Original Article

Physiological and Histopathological Studies on Bisphenol - A Induced Oxidative Stress in Albino Rats: Amelioration by Green and White Tea Extracts

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Abstract

Due to highly exposure of BPA which is harmful substance, we investigate its role in induction of oxidative stress, its effect on pancreatic tissue and some physiological parameters in serum of rats, also make a compartive study of antioxidant role for both green and white tea alcoholic exctracts against this harmful substance. In our experiment 20 male albino rats had been used, their body weights were ranged between 180 and 200 grams. Four equal groups of rats were formed, (five animals each). The first group was treated with corn oil alone, while the second group received 500 mg/kg BPA liquified in corn oil, while the third group received BPA (500 mg/kg) and 300 mg/kg extract of green tea together, and the fourth group received BPA plus 300 mg/kg white tea alcoholic extract tea extract. Animal dosing was orally for 60 days. Level of serum MDA significantly inceased in rat group supplied with PBA while treatment with white and green tea alcoholic extract decreased MDA level. Serum superoxidedismutase (SOD) significantly decreased in PBA group while green tea significantly elevated antioxidant level, rats treated with white tea also showed elevation in serum SOD but it was non significant, indicating higher enhancment of green tea for anioxidant production than white tea. Histopathological examination revealded hemorrahge, apoptosis, necrosis and infiltration of inflammatory cells in pancreatic tissue of PBA of rats group, while treatment with green and white tea alcoholic extract nearly recovered histological architectur of pancreatic islets. Histochemical examination indicated depletion of mucopolysacchride by using a stain named alcian blue periodic- acid schiff stain (AB-PAS), protein also depleted when pancreatic tissue stained with bromophenol blue in rats supplied with PBA, while green tea showed better recovery for protein distribution than white tea.

Keywords: Oxidative Stress, Bisphenol A, Green Tea, White Tea, Rats.

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INTRODUCTION

An industrial chemical precursor named bisphenol A (BPA) is commonly used in the manufacturing of consumer goods such as polycarbonate plastics, epoxy resins, and thermal paper (Vandenberg et al., 2007). Experimental studies into the induction of oxidative stress by BPA, like other aspects of BPA research, have produced plethora of contradictory findings about prooxidant/antioxidant behavior (Babu *et al*., 2013; Chepelev *et al*., 2013), antioxidant deficiency (Huc *et al*., 2012; Ge *et al*., 2014a), malfunction of mitochondria (Moon *et al*., 2012; Kalb *et al*., 2016), and induce death of cells (Ooe *et al*., 2005; Huc *et al*., 2012; Gassman *et al*., 2015; Leem *et al*., 2017).

According to the prior study, BPA has been linked to the formation of amyloid polypeptide in pancreatic islets of human, which results in the loss of cells which produce insulin and ultimately, diabetes mellitus (type 2) (Gong *et al*., 2013). Generation of reactive oxygen species (ROS) by BPA appears to play a part considerably to its toxic effect as well as mutagenic potential, according to mounting data (Seachrist *et al*., 2016; Gassman and Wilson, 2017). A daily BPA dosage of μg/kg/bw to mg/kg/bw were used in animal tests to drastically diminish the total antioxidant capacity of various tissues and organs such as the liver, pancreas, and testes (Hassan *et al*., 2012; Moghaddam *et al*., 2015; Kalb *et al*., 2016), furthermore, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity is reduced

in the brain, epididymal sperm, testes, germ cells, kidney, liver and pancreas (Bindhumol *et al*., 2003; Chitra *et al*., 2003; Kabuto *et al*., 2003; Kabuto *et al*., 2004; Aydogan *et al*., 2008; Jain *et al*., 2011; Hassan *et al*., 2012; Moon *et al*., 2012; Tiwari *et al*., 2012; Wu *et al*., 2013; Moghaddam *et al*., 2015; Kalb *et al*., 2016).

