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Review

Vitellaria paradoxa fruit pulp bioethanol production potential: A review

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In the last decade, bioethanol has become a powerful biofuel for the improvement of environmental pollution such as reduction in greenhouse gas levels. Yet, the source and type of substrate material plays a crucial role in the bioethanol production process due to the different compositional characteristics and availability of monomeric sugars. Different substrates of first, second and third generation fuel sources exist and may be used as reliable and sustainable substrates for bioethanol generation. The current review provides an overview of Vitellaria fruit pulp; its composition and characteristics for ethanol production. This study has examined literature on the background of the *Vitellaria paradoxa*, the characteristics and the potential of the shea nut pulp for fermentation to bioethanol. This review will be useful in harnessing the potentials of the shea pulp as industrially relevant substrate for use independently or in combination with other substrates in microbial fermentation processes for ethanol production.

Key words: *Vitellaria*, shea nut pulp, fermentation, composition, characterization, bioethanol.

INTRODUCTION

Bioethanol is a form of biofuel that has become a major source of bioenergy. Bioethanol is reported as a fuel devoid of pollutants usually mixed with gasoline to run vehicles without modifications to the engine or its design (Doble and Kruthiventi, 2007). It is one of the most commonly used biofuels in the transportation sector and contributes immensely to the reduction of greenhouse gases in the atmosphere (Tesfaw and Assefa, 2014) due to its distinct physico-chemical properties. The United States for instance produced 16.1 billion gallons of clean-burning bioethanol with total consumption rising to 16.2 billion gallons in 2018, 300 million gallons more than 2017 (RFA, 2019). The production and use of bioethanol in automobiles has both economic significance and environmental safety and this remains an important prospect of biofuel generation.

Bioethanol can be produced directly or indirectly from biomass (FAO, 2004; Giampietro et al., 1997; IEA, 2011).

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It is of biological origin excluding material embedded in geological formations and transformed to fossil. Bioethanol is noted to be the most produced biofuel in the world (RFA, 2018) and the production from first generation source (Klanarong et al., 2012; Thalisa, 2010), second generation source (Zakpaa et al., 2010; Suhas et al., 2013) and third generation material sources (Abdul-Mumeen et al., 2016) have received the greatest attention worldwide. Ethanol generated from first generation crops or food crops or energy crops such as maize, cassava and sugar cane and beet has shown numerous benefits but has always done so with myriad of concerns. The large acreage of arable land required for first generation crop production to meet the requisite quantities of ethanol demand is a concern. The main reason is that it poses a huge toll of competition with food and animal feed in addition to other critisms which highlight the raw material processing cost having the abilityto take up to 40% of the total production cost.The use of industrial, agricultural, household and municipal waste or second generation source materials for ethanol production has become the immediate solution to the concerns of using food crops. Residual biomass can contain high carbohydrates content that can be converted to bioethanol. Fruit rinds remain one of the most abundant and affordable raw material source for second generation bioethanol production.

Bioethanol considered as liquid fuel is produced by fermentation - a process by which ethanol is made from sugars (Thomsen et al., 2003). All ethanol fermentation is still based, practically, on the use of the Baker's yeast or *Saccharomyces cerevisiae*, which requires monomeric sugars as the raw material. Fermentation using *S. cerevisiae* produces 0.51 kg of ethanol from 1 kg of any of the C6 sugars: glucose, mannose and sucrose (Thomsen et al., 2003). But *S. cerevisiae* and other microorganisms can also be used to produce ethanol from C5 sugars such as xylose.Ethanol produced by microbial fermentation is used blended or alone, primarily as a substitute for gasoline. Global ethanol usage is expected to increase by 17 billion litters by 2026 and 90% of this increase will take place in developing countries (OECD/FAO, 2017) although bioethanol usage is driven primarily by policies mandating usage levels (FAPRI-MU, 2018).

The *Vitellaria paradoxa* fruit pulp reported to be sweet is a rich source of sugars, minerals and proteins (Maranz et al., 2004) even though the exact monomeric sugars are not known. The shea fruit weighs from 10 to 57 g and its annual production is from 15 to 30 kg/tree (Agbahungba and Depommier, 1989). The pulp constitutes about 60 to 80% (w/w) of the total mass of the shea nut fruit. The *V. paradoxa*fruit pulp with its characteristic soft, smooth and easy to digest macrostructure has not been thoroughly examined for its fermentability to bioethanol. This current assessment describes the biochemical, minerals, soluble sugars, amino acids and the general uses of the *Vitellaria* fruit pulp, the agronomy, production and the potential as feedstock for bioethanol production with a focus on enzyme-assisted and microbial aided fermentation processes. Enzymatic hydrolysis technology prior to fermentation has, in recent times, gained increased attention pertaining to the soluble sugar yield and bioethanol output of targeted substrates. Enzymatic use also allows for reduced cost of hydrolysis in the fermentation of fruit rinds and as a result holds the key to sustainable production of optimal bioethanol in Africa.

THE SHEA NUT TREE

The shea tree (*Vitellaria*) is a member of the Sapotaceae family. It is divided into two subspecies: *paradoxa* and *nilotica* (Moore, 2008). Under the African culture of unwritten facts, known and told by griots, the shea nut tree has been known and used in several different ways for nearly two centuries now. That was the case until in the 18th century when Mungo Park, the British explorer, first came upon it in West Africa in 1796 and described the tree as a useful specie (Wilson, 2019). In 1807, Karl Friedrich Von Gaertner (1772 - 1850), a German Botanist, was the first to classify the shea nut tree as *V. paradoxa* (West African subspecies) and *Vitellaria nilotica* (East African subspecies). Karl Georg Theodore Kotschy (1813 - 1866) Ustron, Poland, an Australian botanist and explorer, reclassified the shea nut tree as *Butyrospermum parkii* for the West African subspecies and *Butyrospermum nilotica* for the East African subspecies.

In Northern Ghana, the shea nut tree, commonly called 'taanga' (Abdul-Mumeen, 2013), was discovered about two centuries ago. The Dogomba women of Northern Ghana were among the first to recognize the significance of the shea tree when they extracted fat from its nuts. During the latter part of the 20th century, shea butter was declared a potential substitute for cocoa butter (Moore, 2008). There was a marked increase in demand for shea butter from the cosmetics and pharmaceutical industries. Thus the shea nut tree was included on the list of tree species constituting African forest genetic resource priorities (FAO, 2014). Therefore, the Cocoa Research Institute of Ghana (CRIG), from 1981 to 1989 was tasked to increase botanical and genetic exploration with research, focusing on diversity, management and propagation of the shea tree (Amissah et al., 2013). Almost five decades down the line, the CRIG is repositioning itself and opening up stations in the three Northern regions to give more definition to the shea nut industry.

The shea tree, an indigenous fruit tree (Figure 1), is perennial and deciduous, and occurs mainly on dry open slopes (Yidana, 2004) and mostly on sandy-loamy soils (Abdulai et al., 2015). The shea tree begins to bear fruit

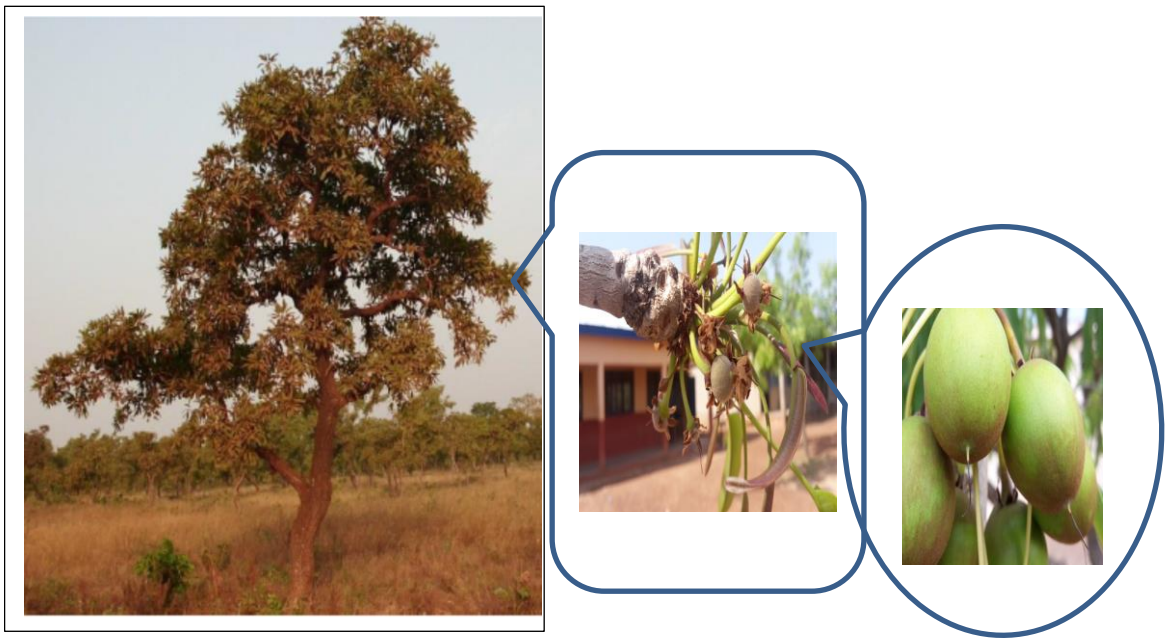

Figure 1. Shea (Vitellaria) tree species, shoots and fruits.

after about 15 to 30 years and can produce good-quality sweet fruits for up to 30 - 250 years (Hall et al., 1996; Dalziel, 1937). The fruits are produced from May to August; being subglobose to ovoid in shape and resemble small avocado fruits. Each fruit is covered with a pulp which is delicious when the fruit is ripe. Olaniyan and Oje (2007) describe the shea fruit to consist of a green epicarp, a fleshy mesocarp and a relatively hard shell (endocarp) which encloses the shea kernel (embryo) sometimes two or more (Ruyssen, 1957). The fruit weighs from 10 to 57 g and its annual production is from 15 to 30 kg/tree (Agbahungba and Depommier, 1989). The pulp of the fruit, which is sweet is widely consumed in areas where the species occurs. It is a rich source of sugars, protein, calcium, ascorbic acid, and iron (Maranz et al., 2004).

