normal control	1.41±1.47^{bc}	2.14±0.10^{C}	6.33±0.50^{bc}	3.19±0.03^{bc}	4.89±1.21^{bc}
Smoked Fish	1.28±0.75^{c}	1.95±0.15^{d}	5.93±0.29^{c}	3.05±0.05^{c}	4.40±0.68^{c}
Smoked Fish+ 100mg/kg Talinum triangulare	2.07±0.31^{b}	2.85±0.43^{b}	6.88±0.42^{b}	4.32±0.53^{b}	5.27±0.71^{b}
Smoked Fish + 200mg/kg Talinum triangulare	2.83±0.43^{a}	3.29±0.41^{a}	7.23±0.82^{a}	5.06±2.10^{a}	6.56±1.17^{a}

All numerical data were expressed as mean± SEM(n=5) significant at p<0.05-0.001
SOD = Superoxide dismuatase, CAT= Catalase, TP = Total proteim, GPx= Glutathione peroxidase,GSH = reduced glutathione.

3.3 The result of the effect of the leaf extract of T. traingulare on liver biomarker of rat fed with smoke fish is presented in Table 3

There was significant increase in the level of ALP, ALT, AST and BIL in group fed with smoked fish which was significant in comparism to the control group (Table 3). While in their counterpart group there was reduction in ALB in group fed smoked fish when compared to Smoked Fish+ 200 mg/kg *T.triangulare* group. Moreover, significant reduction in ALP, ALT, AST, BIL, and GGT was detected in group given smoked fish and 200 mg/kg *T. triangulare*, when compared to Smoked Fish + 100 mg/kg *T. triangulare* group.

Table 3: Effect of *Talinum triangulare* on Liver biomarkers in smoked fish

Parameters	ALP	ALT	AST	GGT	ALB	BIL
Normal control	58.31± 1.13^{b}	80.59± 2.09^{b}	60.28±1.53^{a}	5.30± 0.24^{a}	42.12±2.41^{a}	21.37±1.70^{eb}
Smoked Fish	60.17± 1.82^{a}	83.45±6.20^{a}	61.57±3.01^{a}	5.34 ±0.32^{a}	37.55±0.55^{d}	23.64± 1.87^{a}
Smoked Fish + 100mg/kg talinum triangulare	55.76±3.14^{c}	70.28±3.15^{c}	56.26±2.19^{b}	4.80±0.46^{b}	44.09±0.87^{c}	17.33±2.01^{c}
Smoked Fish+ 200mg/kg talinum triangulare	50.39±1.51^{d}	67.54±1.58^{d}	50.22±1.48^{c}	3.60±0.25^{bc}	49.52±1.20^{b}	16.22±1.65^{d}

All numerical data were expressed as mean± SEM(n=5) significant at p<0.05-0.001

AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, ALP = Alkaline phosphatase, ALB = Alnumin, GGT= Gamma glutamyl transferase

3.4 The result of the effect of the leaf extract of T. triangulare on liver antioxidant enzyme in rats fed with smoke fish

In table 4; in the liver tissues, significant increase in SOD, Catalase, GPx, GSH and total protein was observed in Smoked Fish given 200 mg/kg *T.triangulare* group when compared to control. However, significant reduction of SOD, Catalase, GSH and GPx was detected in rats fed smoked fish only when compared to Smoked Fish + 100 mg/kg *T.triangulare* group.

Table 4: Effect of *Talinum triangulare* on Liver Antioxidant Enzymes in rats fed with smoked fish

Parameters	SOD	CAT	GPx	GSH	TP
Normal control	2.30±1.47^{bc}	3.66±0.10^{bc}	7.01±0.50^{b}	4.13±0.03^{c}	5.47±1.21^{c}
Smoked Fish	2.07±0.75^{c}	3.60±0.15^{c}	6.83±0.29^{bc}	4.05±0.05^{bc}	4.93±0.68^{d}
Smoked Fish + 100mg/kg Talinum triangulare	3.44±0.31^{b}	4.76±0.43^{b}	7.55±0.42^{ab}	5.22±0.53^{b}	6.87±0.71^{b}
Smoked Fish + 200mg/kg Talinum triangulare	4.82±0.43^{a}	5.89±0.41^{a}	8.40±0.82^{a}	6.96±2.10^{a}	7.27±1.17^{a}

All numerical data were expressed as mean± SEM (n=5) significant at p<0.05-0.001

SOD = Superoxide dismuatase, CAT= Catalase, TP = Total proteim, GPx= Glutathione peroxidase, GSH = reduced glutathione.