Additionally, BPA exposure raised ROS levels in rats in a dose-dependent manner, and oxidative stress was connected to metabolic disorder (hyperglycemia and hyperinsulinemia) as well as reproductive destruction, as evidenced by decline of insulin receptor substrate-2 and glucose transporter-8 in testis, two key proteins involved in energy metabolism of testis and process of sperm production (D'Cruz *et al*., 2012). BPA raised lipid peroxidation in adult rats' testis, epididymis and immunity cells (lymphocytes and bone marrow) (Tiwari, 2017). Plant-based herbal medicines are increasingly being used to manage various clinical illness. More emphasis placed on natural antioxidants' ability to protect against drug-induced toxicity, particularly when free radical generation is involved (Frei and Higdon, 2003). Camellia sinensis (L.), a perennial, evergreen, leafy crop that grows in warm, humid climates with plenty of rain, is used to make the influxion tea (Carloni *et al*., 2012). Green and white teas are processed differently. Green tea is made by rolling and steaming young leaves to reduce oxidation, whereas white tea is made from very young tea leaves or buds covered in tiny, silvery hairs that are harvested only once a year in the early spring. The white tea is then steamed and dried right away, after harvesting to prevent oxidation, resulting in a light, delicate flavor (Firuzi *et al*., 2006). Consuming green tea, white tea, or *Pelargonium purpureum* transfusions has been shown to have an antioxidant effect in plasma and organs like heart and lung (Kim *et al*., 2002). They boost immune function while protecting against oxidants and free radicals. Their anti-inflammatory and anti-hemorrhagic properties make them effective against insect bites. (Dudaric *et al*., 2015; Shen *et al*., 2015). In cell cultures and in animal model, a tea component was shown to defend against mutagenic processes produced in various organs, such as the skin, pancreas, and duodenum.

Due to highly exposure of BPA which is harmful substance, we investigate its role in induction of oxidative stress, its effect on pancreatic tissue and some physiological parameters in serum of rats, also make a compartive study of antioxidant role for both green and white tea alcoholic exctracts.

METHODOLOGY

1. Housing of animals and design of experiment

This experiment involved 20 male albino rats (*Ratus novergicus* had been used, their body weights were ranged between 180 and 200 grams. Rats were housed in polypropylene cages with mesh wire tops in a well-ventilated room and given a decent food and pure water ad libitum, with a 12:12 light/dark photoperiod at a temperature of 22±4ºC in Salahaddin university – college of education- department of biology. Four equal groups of rats were formed (5 animals each). The first group was a control group that received just corn oil. The second group received 500mg/kg BPA dissolved in corn oil (Solar Bio Company, Beijing, China); the third group received a mixture of BPA (500 mg/kg) and green tea extract (300 mg/kg). BPA plus white tea alcoholic extract was administered to the fourth group at a dose of 300mg/kg. Our experiment extended for two months, rats were given supplements by gavage. After one hour of BPA administration, green tea extract was administered.

2. Plant extract preparation

Dried green tea leaves and Silver needle white tea were used to prepare the alcoholic extracts. Dried leaves were then powdered with electrical blender and stored until extract preparation. Dried plant powder of about100g was combined with 1000 ml of 95% ethanol alcohol and shaken horizontally for 24 hours before being separated by gauze and centrifuged at 3000 rpm. Filtration was used to obtain the crude extract, which was then accompanied by evaporation of the solvent in a rotatory evaporator at 40°C under low pressure, and was refrigerated (Hernandez-Perez *et al.*, 1994).

3. Animal dissection and serum preparation

Overnight fasting was done on the animals, weighed, at the last part of the experiment, they were sedated with ketamine and xylazine. During dissection of the rats, the pancreas was eliminated and cold saline had been used to wash a portion of pancreas. A cardiac puncture was used to collect samples of blood from the heart then transferred to anticoagulant-free tubes and allowed to clot. For 15 minutes, blood samples were centrifuged at 3000rpm. Sera were gathered and saved at ˉ20°C (Cheng *et al.*, 2005).

4. Histopathological examination

Another section of rat pancreas of all groups was preserved in 10% neutral buffered formalin. To study histological changes, The tissue blocks were deparaffinized and counterstained with hematoxylin and eosin after being treated and sliced with a microtome at a thickness of 5 µm. (H&E). (Alkinani, 2013).