SHEA MORPHOLOGY, AGRONOMY AND PLANTATION

V. paradoxa has a wide range of appearance across sub Saharan Africa. This specie is ellipsoidal or a pyramidal crown in shape, a deciduous fruit tree of medium size with a white scar at one side (Alonge and Olaniyan, 2007; Moore, 2008). The morphological characteristics of the shea nut tree have proved to be significantly different from one tree to another by their characteristics. The height, girth, density, seed length and seed width of the shea tree are all different from one tree to the other (Moore, 2008). Generally the tree can attain a height of about 6.1 m under harsh conditions (Maranz, 2004). A fully matured tree under protected conditions will grow from 10 to 20 m in height and rarely to 25 m (Maydell, 1990; Maranz, 2004). A shea nut tree has a cylindrical trunk of 0.5 to 2.5 m circumference, measuring 3 - 4 m before splitting into numerous branches with thick, fissured bark (Moore, 2008) but can also be of 61 cm girth when ravaged by bush fires (Yidana, 2004). The characteristics of the shea nut fruits and nuts, together with the nut length, leaf length, leaf width and petiole length are among the factors that contribute to the variation among shea nut trees (Enaberue et al*.,* 2014). The elliptically shaped fruit measures 2.0- 8.0 cm long × 1.0 to 4.0 cm wide × 2.3 cm thick (Maranz and Wiseman, 2003; Alonge and Olaniyan, 2007). There is a kernel inside the nut which fits properly into the shell. The kernel is about 3.2 cm large, 2.3 cm wide × 0.1 - 2.1 cm thick in size (Alonge and Olaniyan, 2007; Olaniyan and Oje, 1999).

The shea tree can survive on a range of soil types (Hall et al., 1996) as it grows well in sandy soils, light sandy-loams and loamy soils but not in clay soils (Abdulai et al., 2015). The tree is not adaptable to lands susceptible to flooding (Agyente, 2010). *V. paradoxa* has excellent tolerance for drought and this has been well recognized by its ability to grow in impoverished soils and dry areas such as northern Ghana. *Vitellaria* has an extensively, moderately shallow rooting system. This aids the tree's adaptation to extended dry seasons or areas of unpredictable rainfall (Vermilye, 2004). *V. paradoxa* occurs naturally in the wild and grows slowly by seeds randomly dispersed by humans, birds, bats, wind or by gravitational force. The natural regeneration of *V. paradoxa* may be aided by appropriate land management

practices such as protection from bush fire or grazing of livestock (Kristensen and Lyke, 2003) or good farming practices. As a result *V. paradoxa* is considered a semi-domesticated crop (Boffa, 2015). Research has shown that in most areas of Northern Ghana, centuries of traditional land management has led to semi-domesticated *Vitellaria* plots unconsciously being selected (Lovett and Haq, 2000).

Artificial regeneration of the shea nut tree has not been very successful. Biotechnological improvement of the shea through the manipulation of its genetic code to enhance its long juvenile phase is affected by several factors such as its highly recalcitrant seeds, slow growth and the absence of both efficient conventional vegetative propagation and biotechnological methods. Several vegetative propagation studies have been carried out (Amissah et al., 2013; Opoku-Ameyaw et al., 2002; Sanou et al., 2004; Yeboah et al., 2009) but the shea tree has proven to be recalcitrant, responding unfavorably to all known vegetative propagation techniques. However, *Vitellaria*, once matured, has an average life span of 250 years.

SHEA PRODUCTION AND FEEDSTOCK

The production and harvest of shea (*Vitellaria*) is in the African continent only. The estimated number of productive trees is some several hundred million (Lovett, 2004) and the potential number of shea trees in Africa's shea zone ranges from a couple of a billion (Naughton et al., 2014). *V. paradoxa*or *nilotica* grows across approximately 4 million square kilometers of sub-Saharan Africa (Julia et al*.,* 2015) and stretches along almost 19 countries in west and central Africa (Scholz, 2009). The shea nut tree becomes therefore the largest tree population size of the economic tree species in the region. Africa produces about 1.76 million metric tons of raw shea nuts annually (Mohammed et al., 2013). There was an estimated 94 million shea nut trees in Ghana which were projected to produce at least 60,000 metric tons of shea nuts per annum for the production of all shea butter processed locally (Ofosu, 2009). The thickest of shea nut trees is in the Northern Savannah areas, covering over 80% of the woody vegetation (Lovett and Haq, 2000) and offering Ghana the potential to produce 90% of the world's shea nuts (Techno Serve Ghana, 2004).

The shea nut pulp (SNP) constitutes about 60- 80% of fruit weight of the shea nut fruit. During the processing of the shea nut fruit for shea butter extraction, the SNP is first removed by a process known as depulping through unguided fermentation (Abdulai et al., 2015) in mass quantities. For instance, for every 1000 Kg of wet mass of shea fruits picked, about 600 to 800 Kg of wet mass of SNP is generated. The quantum of waste generated is thus huge and therefore the shea nut pulp is best described as an industrial *residue* or forestry waste in Abundance (Figure 3).

OVERVIEW OF ATTRIBUTES AND COMPOSITION OF SHEA NUT FRUIT

The shea nut fruit is a berry, it is hard when raw and soft when ripe but generally green from outside when raw or ripe. The shea nut fruit (SNF) is a naturally profiled layer of four. It consists of a thin epicarp and a soft mesocarp enclosing a single seed, sometimes two or more (Ruyssen, 1957). The thin epicarp and the soft mesocarp constitute the pulp which is very sweet and highly nutritious when ripe (Maranz et al., 2004). The pulp is widely consumed in areas where the shea tree species occurs. It is a rich source of sugars, proteins, calcium, ascorbic acid, and iron (Maranz et al., 2004). The pulp surrounds a relatively large oily-rich oval, brown seed, referred to as shea nut (Figure 2) from which shea butter is extracted (Mohammed et al., 2013; Moore, 2008).

The enclosed nut has a shiny, smooth surface and comprises about 50% of the fresh weight of the fruit (Maranzand Wiseman, 2003). The shea nut has 2 layers: a brown testa or shell and an endosperm or (an oil-bearing) kernel from which shea butter is extracted. SNP is a polysaccharide and has been examined to consist of many biochemical constituents or nutritional elements such as carbohydrates, protein, lipids and fibre (Enaberue et al., 2014; Aguzue et al., 2013; Okullo et al., 2010; Ugese et al., 2008b; Mbaiguinam et al., 2007; Ojo and Adebayo, 2013; Omujal, 2009). The presence of carbohydrates in the SNP which is mostly deduced by difference varies across the shea regional zones from a minimum of 8.10 g/100g to 62.68 g/100 g (Table 1). The difference in carbohydrate levels of the SNP is explained by the fact that soil variation impactsshea nut fruit composition (Abdulai et al., 2015) as well as the stage of harvest and the source or location (Ugese et al, 2008a; Mbaiguinam et al., 2007). The presence and levels of carbohydrates suggest that the SNP contains monosaccharide units such as glucose or its isomers, once it is treated to produce as such. In addition, SNP contains some amount of protein ranging between 4.2 g/100g to 5.6 g/100 g, Table 1, suggesting that the SNP will contain traces of protein biosynthetic precursors, amino acids. Likewise, the amino acid profile (Table 1) will differ from place to place (Mbaiguinam et al., 2007; Dakora and Naab, 2014) mainly due to differences in the location, harvest stage and soil variation (Abdulai et al., 2015). SNP is hydrolysable and has been reported to produce soluble sugars, glucose and fructose especially, also contains sucrose and Mannitol (Dakora and Naab, 2014). The sucrose and glucose levels have been reported very high, 151 g/100g and 157 g/100g respectively, as well as the levels of fructose (145g/100g) and Mannitol (139g/100g). The literature reports several mineral compositions (Table 1) of the SNP; Dakora and Naab (2014) found 6 macronutrients (P, K, Ca, Mg,