3.5 The **result of the effect of the leaf extract of T. triangulare on lipid peroxidation (MDA) in liver of rats fed with smoke fish (Fig 2).**

There was significant increase in MDA of rats fed with smoke fish when compared to the control group.

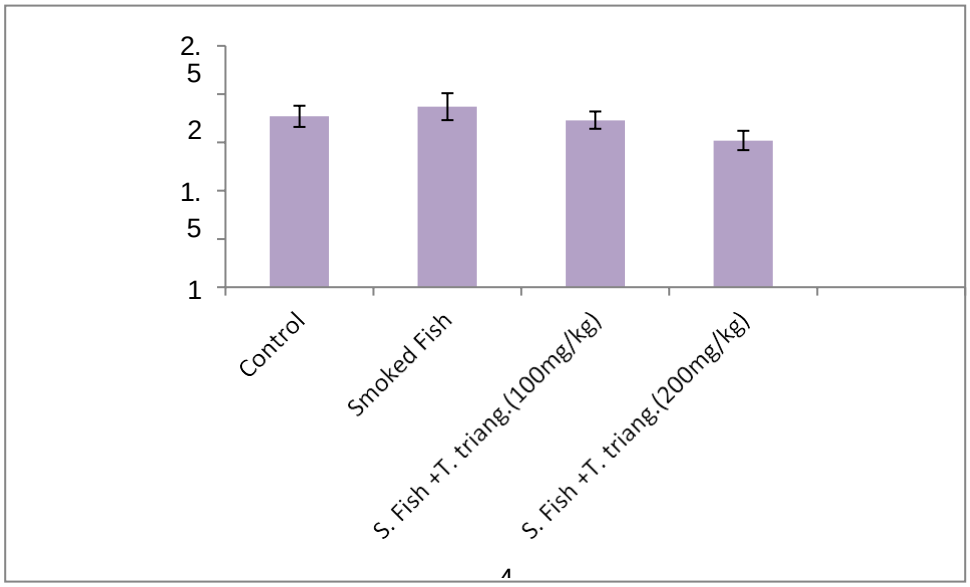

Fig 2: **Total malondialdehyde concentration in liver tissue**

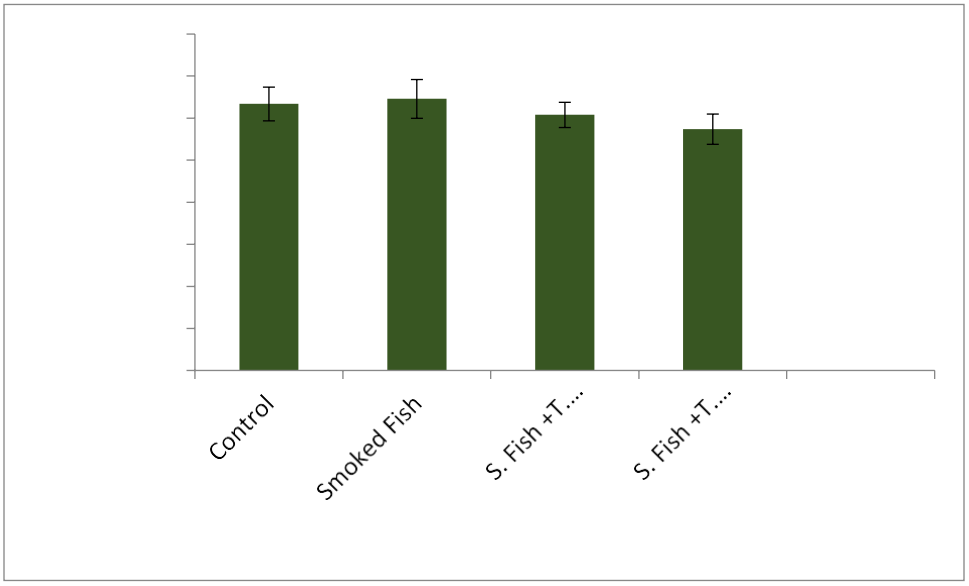

Fig 1: Total malondialdehyde concentration in kidney tissue.