For histochemical study, mucopolysaccharides and carbohydrates are detected by using AB-PAS stain kit. On glass slides, tissue slices were gathered. Xylol was used for deparaffinization, then the tissue was rehydrated by graded series of alcohol. The stained area was then rinsed three times in running tap water for 1-2 minutes after being stained in AB solution (PH. 2.5). After that, for 5 minutes, the slides were submerged in a periodic acid solution before being rinsed several times with deionized water, after being placed in Schiff reagent for 10-20 minutes, it was rinsed in tap water for 10 minutes. The specimen was then stained for 1-2 minutes in hematoxylin solution. Before being immersed in

Scott bluing solution for 2-5 minutes, slides were distinguished with acid alcohol solution for 2-3 seconds. Followed by 5 minutes of washing under running water, and rinsing in deionized water. Dehydrated by alternating between 95 percent and absolute alcohol for two minutes each. The piece was then cleared in two changes of xylene, mounted with Canada balsam, and covered. Additionally, a stain named aqueous bromophenol blue used in our study for the detection of total proteins, Bromophenol blue solution (0.1%) was obtained by adding 0.1 gram of bromophenol blue powder to 100 mL double distilled water. Glass slides were used for gathering the tissue slices, then xylol used for removing paraffin and rehydrated to deionized water with graded alcohol. The Slices were colored for 5 minutes. At room temperature in bromophenol blue solution. After being washed in double distilled water, the specimen was examined under a light microscope (Subramonm, 1982).

5. Data analysis

Statistical analyses were carried out using the Graph Pad Prism software version 6.0. (GraphPad, San Diego, CA). The data is presented as means with standard errors of the mean (mean ± SEM). Before ANOVA, the data's normality and homogeneity were confirmed, One-way ANOVA and Turkey's test were used to assess differences among test group. P0.05 was chosen as the statistically significant level.

3. Results and Discussion

1. Phisyological study

As shows in table 3.1 the level of serum MDA significantly inceased in rat group supplied with PBA (56.52± 1.05) as copmared with control rats, while treatment with white and green tea alcoholic extract significantly decreased oxidative stress marker and respectively were (45.27± 0.6398, 41.65± 0.5225). On the other hand, serum anioxidant level (SOD) decreased significantly in the PBA group versus control rats at =P<0.05. and the level was (463.7± 2.281), while green tea significantly elevated antioxidant level 500.8± 2.903 in comparison with BPA group, rats treated with white tea also showed elevation in serum SOD but it was non significant 497.5± 1.67 indicating higher enhancment of green tea for anioxidant production than white tea.

Table 3.1: The impact of BPA, green and white tea alcoholic extracts on oxidative stress markers (MDA and SOD) in the serum.

Groups (rats) / Level in serum:	Control	Bisphenol A	Green tea alcoholic extract	White alcoholic extract
MDA (nmol/L)	42.7 ±0.5398 a	56.52± 1.05 b*	45.27± 0.6398 ac*	41.65± 0.5225 ac*
SOD (nmol/ml)	672.8± 2.531 a	463.7± 2.281 b*	500.8± 2.903 a c*	497.5± 1.67 d b

• Data presented as mean ± S.E. (n=6 in each group)

• The same letters indicate that there are no statistical differences

• The various letters denote statistical differences. * =P<0.05.

The markers of oxidative damage, 8-OHdG for nucleic acids, protein carbonyls for proteins, and malondialdehyde (MDA) for lipids, in the existence of BPA, were elevated (Peluso *et al*., 2016; Huang *et al*., 2020), this is also supported by our study. This biomacromolecular damage, particularly BPA exposure leads to DNA damage, is indeed one of the pathways that causes cell apoptosis (Huang *et al*., 2020). Catalase, SOD, glutathione reductase, and glutathione peroxidase are examples of enzymatic antioxidants (Hassani *et al*., 2017). The mitochondria produce SOD and glutathione peroxidase and they prevent oxidative damage against O2•- and H2O2, respectively (Nelson & Cox, 2012).

Additionally, it has been demonstrated that BPA reduces the manufacturing of antioxidant enzymes like SOD, glutathione reductase, glutathione peroxidase, and catalase, which in turn aids in the development of oxidative stress (Meli *et al.*, 2020). Studies conducted show that the structural analogues used to replace BPA also induce the formation of ROS such as •OH, which is highly reactive (Mokra *et al*., 2018).