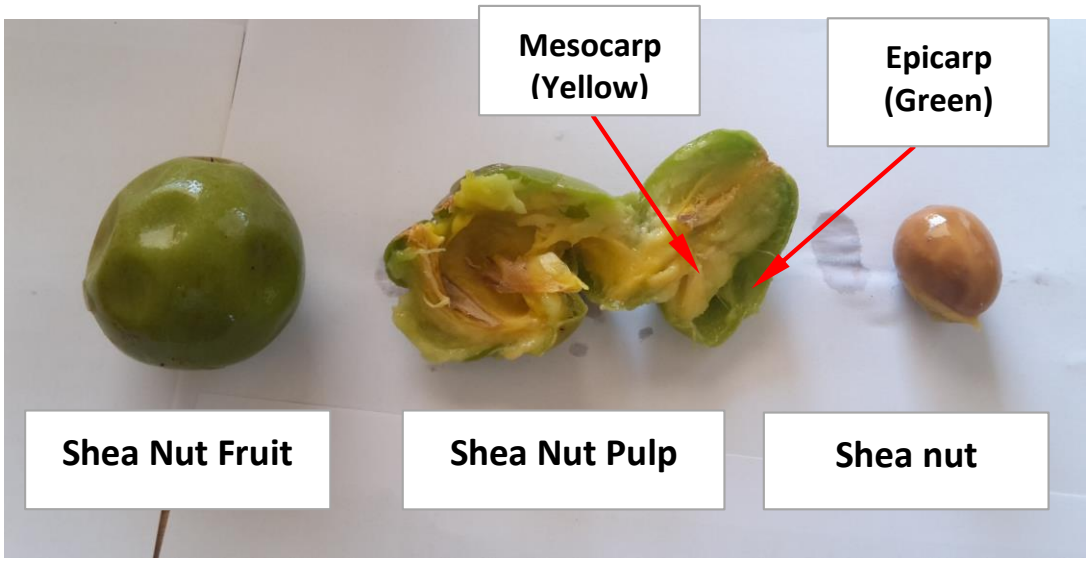

Figure 2. Shea nut fruit, pulp and the nut (seed).
Source: Abdul-Mumeen et al. (2019).

S and Na) and 6 trace elements (Fe, Cu, Zn, Mn, B and Al) in the SNP.

SNP FOR BIOETHANOL PRODUCTION

It has been demonstrated that the SNP is a potential source of reducing sugars (Table 1). The major source of bioethanol is the conversion of bioethanol from reducing sugars, by fermentation. Unfortunately, there has not been any report on converting the SNP to bioethanol by any means possible yet bioethanol has become one of the safest fuels for combating climate change factors. Bioethanol is a form of biofuel that generates bioenergy and it is one of the most commonly used biofuels in the transport sector to reduce greenhouse gases (Tesfaw and Assefa, 2014). The FAO (2004) defines biofuel as "fuel produced directly or indirectly from biomass". Biomass is a material of biological origin, excluding material embedded in geological formations, transformed to fossil (FAO, 2004). Biofuel is also considered as any solid, liquid or gaseous fuel that is produced from biomass (Giampietro et al*.,* 1997; IEA, 2010). Therefore, bioenergy is all the energies derived from biofuels. Bioethanol is mainly obtained by the processes of fermentation.

Bioethanol is produced by the fermentation of materials of sugar or starch source. The most common substrates are sugar cane, corn, wheat, sugar beet, seaweeds and fruit pulps. Cellulosic biomass such as grasses, woody crops, and organic wastes can also be used to produce bioethanol through advanced processing. Several studies (Grohman, 1995; Hammond, 1996; Grohmann, 1998; Sharma et al., 2007; Tesfaw and Assefa, 2014) investigating cellulosic biomass have been carried out for bioethanol production. Bioethanol production from green seaweeds (Abdul-Mumeen et al., 2016), banana and citrus waste (Sharma et al., 2007) has also been investigated but limited literature is available on ethanol production from the SNP. The SNP is in abundance and with its characteristic soft, smooth and easy to digest macrostructure, requires no any special treatment prior to fermentation. Therefore, the energy needs of the bioethanol production process using the SNP as substrate may be curtailed and as a result, the pulp remains a huge potential for bioethanol production.

MICROBIAL/ENZYMATIC SACCHARIFICATION OF SHEA NUT PULP

Fruit pulps like the shea nut pulp are composed of carbohydrates or sugars, best described as monosaccharides, disaccharides and polysaccharides. Monosaccharides are simple sugars that cannot be broken down further while disaccharides and polysaccharides have glycosidic bonds between every 2 simple sugar molecules. They require further breakdown by the requisite mechanisms to obtain reasonable amounts of monomeric units, prior to fermentation. According to Thomas et al. (1993), the sugar content in fuel ethanol fermentation can be categorized to normal when the sugar composition is between 20-22% of the substrate or very high when the sugar level is greater than 27% of the total substrate weight.

Several saccharification methods have been used to breakdown the glycosidic bonds holding together, monomeric units in polysaccharides. The methods could be physical, chemical or biological breakdown of

Table 1. Summary of the biochemical characteristics, mineral content, soluble sugars and the amino acid profile mapping of shea nut pulp (SNP).

Characteristics	Range of values			References
	Min	Mean	Max	
Biochemical composition (g/100 g)				
Carbohydrates	8.10	22.60	62.68	Enaberue et al. (2014) Aguzue et al. (2013) Okullo et al. (2010) Ugese et al. (2008b) Mbaiguinam et al. (2015) Ojo and Adebayo (2013) Omujal (2009)
Crude protein	4.20	5.20	5.60	
Crude lipid	1.30	1.30	34.53	
Crude fiber	42.20	42.20	42.20	
Ash	4.92	5.06	5.20	
Energy	248.16	250.25	252.29	
Mineral (mg/100 g)				
Na	10.79	19.30	52.30	
Ca	0.19	70.30	117.30	
Mg	0.50	21.78	57.20	
K	1.40	51.38	830.30	
Cu	0.14	0.62	1.10	Enaberue et al. (2014) Aguzue et al. (2013) Okullo et al. (2010) Ugese et al. (2008b) Mbaguinam et al. (2007) Dakora and Naab (2014) Omujal (2009) Ojo and Adebayo (2013)
Fe	0.01	14.15	28.29	
Mn	0.20	0.64	1.07	
P	0.07	35.74	71.40	
Zn	0.50	2.25	4.00	
Ni	-	0.86	-	
Cd	-	0.04	-	
Co	-	0.80	-	
S	-	0.05	-	
B	-	0.90	-	
Al	-	14.26	-	
Soluble sugar (g/100 g)				
Fructose	40	87	145	Dakora and Naab (2014)
Mannitol	47	91	139	
Glucose	51	103	157	
Sucrose	38	96	151	
Amino acids (g/100 g)				
Alanine	2.21	63.32	120.00	Mbaiguinam et al. (2007) Dakora and Naab (2014)
Arginine	2.93	91.40	174.00	
Asparagine	6.03	95.05	172.00	
Cysteine	0.97	1.12	1.28	
Glycine	1.93	2.18	2.44	
Glutamine	4.98	5.59	6.28	
Histidine	1.03	1.23	1.37	
Isoleucine	1.87	17.81	30.00	
Leucine	2.88	27.82	47.00	
Lysine	1.67	1.79	1.91	
Methionine	0.07	0.09	0.12	
Phenylalamine	1.29	1.44	1.65	
Proline	3.56	599.84	1189.00	
Serine	1.71	42.57	80.00	
Threonine	1.53	13.80	23.00	
Tyrosine	1.41	14.62	25.00	
Valine	2.25	29.88	53.00	

polysaccharides into their base monomer units. Any such treatment; acidic or alkaline, enzymes or microorganisms, size reduction or softening by beating, or thermal application aimed at breaking down the cell wall, hemicellulose, cellulose or lignin for the release of soluble sugars; pentose or hexose, is also referred to as pretreatment. That is, in fermentation processes, the terms saccharification, hydrolysis and pretreatment are sometimes used interchangeably. Microbial or enzymatic hydrolysis is by far the mildest and the most environmentally safe process for the release of monomeric sugars from fruit pulps (Figure 2).

Enzymes are vegetable or animal extracts or just microorganisms. They have been used as such throughout civilization. Microbes or their enzymes have been widely used for breaking glycosidic bonds in complex sugars to produce monomeric sugars. Some plant materials such as lignin may be very recalcitrant to microbial or enzymatic attack. The production of bioethanol from maize agro-wastes (lignocellulose) with cellulase as the saccharifying agent is crucial and relatively expensive cost-wise since enzyme cost alone contributes about 40% (zakpaa et al., 2010; Howard et al., 2003; Miyamoto, 1997) of the production cost.

Zakpaa et al. (2010), in search of low cost saccharifying organisms for corncob, assayed cellulolytic isolates on corncob based broth media. *Aspergillus niger* had the highest significant filter paper activity (0.37 FPU/ml), carboxymethyl cellulose activity (0.7025 U/ml) and protein concentration (5.62 mg/ml) although *Trichoderma, Penicillium, Mucor, Fusarium Rhodotorula, Acremonium and Coccidioides* were all isolated and assayed for their saccharification potentials (Table 2). Thus, the use of cellulase-producing organisms (Bon and Ferarra, 2007) is one way of reducing the higher production cost which also remains one of the ways to increasing available sugar in the fermentation media. Suhas et al. (2013) utilizing fruit rinds from four fruits (Pineapple, Jackfruit, Watermelon and Muskmelon) as possible source of cellulosic ethanol under anaerobic conditions, employed *Trichoderma viride* for saccharification of the powdered substrate. Significant amounts of reducing sugars were obtained at the end of the saccharification process, with the microbe being most effective on jackfruit and pineapple rinds, resulting in a monomeric sugar recovery of 10.28 mg/ml and 10.18 mg/ml respectively.