Histopathology of kidney

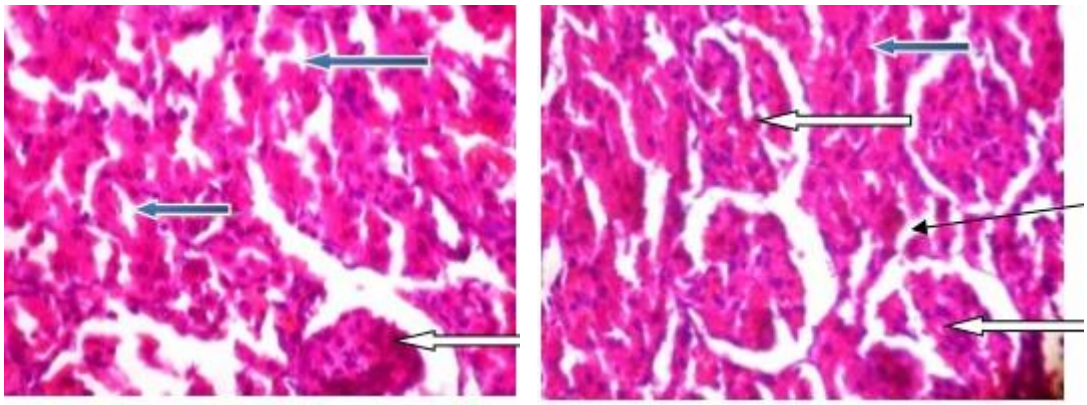

Fig 3: Photomicrographs of kidney sections stained by Haematoxylin and Eosin of rats Chow pellets with distilled water only for 28 days showing normal architecture as seen in lower magnification x100, the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), the interstitial spaces appear normal (slender arrow).

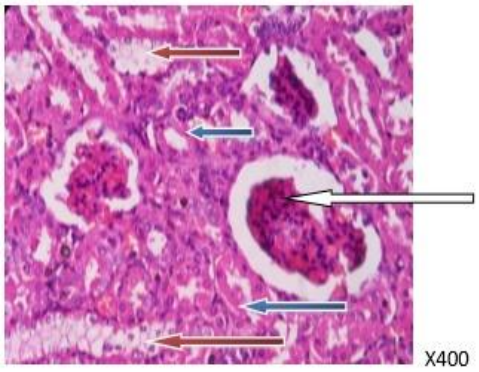

Fig 4: Photomicrographs of kidney sections stained by Haematoxylin and Eosin of rats exposed smoke fish for 28 days show moderate architecture as seen in lower magnification x 400, the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), some renal tubules appear mildly degenerated (red arrow), while other tubules appear normal (blue arrow), the interstitial spaces show mild vascular congestion (slender arrow).