Over a period of 1–4 weeks, green tea and microencapsulated extracts have also been shown to reduce oxidative status biomarkers when consumed on a regular basis (Cabrera, and Giménez, 2010), This is also agreed with our results. According to Serafini *et al*., (1996) it has been found that tea ingestion increases serum antioxidant levels, green tea has a greater antioxidant capacity than other tea forms and other plant products, according to several studies. However other studies found that white tea which is one of the types of tea obtained from the tea shoots in the form of buds. does not go through fermentation process; thus, its catechin content is higher than green tea (Textiera *et al*., 2012).

2. Histopathological study

For histopathological study of bisphenol- A induce oxidative stress in rats and protection by green and white tea, sections of pancreas stained with 3 stains: normal histological stains H and E, AB- PAS and bromophenol blue stain.

In fig. 1 H and E sections through pancreas of control rat shows normal histological feature of islets and acinar cells, while of pancreas in rats infected with bisphenol A BPA (500 mg/kg) shows atrophy of islets, necrosis, and inflamatory cell infiltration fig 2., piknosis as a sign of apoptosis and karyolysis also detected in the same group fig 3. Additionally, conjested blood vessel, vacuolization of islets observed fig 4.

Oxidative stress is a physiological event that occurs when the reactive oxygen and nitrogen species (RONS) are produce and the body capacity to detoxify those reactive products is shifted out of balance (Preiser, 2012). BPA is a chemical found in many polycarbonate plastics and epoxy resins; it is a recognized endocrine disruptor and it has been related to oxidative stress (Meli *et al*., 2020), also raises the expression of stress response genes, which raises the expression of oxidative response and cellular damage genes (Garcia-Espineira *et al*., 2018). Prior research has linked many gross morphological changes in adult animals, like islet hypertrophy, necrosis of the cells, amyloid deposits, irregular islet architecture, and -cell degranulation have all been linked to altered hormone manufacture and, as a result, lowered pancreatic function. (Weir and Bonner-Weir**,** 2013), these alterations also revealed in our research.

High concentrations of ROS have been linked to the damage of lipids, nucleic acids, carbohydrates, and proteins due to their high reactivity with these structures (Ozcan & Ogun, 2015; Pizzino *et al*., 2017). This is agree with our study since depletion of protein and carbohydrate observed in pancreas of rats taken BPA by using PAS and bromophenol blue respectively.

On the other hand, rats treated with green tea alcoholic extract (300 mg/kg) plus bisphenol A BPA (500 mg/kg) shows approximately normal histomorphological artitecture of islets and acinar cells. Fig5. rats treated with white tea alcoholic extract in the same dose as green tea and bisphenol A BPA shows approximately normal hisomorhological feature of pancreas with little congestion of blood vessel fig. 6.

For histochemical study AB PAS stain used for detection of mucopoly saccharide, Section through pancres from control rat stained positively purple with AB PAS stain shows normal distribution of mucopoly saccharide fig 7 while pancreas of rats infected with bisphenol A stained weakly with AB PAS stain indicated mild mucopolysaccharide aboundance fig 8. Furthermore, treatment of rats with white tea was more effective than green one in retention of mucopolysaccharide to normal distribution fig. 9 and 10.

Another special stain used in our study, named bromophenol blue for protein detection. As shows in Fig. 11 Section through pancres from control rats positively stained with bromophenol blue indicates normal distribution of protein since the slide colour is heavy blue, but pancreas of rats infected with BPA stained negatively with light blue as A sign for poor availability of protein Fig 12. Finally pancreas of rats treated with green and white tea extracts positively stained blue colour, White tea outperformed green tea in terms of effectiveness. It recovers protein distribution in comparision with green one, indicating that white tea has a higher antioxidant activity than green tea. fig 13 and 14.

Latest researches have found that green and white teas contain anti-cancer, anti-metabolic syndrome, anti-type 2 diabetes, anti-cardiovascular, and anti-neurodegenerative characteristics. These antioxidant and anti-inflammatory features are accompanied with the strong antioxidant and anti-inflammatory activities of xanthic bases (caffeine and theophylline), essential oils (green and white tea have the highest content), minerals (F, Mn, Cr), L-theanine, and, most notably, catechins and other phenolic substances (Cabrera and Giménez, 2010; Cooper, 2012).