Microbial saccharification of sugary substrates is common in the natural environment. SNP easily decays from microbial attack of its high sugar quantities (Caroline et al., 2009). The fungal attack of SNP does not only deteriorate the pulp but also affects the oil content of the oil bearing nut and must be removed during shea butter processing to prevent further fungal growth (Caroline et al., 2009).

Many fungal species have been identified to be associated with the saccharification of SNP. Eight fungi species during the bio-deterioration of the shea nut pulp were isolated from the fruit natural environment (Ojo and Adebayo, 2013). *Aspergillus flavus, Aspergillus niger, Botrydiplodia theombromae, Botryosphaeria* spp*., Colletotrichum gleosphoriedes, Lisidiplodia spp., Pseudofasicocum* spp*. and Trichoderma viridae* were mentioned (Ojo and Adebayo, 2013). *Aspergillus niger* developed the most extensive saccharifiable ability when the microbes were inoculated directly on the shea nut fruit. Nwufo and Mba (1987) also mentioned *Aspergillus niger*as part of the fungi found associated with the decomposing seeds of African shea butter fruit in Nigeria. Similarly (Aculey et al., 2012) noted during an investigation of the deteriorating parboiled shea nut kernels that the frequently encountered moulds were of *Aspergillus* and the *Rhizopus species*. Thus, *Aspergillus niger* has by far proven to be causing the most rot once inoculated alongside other fungi species, common at shea nut pulp environments, producing the highest significant filter paper activity, carboxymethyl cellulose activity and protein concentration.

The use of saccharifying microbes during simultaneous fermentation processes however occurs, however, with some demerits. In many situations, the most secreted proteins by the microorganisms are not that particularly thermostable or that the native ß-glucosidase released in the fermentation media is sufficient for the hydrolysis of most substrates. Once produced in the fermentation medium, sometimes the native GH61 proteins are not highly expressed and are not particularly active in the medium to cause the needed breakdown of the substrate. Other organisms during the fermentation can produce enzymes that are individually superior.

CONDITIONS FOR SUBSTRATE FERMENTATION WITH *SACCHAROMYCES CEREVISIAE*

A substrate for bioethanol production refers to any plant material or algae that have the potential of releasing soluble sugars in solution for fermentation to proceed. Such biomasses as forestry wastes, corn stalk and cobs, wheat straw, grasses and rice straw have been mentioned. Fermentation is the core process in ethanol production from a given substrate. Fermentation occurs through the activity of a variety of microorganisms including fungi, bacteria, and yeasts. Ethanol production from kinnow waste and banana peels by simultaneous saccharification and fermentation using cellulase and co-culture of *S. cerevisiae* G and *Pachysolen tannophilus* MTCC 1077 has been carried out by Sharma et al. (2007) at optimized conditions. Certain fermentation parameters such as inoculum, enzyme and substrate concentration besides optimum pH, temperature, time, agitation among others play an important role in obtaining good ethanol yield (Sharma et al., 2007). The biomass after enzymatic saccharification containing 63 gL^{-1} reducing sugars was

Table 2. Saccharifying abilities of different fungi species associated with the deterioration of shea nut pulp, corn cobs and shea nut kernels.

Substrate	Microrganism	Saccharifying ability	References
Shea nut pulp	*Aspergillus niger*	Most extensive	Ojo and Adebayo (2013) Aculey et al. (2012) Nwufo and Mba (1987)
	Rhizopus species	Most extensive	
	Aspergillus flavus	Extensive	
	Botryodiplodia theobromae	More extensive	
	Botryosphaeria spp	Extensive	
	Collectotrichum gloeosporioides	Extensive	
	Lisidiplodia theobromae	Extensive	
	Pestaliopsis spp.	Extensive	
	Pseudofasicocum spp.	Extensive	
	Trichoderma viridae	Extensive	
Corn cob	*Aspergillusniger*	Highest	Zakpaa et al. (2010)
	Trichodermaviridae	Higher	
	Penicillium	High	
	Mucor	High	
	FusariumRhodotorula	High	
	Acremonium	High	
	Coccidioides	High	
Shea nuts and kernel	*Aspergillusniger*	Most extensive	Esiegbuya et al. (2014)
	Aspergillusflavus	Most extensive	
	Aspergilluspersii	Most extensive	
	*Mucor*sp	Extensive	
	*Fusarium*sp	Extensive	
	*Phoma*sp	Extensive	
	*Xylaria*sp	Extensive	

fermented with both hexose and pentose fermenting yeast strains, resulting in ethanol production, ethanol yield and ethanol fermentation efficiency of 26.84 and 0.426 gg^{-1} and 83.52% respectively. Suhas et al. (2013) carried out fermentation on fruit rinds using *S. cerevisiae*. The amount of ethanol produced after fermentation was analyzed by gas chromatography and found to be the highest for jackfruits and pineapple rind fruits with yields of 4.64 and 4.38 g/L respectively. Coculturing *S. cerevisiae* with other yeasts or microbes is targeted to optimize ethanol production, shorten fermentation time, and reduce process cost.

To increase the yield of ethanol by microbial fermentation, the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology remains essential (Benjamin et al., 2014). Also, one of the efforts to increase the production of ethanol is the engineering of the microbial genetic composition or the modification of fermentation media, or combination of both (Chan-u-tit et al., 2013; Deesuth et al., 2012; Krause et al., 2007; Nikolić et al., 2009; Takagi et al., 2005; Xueet al., 2008). A considerable amount of literature has been published on microbial fermentation. These studies (Benitez et al., 1983; Diwanya et al., 1992) have suggested that an ideal microorganism for bioethanol production must have rapid fermentative potential, is thermo-stable, has improved flocculating ability, appropriate osmo-tolerance and can withstand high ethanol concentrations.

Recent research findings suggest that *S. cerevisiae* is one of the widely studied and used yeasts at both industry and household levels with bioethanol generated as the main fermentation product (Tesfaw and Assefa, 2014). Over the past decade, most research on the use of the right microorganism for fermentation process had emphasized the use of *S. cerevisiae* (Zakpaa et al., 2009; Hossain et al., 2011; Benjamin et al., 2014; Abdul-Mumeen et al., 2016).

S. cerevisiae is chosen for most fermentation experiments since it is a well understood fermentative organism (Lamb et al., 2018). *S.cerevisiae*, a natural evolution meant for efficient consumption of sugars especially sucrose, remains one of the most important cell factories due to its robustness, stress tolerance,

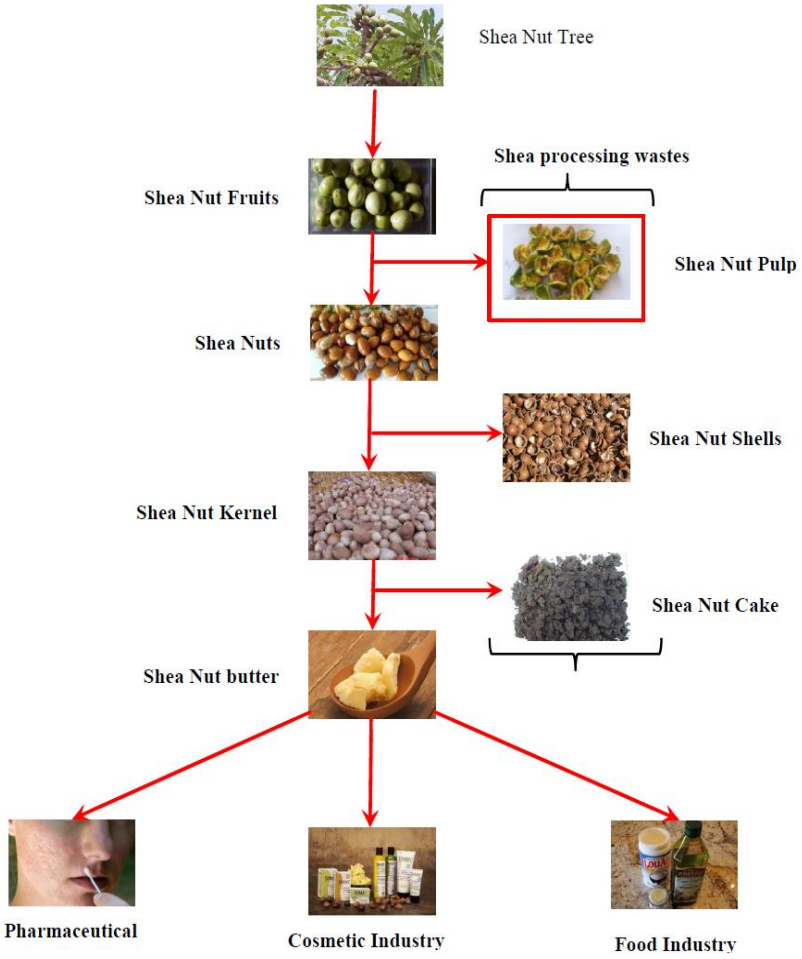

Figure 3. Various waste substances generated from shea butter processing.

genetic accessibility, simple nutrient requirements and long history as an industrial workhorse. Fermentation performance of the yeast *S. cerevisiae* is however influenced, among others, by growth media composition (Djajasoepena et al., 2015). Complex nitrogen source media tend to give better fermentation performance. Djajasoepena et al. (2015) confirm the effect of growth media composition, especially media with complex nitrogen source tends to increase fermentation performance of the yeast *S. cerevisiae.* Paul (2010) suggests the growth curve of *S. cerevisiae* with the right media composition at 30°C for 12 h with absorbance reading at 600 nm to be the result, as shown in Figure 4.