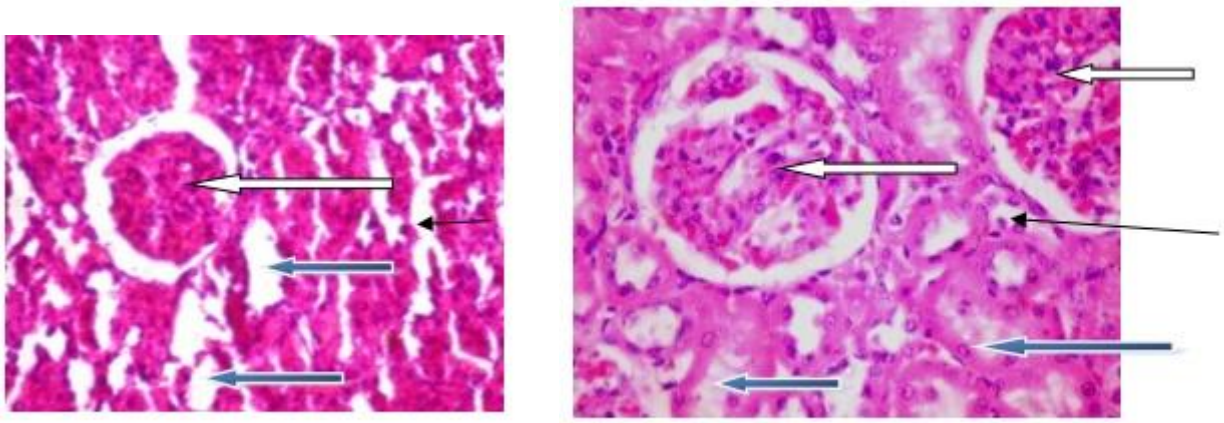

Fig 5: Photomicrographs of kidney sections stained by Haematoxylin and Eosin of rats Fed smoked fish and 200mg/kg T.triang daily for 28 days show normal architecture as seen in lower magnification x400, the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), the interstitial spaces appear normal (slender arrow).

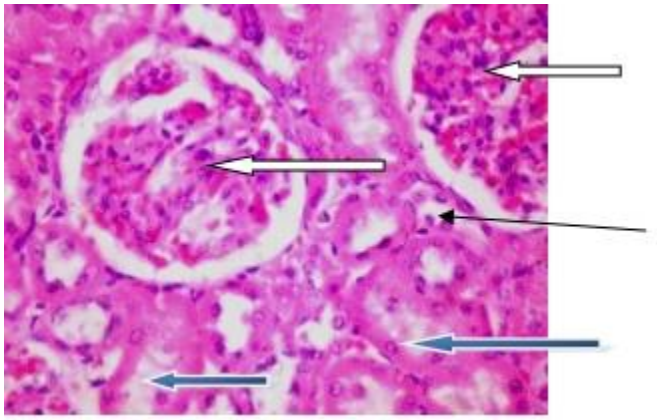

Fig 6: Photomicrographs of kidney sections stained by Haematoxylin and Eosin rats Fed smoked fish and 100mg/kg T.triang daily for 28 days show normal architecture as seen in lower magnification x100, the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), the interstitial spaces appear normal (slender arrow).

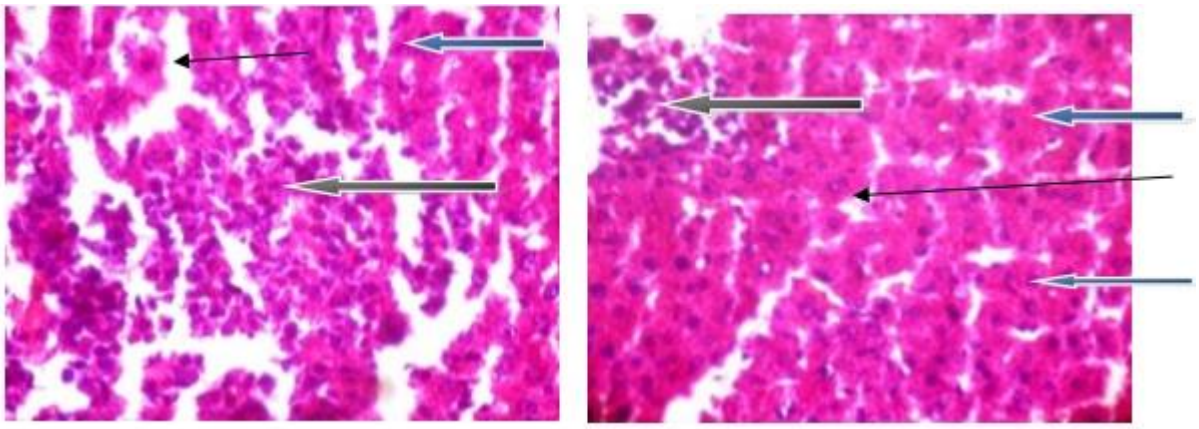

Fig 7: Photomicrograph of a liver section stained by Haematoxylin and Eosin of rats Chow pellets with distilled water only for 28 days showing normal central venules without congestion (white arrow), the parenchyma of the liver show focal areas of moderate inflmmatory cells aggregate (black arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow).