On the other hand, white teas have been found to have more antielastase, anticollagenase, and antioxidative action than green teas, implying that their ability to enhance strong and elastic skin while also alleviating inflammation and rheumatoid arthritis has piqued the interest of tea drinkers. (Thring *et al*., 2009), our data is somewhat support with this study since pancreas of rats treated with white tea showed better relevant of normal distribution of protein and carbohydrate by using special stains mentioned previously, both types of tea (green and white succeded in reducing inflammation rate.

Furthermore, Suthar *et al*., (2014) reported that BPA causes cytotoxicity in human erythrocytes by producing hydroxyl radicals in the same way that hydrogen peroxide does. Because of their potent antioxidant properties and ability to reduce oxidative stress, green tea extracts have a protective effect against BPA-induced cytotoxicity. Infact our research is a novel study performed on oxidative stress induced by BPA in pancreas and used special stains for detection of protein and carbohydrates. Most studies are performed on glucose metabolism which is linked with pancreas for example, Song *et al*., (2014) revealed that BPA is estrogenic, elevates insulin release from beta-cells of pancreas, and induces insulin resistance by interfering with insulin transduction of target cells.

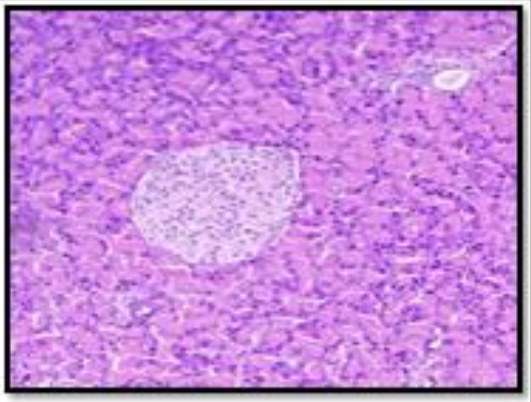

Figure 1: H and E photomicrograph of pancreas from control rat shows normal histological feature of islets and acinar cells.

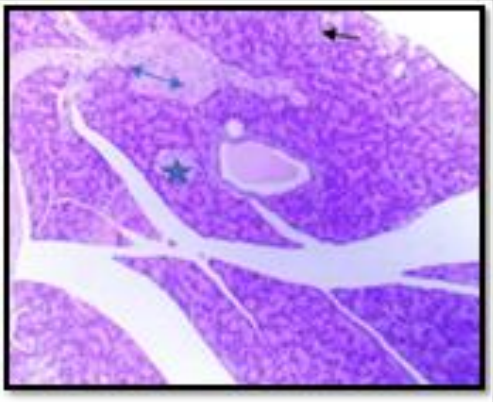

Figure 2: H and E photomicrograph of pancreas in rats infected with bisphenol A BPA (500 mg/kg) shows atrophy of islets ★ and necrosis ←, inflamatory cell infiltration ↔.

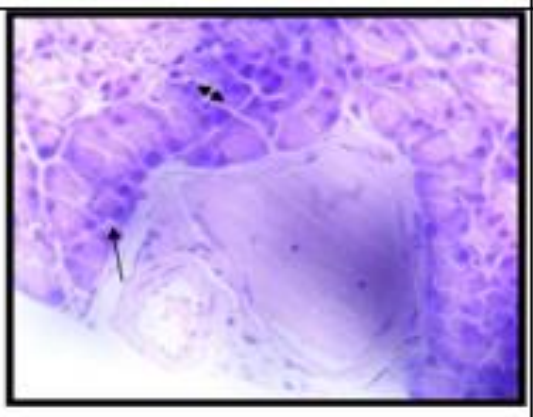

Figure 3: H and E photomicrograph of pancreas in rats infected with bisphenol A BPA (500 mg/kg) shows piknosis ↕ as a sign of apoptosis and karyolysis ↗.

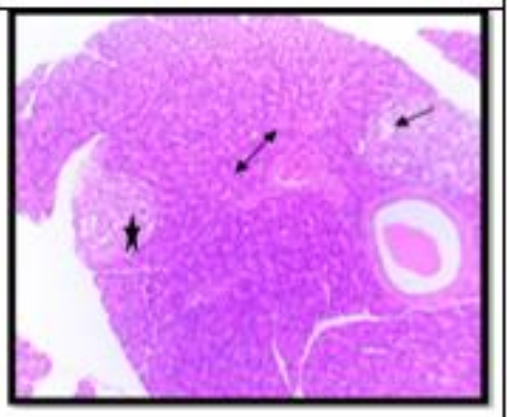

Figure 4: H and E photomicrograph of pancreas in rats infected with bisphenol A BPA (500 mg/kg) shows conjested blood vessel ★, vacuolization of islets and inflammatory cell infiltration ↗.