For the several good factors about *S. cerevisiae*many researchers (Lamb et al., 2018; Tropea et al., 2014; Almeida and Angelis, 2016; Suhas et al., 2013; Togarepi et al., 2012; Ofosu-Appiah et al., 2016; Sharma et al., 2007) have relied on the microbe for the fermentation of different substrates for ethanol generation, Table 3. *S. cerevisiae* is superior to bacteria, other yeasts, and filamentous fungi in various physiological characteristics regarding ethanol production in industrial context. It tolerates a wide range of pH (Lin et al., 2012) operates at optimum acidity (Ortiz-Muñizet al., 2010) and its robust. It also tolerates ethanol better than other ethanol producing microorganisms (Prasertwasu et al., 2014). The use of *S. cerevisiae* in fermentation is safe and less susceptible to infection since it is extensively used for human consumption.

With the use of *S. cerevisiae* on dried *Ziziphus mauritiana* (Chinese date) fruit pulp for instance, at pH of 6, with optimum temperature at 30ºC, the yeast concentration of 8 g/20g (0.4 g/g) fruit pulp yielded the optimum rate of fermentation after the stipulated seven days, Table 3. Using a free cell batch fermentation process, *Zymomonas mobilis* reached 59.95% of the theoretical yield. Immobilized cells reached 68.53% using a batch and 74.49% using a continuous fermentation process. Under the same conditions, *Saccharomyces cerevisiae* reached 70.03, 77.10 and 78.47% of the theoretical yield respectively. Higher yields were achieved for both microorganisms using mixed culture fermentation, compared to pure cultures. Under the same conditions for both pure cultures, mixed cultures reached

Table 3. Review of fermentation conditions, mechanisms and ethanol yield.

Microorganism	Substrate	Fermentation condition			Fermentation Vol. (ml)	Microb/substrate conc.	Max ethanol yld	References
		pH	Temp (°C)	Duration/day				
Escherichia coli KO11	*Laminaria Japonica*	-	-	-			0.40 g/g	Kim et al. (2011)
Saccharomyces cerevisiae	Sorghum Pito Mash	pH 6.0	30	4	500	10 ml/50 g	3.03 g/L	Ofosu et al. (2016)
Zymomonas mobilis	Sorghum Pito Mash	pH 5.5	35	3	500	10 ml/50 g	3.63 g/L	
S. cerevisiae	*Ziziphus mauritiana*	pH 6	30		500	8.0 g/20 g	63.00 g/L	Togarepi et al. (2012)
S. cerevisiae and *Pachysolen tannophilus MTCC 1077*	Kinnow waste and banana peels	6% 4%	30	-	500	8 g/25 g	0.43 g/g	Sharma et al. (2007)
Saccharomyces cerevisiae	Jackfruit Rind	-	25	4	250	15 ml/50 g	4.64 g/L	Suhas et al. (2013)
Saccharomyces cerevisiae	Pineapple Rind	-	25	4	250	15 ml/50 g	4.38 g/L	
Zymomonas mobilis	Sugarcane juice		-	-	250	0.8 ml/50 g	59.95%	Almeida and Angelis (2016)
Zymomonas mobilis	Sugarcane juice		-	-	250	0.8 ml/50 g	68.53%	
Zymomonas mobilis	Sugarcane juice		-	-	250	0.8 ml/50 g	74.49%	
Saccharomyces cerevisiae	Sugarcane juice		-	-	250	0.8 ml/50 g	70.03%	
Saccharomyces cerevisiae	Sugarcane juice		-	-	250	0.8 ml/50 g	77.10%	
Saccharomyces cerevisiae	Sugarcane juice		-	-	250	0.8 ml/50 g	78.47%	
Zymomonas mobilis/S. cerevisiae	Sugarcane juice		-	-	250	(0.4/0.4) ml/50 g	70.86%	
Zymomonas mobilis/S. cerevisiae	Sugarcane juice		-	-	250	(0.4/0.4) ml/50 g	79.07	
Zymomonas mobilis/S. cerevisiae	Sugarcane juice		-	-	250	0.4 ml each/50 g	80.86%	
Saccharomyces cerevisiae	Pineapples waste	4.5	30	-	2.5	20 ml/1.5 L	3.90%	Tropea et al., 2014
Saccharomyces cerevisiae	*Saccharina latissima*	6.8	30	2	25	1 g/L	0.42 g/g	Lamb et al., *2018*

70.86, 79.07 and 80.86% of the theoretical yield respectively.

CONCLUSION

Shea nut pulp could be unique source of valuable monomeric sugars that have significant importance to the bioenergy sector for renewable energy generation by fermentation. The ordinary fermentation processes previously relied on the use of chemical pretreatments of the substrate under harsh conditions. To maintain a high glucose yielding substrate and to evade chemical use for the pretreatment, a milder and more selective fermentation process is required. Currently, research is focused on the nutritional and mineral composition of the shea nut pulp but several enzymes and microorganisms have also been identified to cause severe deterioration to the fruit skin in its natural environment.

Some studies have covered the use of commercial enzymes or microbial consortium in simultaneous saccharification and fermentation processes. Although commercial enzyme mixtures have generally been developed for terrestrial plant biomass processing, the use of indigenous microbial consortia can be cost effective with equal yield or better. This further allows for reduction in chemicals use in bioethanol production process and thus holds enormous potential for creation of sustainable

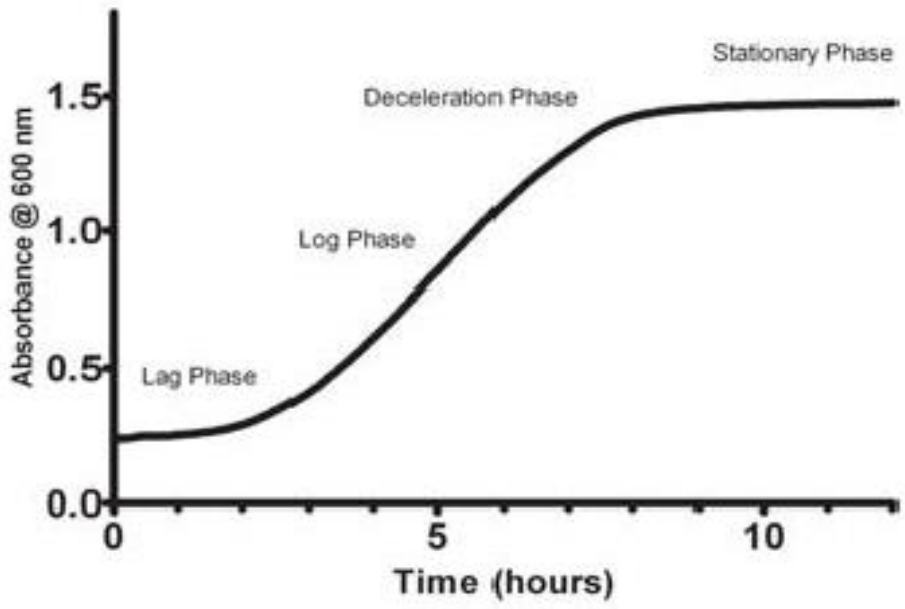

Figure 4. Typical yeast growth curve. *S. cerevisiae* grown in YPD media at 30°C for 12 h with data measurements every 2 min.
Source: Paul (2010).

ethanol processing from SNP substrate.