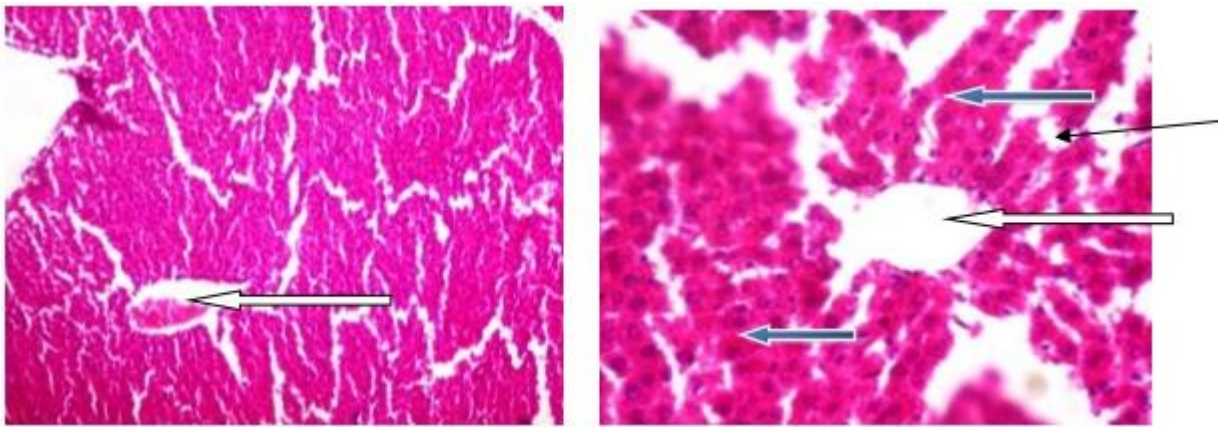

Fig 8: Photomicrograph of a liver section stained by Haematoxylin and Eosin showing of rats administered smoke fish shows normal central venules without congestion (white arrow) , mild portal congestionthe morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen

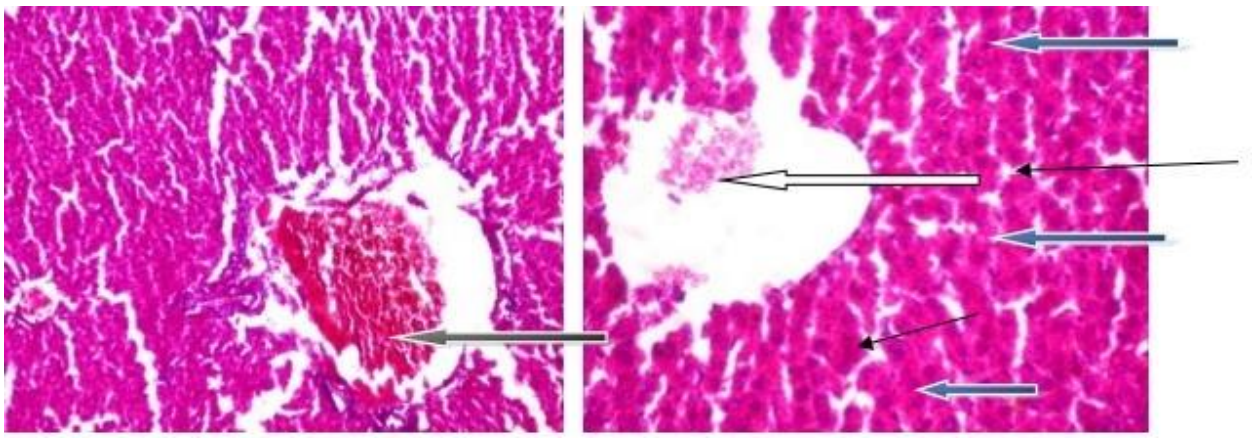

Fig 9: Photomicrograph of a liver section stained by Haematoxylin and Eosin of rats administered smoke fish and 100mg/kg talinum traingulare showing mild congestion of portal vein (black arrow), normal venules are seen (white arrow) the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow).