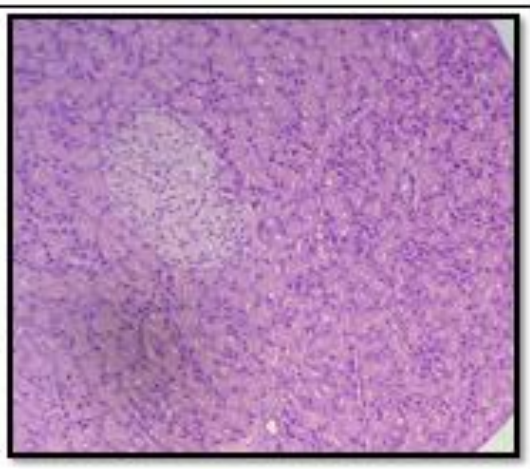

Figure 5: H and E photomicrograph of pancreas from rats treated with green tea alcoholic extract (300 mg/kg) and bisphenol A BPA (500 mg/kg) shows approximately normal histomorphological artitecture of islets and acinar cells.

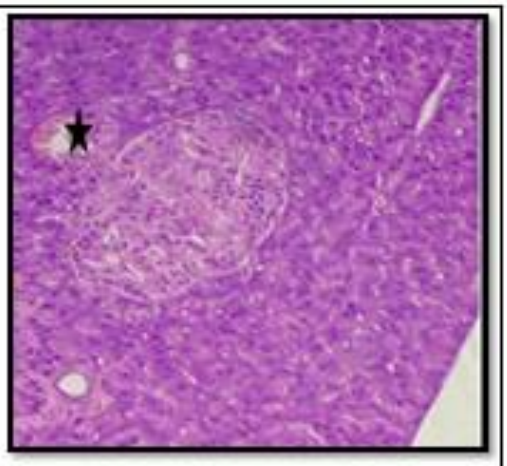

Figure 6: H and E photomicrograph of pancreas from rats treated with white tea alcoholic extract (300 mg/kg) and bisphenol A BPA (500 mg/kg) shows approximately normal histomorphological artitecture of islets and acinar cells, little conjested blood vessel ★ .

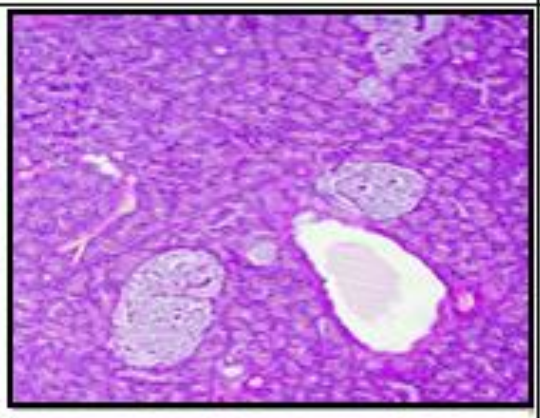

Figure 7: Section through pancres from control rat stained positively purple with AB PAS stain shows normal distribution of mucopoly saccharide.

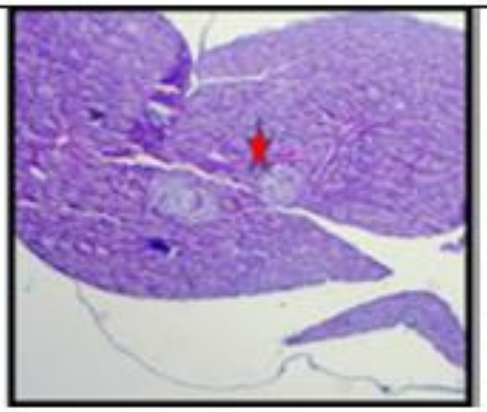

Figure 8: Section through pancres from BPA infected rat stained weakly stained with AB PAS stain shows mild mucopolysaccharide ★ .