RECOMMENDATIONS

A research conducted into the potentials of the Ghanaian shea nut pulp for use as substrate for the production of fuel ethanol will be of enormous benefit to renewable energy policy targets. This can be done by either using enzymes directly or by microbial consortia to hydrolyze the dry or fresh shea nut pulp at optimum conditions for optimal bioethanol yield. Such a conversion will find more uses for the shea nut waste away from its environmental nuisance at the shea butter processing centers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluationof *in vivo* toxicity of rice husk used as fuel for cooking in households

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This study meant to assess the toxicological impact of nourishment cooked or water overflowed with a fan-helped top-lit-updraft rice husk fuelled gasifier stove named Paul Olivier 150 (PO150). Refined water was bubbled for 1 h utilizing this stove in an opened pot and shut room. This water was then cooled to room temperature before being managed to the rodents with body loads going from 70 to 110 g. Two kinds of tests were performed: Acute and sub-chronic toxicity tests. For the acute toxicity study, an extraordinary portion of 2 ml/100 g body weight (BW) of bubbled water was managed orally to the rodents. The creatures were watched for harmful indications and mortality day by day for 14 days. In a sub-chronic toxicity study, the bubbled water, at dosages of 0.5, 1 and 2 ml/100 g BW were orally managed day by day for 28 days to rodents. Following 28 days, the rodents were yielded, Blood tests were gathered for hematological, biochemical and histological assessment. The control rodents were managed in refined water. The example of refined water overflowed with rice husk fuelled gasifier stove indicated no proof of single-portion toxicity (2 ml/100g) when studying acute toxicity. For the sub-chronic toxicity study, bubbled water at dosages of 0.5, 1 and 2 ml/100 g indicated huge contrast in certain parameters, for example, creatinine in guys (71.81 mg/dL), uric corrosive (2.75 mg/dL) and complete bilirubin (0.08 mg/dL), monocytes (0.49 103/µL)and granulocytes in females (2.70 103/µL) contrasted with the control gathering (64.16 mg/dL, 2.25 mg/dL, 0.19 mg/l, 0.37 103/µL and 1.80 103/µL for every parameter separately) however, the information did not ascend to the level for the responses to be viewed as a poisonous impact. These demonstrated that cooking in an open pot with a rice husk fuelled PO150 gasifier stove doesnot cause toxicity at the dosages considered.

Key words: Acute toxicity, sub-chronic toxicity, biochemical analysis, hematological parameters, histopathology, rice husk.

INTRODUCTION

The adoption of fire in such a large number of years before was without a doubt one of the most remarkable

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advancements in mankind's history. Fire for cooking has made the utilization of a lot more extensive assortment of staples and incredibly improved sanitation. Fire for warming has permitted people to grow their zones of home to higher scopes and rises, and it has in a general sense changed the examples of social advancement. In any case, with fire additionally came the main anthropogenic contamination, proven by the ash despite everything found in ancient caverns (GEMS, 1990). About 3 billion individuals around the world, and a dominant part of families in creating nations, depend on strong powers, (for example, wood, waste, crop deposits, coal, and charcoal) with almost no entrance to current fills for cooking and other family unit vitality needs (Lim et al., 2012; Smith et al., 2012). The kinds of fuel utilized for household needs, for example, cooking and warming can be ordered into non-strong and strong energizes (Torres et al., 2008).

Rice husk (strong fuel) establishes about 20% of the heaviness of paddy and is made out of cellulose (half), lignin (25-30%), silica (15-20%), and dampness (10-15%) (Bhupinder, 2018). As per the United States Department of Agriculture (USDA), paddy creation in Sub-Sahara Africa (SSA) in 2018 was 26.5 million tons (IRRI RICESTAT, 2019). In view of paddy creation evaluations in 2018, some 5.3 million tons of rice husk was delivered yearly in SSA and this can be an ideal wellspring of the sustainable power source. Be that as it may, the husk right now for the most part discarded by copying in the field of streets or potentially dumping in waterway beds prompting significant levels of land, water, and air contamination. The rice husk can deliver around 3000 kcal per kilogram of warmth vitality (Anderson et al., 2008). Burning and gasification remain the most significant feasible alternatives of utilizing rice husk as fuel in SSA whereby, the rice husk can be utilized natural (Ndindeng et al., 2019) or prepared into briquettes or pellets (Ndindeng et al., 2015).

Poisonous wellbeing impacts of strong powers were distinguished as ahead of schedule as the late eighteenth century when coal residue was perceived as a reason for scrotal disease in fireplace clears (Brown et al., 1957). In the twentieth century, both coal and biomass powers were subjects of escalated examinations on their conceivable negative wellbeing impacts. The consequences of these examine recommend that a few constituents of biomass smoke emanations have aggravating, incendiary and cancer-causing properties (Wei-Yen and Seow, 2012). Smoke outflows have cancer-causing and mutagenic properties in examines directed on *in vitro* frameworks and creature models. At the populace level, there is epidemiological proof that biomass fills are related to respiratory and cardiovascular ailments, for example, lower respiratory tract contaminations, chronic obstructive lung ailment and coronary illness (Dherani et al., 2008; Black et al., 2010). The fragmented burning of these strong energizes brings about a significant part of the fuel vitality being discharged as conceivably lethal toxins, including particles of fluctuating sizes, carbon monoxide (CO), nitrogen dioxide, unstable and semivolatile natural mixes (e.g., formaldehyde and benzo[a]pyrene), methylene chloride, and dioxins (Naeher et al., 2007). The utilization of strong energizes, basically for cooking, has been evaluated to be answerable for > 3.5 million unexpected losses for each year (in addition to an extra 0.5 million passing from open-air contamination because of family fuel use) and 110 million handicap balanced life years (DALYs) (Lim et al., 2012).

Ndindeng et al. (2019) assessed five diverse rice husk top-lit updraft (TLUD) gasifier cook-stoves for use in cooking tasks in Africa. This study showed that fan-helped cook-stoves particularly PO150 recorded better warm and discharge files and more secure to utilize contrasted with the characteristic draft gasifier stove (Mayon). Despite the fact that PO150 administrator may securely utilize the stove for cooking in all around ventilated conditions, questions despite everything exist regarding whether a few mixes transmitted by the stove can taint the nourishment being cooked and apply poisonous impacts on purchasers. Be that as it may, as far as anyone is concerned, no study on the *in vivo* toxicity of rice husk as a fuel has been depicted in the writing. In this manner, in the present examination, we planned to explore the toxicity (both oral acute and sub-chronic) of refined water bubbled on a P0150 gasifier utilizing rice husk as fuel on rodents.

MATERIALS AND METHODS

Operation of the stove and preparation of the water sample

PO150 gasifier operates on bunch mode and on a constrained draft framework which is a fan with anenergizing limit of 0.78 kg per cluster and a group enduring 30 min. At the point when the rice husk in the gasifier was totally spent, the biochar was disposed of before new fuel was placed in the gasifier to start another vitality generation process. So as to lessen the fuel topping off time and guarantee smooth cooking of dishes whose cooking time is higher than the most extreme consuming time for bunch type gasifiers, stove exchanging was utilized as recently portrayed (Ndindeng et al., 2019). Quickly, two PO150 gasifiers, A1 and A2 were delivered and utilized for the study. When A1 was running, A2 was loaded up with rice husks and set close by. The gasifier A2 was lit when the consuming time in A1 was 5 min to halting time. When A1 halted, the pot was moved to A2 and the biochar in A1 chamber disposed of, new fuel-filled and set close by.

Refined water was heated up each day during the time of the trial (28 days). The fan-helped gasifier PO150 (Figure 1) was put in a stay with a window and entryway shut.

Five liters (5 L) of refined water were placed in an open pot with 10L of limit. Since the preparing time of nourishment differs (10 min to 2 h), the time the water continues bubbling on the stove is basic, and the more it is extended, the better it will be to take into account any potential poisons from the stove emanations to break up in the water. The water was in this manner permitted to bubble for about 1h from the breaking point on the stove utilizing the rice husk as fuel. This water was then cooled to room temperature before being

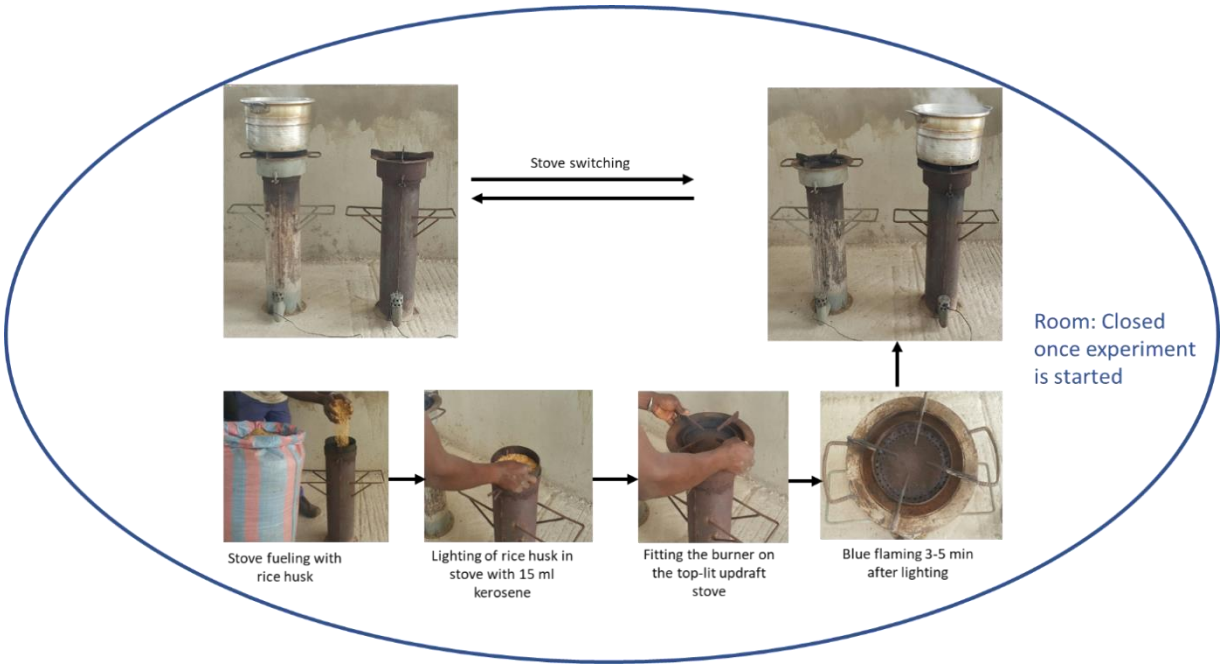

Figure 1. Schematic presentation of the setup for boiling water with a PO150 fan-assisted rice husk fuelled gasifier stove.

controlled to the rats.