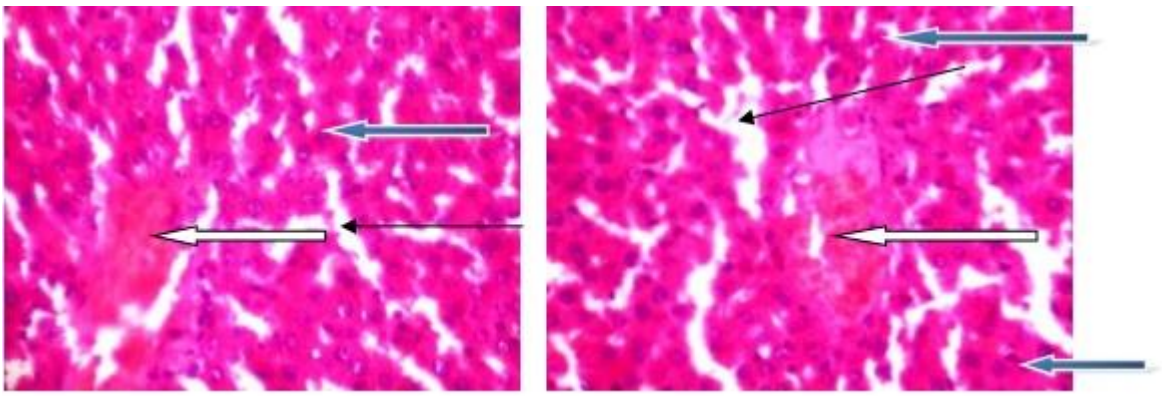

Fig 10: Photomicrograph of a liver section stained by Haematoxylin and Eosin of rats administered smoke fish and 200mgkg talinum traingulare showing central venules with mild congestion (white arrow), the morphology of the hepatocytes appears normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow).

DISCUSSION

The present study investigated the protective effect of the hydroethanolic extract of *T.triangulare* leaf on smoked fish-induced oxidative stress. Nephrotic damage to the kidneys of rats studied showed that there were significantly elevated levels of some kidney biomarkers (Urea, Creatine kinase and uric acid) in rats fed with smoked fish. Which led to their accumulation in the blood stream and subsequent leakage into the urine. These significant derangements observed for these biomarkers are pointers to Kidney damage accompany with lipid peroxidation and compromised membrane integrity (Ayala *et al.,* 2014).

Similarly, the observed increase in the liver enzymes is an indication of the oxidative damages to the hepatic tissues, resulting in elevated concentration of the enzyme in the blood stream. However, the oxidative damages on both liver and kidney tissues was mitigated significantly by treatments with leaf extract *T.triangulare* (100 mg/kg b. wt. and 200 mg/kg body to weight). The dosage of 200 mg/kg body to weight exhibited significant ameliorative properties than 100 mg/kg of *T.triangulare* (Afolabi and Oloyede, 2014).

Rat fed with smoke fish showed significant increase in oxidative stress, which was significantly mitigated by the administration of the leaf extract of *T.triangulare* leaf extract it was mitigated/reduced. *T.triangulare* demonstrated is a good antioxidant with 200mg /bd w.t showing more effectiveness. Administration of the leaf extract of *T.triangulare* significantly increased the amount of increase in SOD, catalase, GPx and GSH, which shows it a good antioxidants by ameliorating oxidative stress. The work is in accordance to result gotten by Afolabi and Oloyede (2014).

The results of the Histological studies validate our findings on the effect of leaf extract *of T. triangulare* treatment on smoked fish-induced oxidative stress as there were normal Kidney photomicrograph plate had normal architecture. It reverses the damages caused by oxidative stress.

CONCLUSION

Smoking and roasting of fish as a means of processing and preservation of fish. Exposure over a naked fire over a continuous period of time can produces genotoxic compounds such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, heterocyclic aromatic amines (HAAs) which can induce oxidative stress. When roasting meat and fish over intense heat over a direct flame, fat drips onto the fire and produces flames with PAHs and HAAs. Continuous consumption over duration of time can induce oxidative stress.

T. triangulare proved to be a good antioxidant potential to mitigate against lipid peroxidation in rat kidneys and liver induced by MDA contained in smoked fish and the histopathological studies showed it had restored the kidney architecture. Consuming antioxidants from plants such Talinum triangulare (water leaf) 200mg/kg helps fight a variety of metabolic disorders. Talinum triangulare has shown to be a good antioxidant by ameliorating damages caused by oxidative stress from smoked fish.

RECOMMENDATIONS

Based on our findings 200mg/kg Talinum triangulare (Water leaf) showed a significant kidney and liver protective effect against smoked fish-induced oxidative stress in the kidney and liver. People should be educated on the impact of exposes or roasting of fish on naked fire over continuous period of time.

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