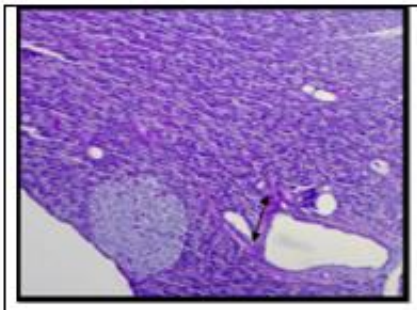

Figure 9: Section through pancres from rats treated with green tea alcoholic extract (300 mg/kg) and bisphenol A BPA (500 mg/kg) shows moderately mucopolysaccharide retention when stained with AB PAS satin .

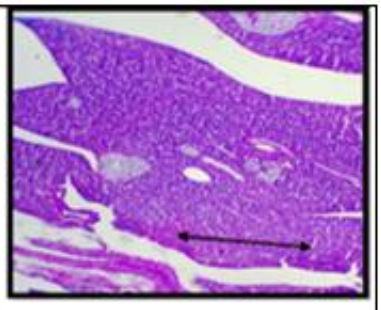

Figure 10: Section through pancres from rats treated with white tea alcoholic extract (300 mg/kg) and bisphenol A BPA (500 mg/kg) shows positively stained with AB PAS satin (purple) indicates normal distribution of mucopolysaccharide .

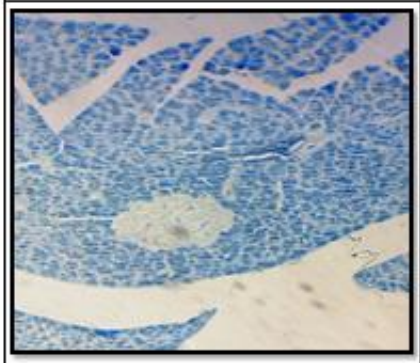

Figure 11: Section through pancres from control rats shows positively stained with bromophenol blue indicates normal distribution of protein since the slide colour is heavy blue.

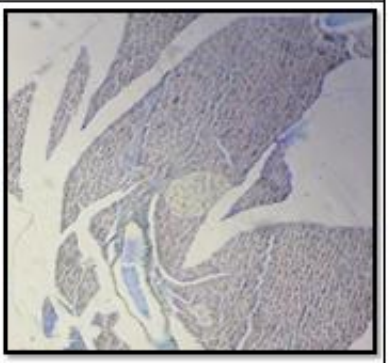

Figure 12: Section through pancres of rats infected with BPA (500 mg/kg) negatively stained with bromophenol blue indicates weak distribution of protein.

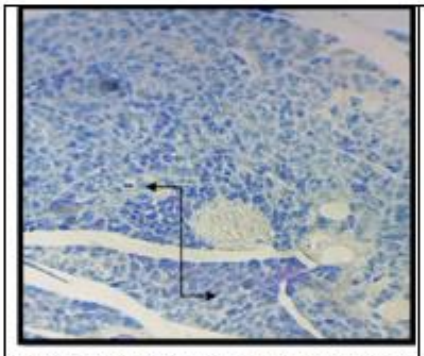

Figure 13: Section through pancres of rats treated with green tea alcoholic extract (300 mg/kg) and BPA (500 mg/kg) shows positive staining with bromophenol blue indicates approximately normal distribution of protein since the slide colour is heavy blue .

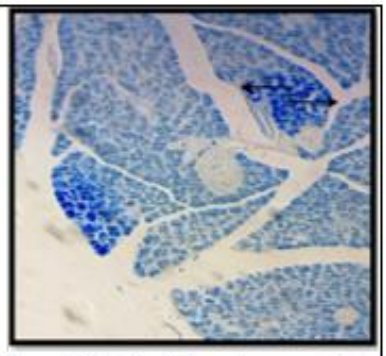

Figure 14: Section through pancres of rats treated with white tea alcoholic extract (300 mg/kg) and BPA (500 mg/kg) positively stained with bromophenol blue indicates nearly normal distribution of protein since the slide colour is dark blue .

CONCLUSIONS

We concluded from this study that BPA caused oxidative stress in rat pancreas and serum while green tea show protective role against BPA stress even more than white tea extract.

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