Experimental animals

For the evaluation of rice husk-related toxicity, 48 albino rats of Wistar breed (20 males and 28 non-pregnant females), aged about 6 weeks, and body weights ranging from 70-110 g at the beginning of the experiment, were used. They were purchased from the Animal House of the Laboratory of Animal Physiology, Department of Biochemistry, University of Yaoundé I, and bred at room temperature for a 12 h' light/dark photoperiod cycle. A seven (7)-day adaptation period was observed before the experiment. They were kept in their plastic cages where they received the standard diet and water *ad libitum*. The litter used was sawdust, renewed twice per week to ensure good hygienic status of animals. Authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. Nº. FWA-IRD 0001954).

Grouping of animals

The 48 *Wistar* albino rats were randomly divided into 6 groups of 8 animals each. Group 1 (consisting of 8 females) was used for the acute toxicity assessment Female rats were used because literature surveys of conventional LD_{50} tests show that, although there is little difference in sensitivity between the sexes, in cases where differences are observed females are generally slightly more sensitive (OCDE, 2001). And the other 5 groups (8 rats per group, made up of 4 females and 4 males) were used for the sub-chronic toxicity.

Acute toxicity study in rats

For acute toxicity testing, in rodents, the volume of administered substance should not normally exceed 1 mL/100 g of body weight. However, for aqueous solutions, 2 mL/100 g body weight (bw) can be considered. In this study, distilled water boiled with rice husk was given to the rats at the unique dose of 2 ml/100g bw, according to the Organization for Economic Cooperation Development (OECD) guidelines 423(OCDE, 2001). Eight (8) healthy Wistar female rats were randomly divided into 2 groups (4 females per group).On the day of the experiment, food but not water was withheld overnight. Group 1 (Normal control group) received distilled water, given orally. Group 2 (The Experimental group) orally received unique dose of 2 ml/100g bw distilled water that was boiled with rice husk. Food was withheld for a further 4 h after giving the water. Animals were weighed every 2 days and were observed individually for general behaviour and body weight changes, toxic symptoms, and mortality during the first 30 min, periodically during the first 4 h after the administration of the unique dose of 2 ml/100 g bw boiled water, for a total of 14 days. During this period, signs of toxicity including change in coat, motility, tremors, mass, grooming, sensitivity to noise after metal shock, stool appearance, mobility and death were observed. The rats were sacrificed by cervical dislocation, and their organs were excised (heart, liver, spleen, lungs, kidneys), and weighed using an analytical balance.

Sub-chronic toxicity study in rats

The sub-chronictoxicity study was carried out on the rats according to the Organization for Economic Cooperation and Development (OECD guideline 407 for testing of chemicals on sub-chronic toxicity with slight modifications); which stated that the volume given to rats should not normally exceed 1mL/100g of body weight, however in the case of aqueous solutions, 2 mL/100g body weight (bw) can be considered (OCDE, 2008). Forty (40) healthy Wistar rats were weighed, orderly marked, and randomly divided into 5 groups (4 males and 4 females per group). Group 1 (Control group) received distilled water by oral gavage throughout the course of the

study. The experimental groups (2–4) were orally administered samples of distilled water boiled with rice husk stove as follows: Low dose (0.5 ml/100g), medium dose (1 ml/100g) and high dose (2 ml/100g) body weight/day, respectively, for 28 days. The body weight was measured every 2 days and signs of toxicity were noted daily. At the end of 28 days, groups 2- 4 were sacrificed while the physiological condition of the rats of group 5was restored for another 2 weeks (with food and water supplied *ad libitum*). Group 5 was orally administered samples of distilled water boiled with high dose (2 ml/100g) for 28 days but not sacrificed at the end of 28 days as Groups 1-4. Group 5 was observed for additional 14 days and sacrificed on the 42nd day. Surviving rats were anesthetized with carbon dioxide and blood samples were obtained from the eyes of the rat using capillary tubes for hematological and serum biochemical studies. After blood collection, the rats were sacrificed by cervical dislocation.

Measurement of body and organ weights

The animals were weighed every 2 days and the percentage weight gain was calculated using the formula:

$$\text{Weight gain } (\%) = \frac{W_f - W_i}{W_i} \times 100$$

Where W_f: final weight; W_i: initial weight.

All the animals in this study were subjected to general autopsy. Animals were pinned down in a dissection tray by placing them with ventral side up. The abdominal skin was lifted with forceps and cut through with scissors. The scissor was inserted under the skin and moved towards the cephalic direction. The rats were cut along the body midline, from the public region to the lower jaw. A lateral cut was made about halfway down the ventral surface of each limb. The liver, heart and kidneys were removed, cleaned, and kept in the refrigerator. The relative weight of the liver, heart and kidneys was determined by the formula:

$$\text{Relative organ weight} = \frac{weight\ of\ organ}{Animal\ weight} \times 100$$

Biochemical parameters

Blood samples were collected in nonheparinized tubes and centrifuged at 3000 rpm to obtain the serum that served for the assessment of the parameters for liver and kidney functions. The experiment was performed in accordance with protocols provided with commercial kits, Fortress Diagnostics, reviewed in October 2007. The levels of aspartate amino transferase (AST), alanine amino transferase (ALT), creatinine, uric acid, total bilirubin and proteins were assessed. Creatine, uric acid and total bilirubin and protein were analyzed using the method described by Timothy et al. (2015). Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) assays in serum were assayed using the colorimetric test of Reitman and Frankel (1957) as published by Rodier and Mallein (1983).

Hematological analysis

The following haematological parameters were evaluated with the help of a "Hospitex Diagnostics Hema Screen 18" Automated Analyzer from the Haematology Laboratory of the Yaoundé Central Hospital: white blood cell count (WBC), haemoglobin (Hb), red blood cell counts (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), hematocrit (HCT), lymphocytes (LYM), monocytes (MON), granulocytes (GRA) and platelet count (PLT).

Histopathological study

The liver, heart and kidney stored in formalin 10% for 3 weeks, were cut into small pieces of 5 to 10 mm. The tissues were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin wax melted at 60°C. Serial sections (5 µm thick) obtained by cutting the embedded tissue with microtome, were mounted on 3- aminopropyl triethsilane coated slides and dried for 24 h at 37°C (Baravalle et al.,2006). The sections on the slides were deparaffinised with xylene and hydrated in a descending series of alcohol. They were then stained with Mayer's haematoxylin and eosin dyes, dried and mounted on a light microscope (X100 and X200) for histopathological examination.

Statistical analysis

The data was analyzed using the software, Excel and Graph Pad. Quantitative data were presented as mean ± standard deviation (SD) on graphs and tables. One-way Analysis of Variance (ANOVA) was used to compare the means between the groups. This was accompanied by the post hoc Tukey's multiple comparison tests to determine significant differences between values. The value of $p < 0.05$ was considered statistically significant.

RESULTS

Acute oral toxicity test

In all eight female animals used for the test, no signs of toxicity or death were observed among the rats during the 14 days of the acute toxicity experimental period, after the administration of a single oral dose of 2 ml/100g of distilled water, boiled with rice husk as fuel. The average gain in body weight of the rats was 10.2 ± 2.32%, 40.75 ± 7.78% and 60.75 ± 8.78% for days 2, 8 and 14, respectively. The body weight gradually increased within the normal range of body weight gain. After 14 days of testing, all the rats were subjected to gross necropsy. The pathological studies on the liver and kidneys of the rats tested showed no significant abnormal changes in colour, size, shape and texture compared to the control. This result suggests that water boiled with rice husk as fuel was not toxic, after an acute exposure.

28-day sub-chronic oral toxicity study

Effects on the behavior, organ and body weight

The administration of various doses of water boiled with rice husk as fuel (0.5, 1 and 2 ml/100g bw) for 28 days, had no significant change ($p > 0.05$) on the body weight of either male or female rats compared to the controls (Figure 2A and 2B).No deaths or obvious clinical signs of toxicity in the rats were observed in all the groups, including the group that received the highest dose of 2 ml/100 g bw. Figures 3A and 3B show the relative organ

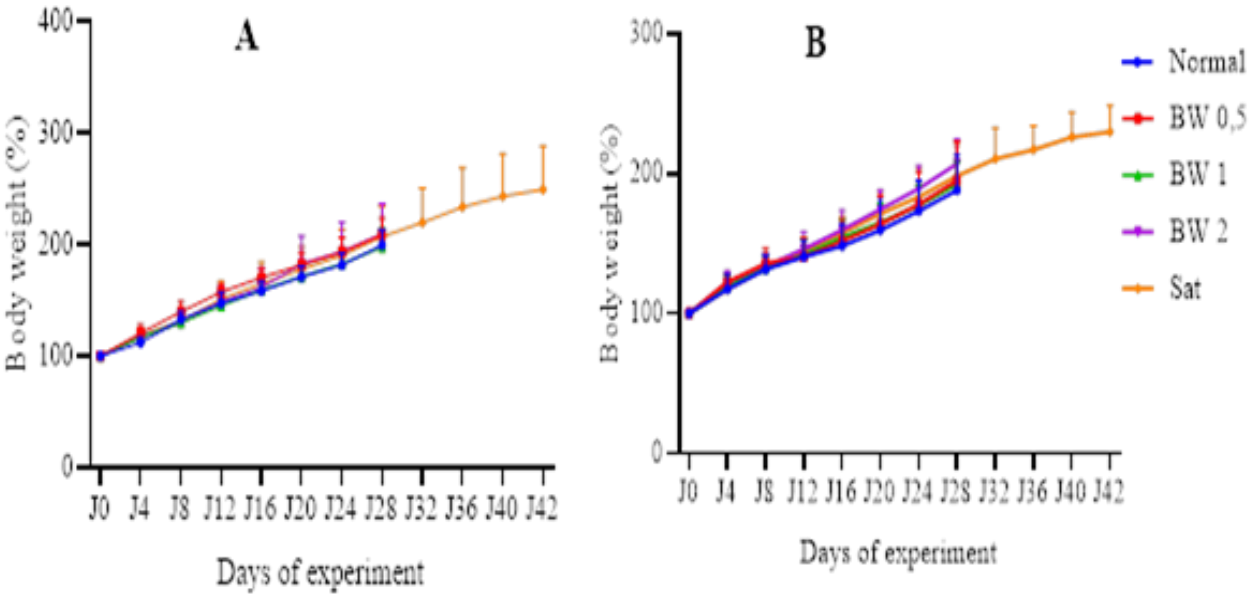

Figure 2. Effect of water boiled with rice husk on the body weight of male (A) and female (B) wistar rats. n = 4. Each value represents mean ± SD. Control: healthy rats that received distilled water; Sat: Rats that received 2 ml/100 g bw of boiled water and observed 14 days after the end of the experiment, BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/ 100 g, 1 ml/100 g and 2 ml/100 g bw respectively.

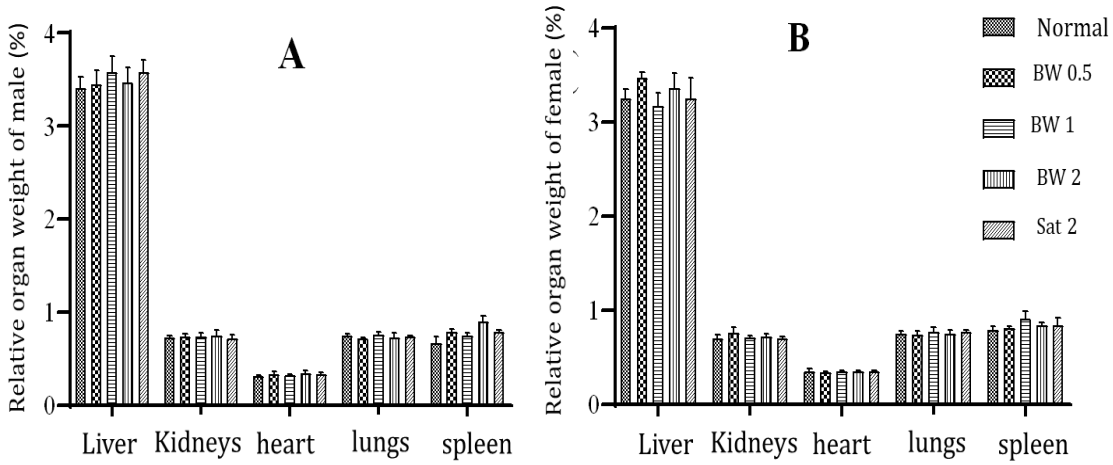

Figure 3. Relative organ weights of male (A) and female (B) rats that received different doses of the boiled water for 28 days. n = 4. Each bar represents mean ± SD. Control: Healthy rats that received distilled water; Sat: Rats that received 2 ml/100g bw of boiled water and observed 14 days after the end of the experiment, BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/100 g, 1 ml/100 g and 2 ml/100 g bw respectively.

weights of the male and female rats after 28 days of administration of the boiled water sample. The relative organ weight of the liver and kidneys, heart, lungs and spleen evaluated and calculated at necropsy in the treated groups did not show any significant difference ($p > 0.05$) from the control.

Effects on biochemical and haematological parameters

The results of the biochemical parameters are recorded on Table 1. The results for male rats showed no significant difference in some parameters (ALT, AST,

Table 1. Biochemical profile of male rats that received different doses of boiled water for 28 days.

Parameter	Group				
	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/100 g)
AST (UI/L)	134.44 ± 7.877	144.13 ± 10.24	136.37 ± 2.59	144.40 ± 5.89	134.17 ± 4.67
ALT (UI/L)	36.75 ± 2.40	43.06 ± 2.36	41.08 ± 6.04	38.07 ± 10.22	40.70 ± 2.84
Creatinine (mg/dL)	64.16 ± 2.43	65.93 ± 1.93	71.81 ± 1.09*	67.28 ± 1.72	63.89 ± 1.86
Uric acid (mg/dL)	2.44 ± 0.10	2.24 ± 0.07	2.2 ± 0.16	2.13 ± 0.10	2.11 ± 0.18
Protein (mg/dL)	2.17 ± 0.05	2.17 ± 0.05	2.28 ± 0.03	2.22 ± 0.06	2.17 ± 0.10
Bilirunin (mg/l)	0.19 ± 0.01	0.19 ± 0.03	0.23 ± 0.04	0.26 ± 0.01	0.18 ± 0.02

Table 2. Biochemical profile of female rats that received different doses of boiled water for 28 days.

Parameter	Group				
	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/100 g)
AST (UI/L)	171.10 ± 4.95	154.85 ± 9.05	147.08 ± 7.68	143.64 ± 11.00	160.98 ± 2.65
ALT (UI/L)	52.77 ± 4.43	50.35 ± 7.95	42.82 ± 2.14	44.51 ± 3.26	48.17 ± 3.77
Creatinine (mg/dL)	67.55 ± 0.39	72.01 ± 2.86	65.59 ± 4.43	69.11 ± 3.65	66.01 ± 1.24
Uric acid (mg/dL)	2.25 ± 0.19	2.45 ± 0.09	2.29 ± 0.17	2.75 ± 0.13 *	2.27 ± 0.15
Protein (mg/dL)	2.23 ± 0.03	2.22 ± 0.12	2.06 ± 0.05	2.23 ± 0.07	2.17 ± 0.06
Bilirubin (mg/l)	0.19 ± 0.01	0.19 ± 0.03	0.15 ± 0.03	0.08 ± 0.02*	0.10 ± 0.04*

Values are expressed as mean ± SD; * Significantly different from the control group ($p < 0.05$). AST: Aspartate Amino transferase, ALT: Alanine amino transferase, n = 4. Control: healthy rats given distilled water; Sat: Rats that received 2 ml/100g of boiled water and observed 14 days after the end of the experiment, BW 0.5, BW1 and BW 2 represent groups of rats that received boiled water doses at doses of 0.5, 1 and 2 ml/100 g bw respectively.

total protein, urea and total bilirubin) at all treatment doses except creatinine which significantly ($P<0.05$) increased at the dose of 1 ml/ 100g compared to the control.

In the female rats, uric acid and total bilirubin recorded significant differences, at the dose of 2 ml/100g administered. The analyses showed significant increase ($P<0.05$) in uric acid while total bilirubin significantly decreased, with more decrease14 days after the end of the experiment compared to the control group.

The effect of the boiled water sample on the haematological indices of the rats was examined at the end of the experiment (Table 2a and b). Analysis of variances showed no significant difference in most of the parameters except of platelets ($P<0.05$) compared to the control in the male rats. However, in the female rats, there was no significant difference on several parameters such as red blood cell counts, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell counts, lymphocytes, and platelet. On the other hand, for monocytes the analyses showed a significant increase at the 2 ml/100 g dose ($P<0.05$). The number of granulocytes also increased significantly ($P<0.05$) at all doses, when compared to the control.

Histopathology study

Figures 4 and 5 showed the histopathology profile of male and female rats respectively after 28 days of administration of the water sample. Light microscopic examination of sections of the kidney, liver, and heart of rats from the control group and those given a low dose (0.5 ml/100g bw), medium dose (1 ml/100g bw) and high dose (2 ml/100g bw), showed a normal histology.

DISCUSSION

As an initial step, an oral acute toxicity study was directed, it was seen that independent of the sex and the treatment, an expansion in weight of a similar sufficiency was seen with no measurable contrasts ($p<0.05$) between the experimental groups. Weight increment means that development. The sensible homogeneous expanding pattern of body and organ weight in all the rodent's gatherings can be taken as a sign of the low effect of various medicines on creature taking care of and wellbeing. Njayou et al. (2010) saw that body weight may increment or decline with connection to sex, non-attendance of toxicity or instigated anorexia. The

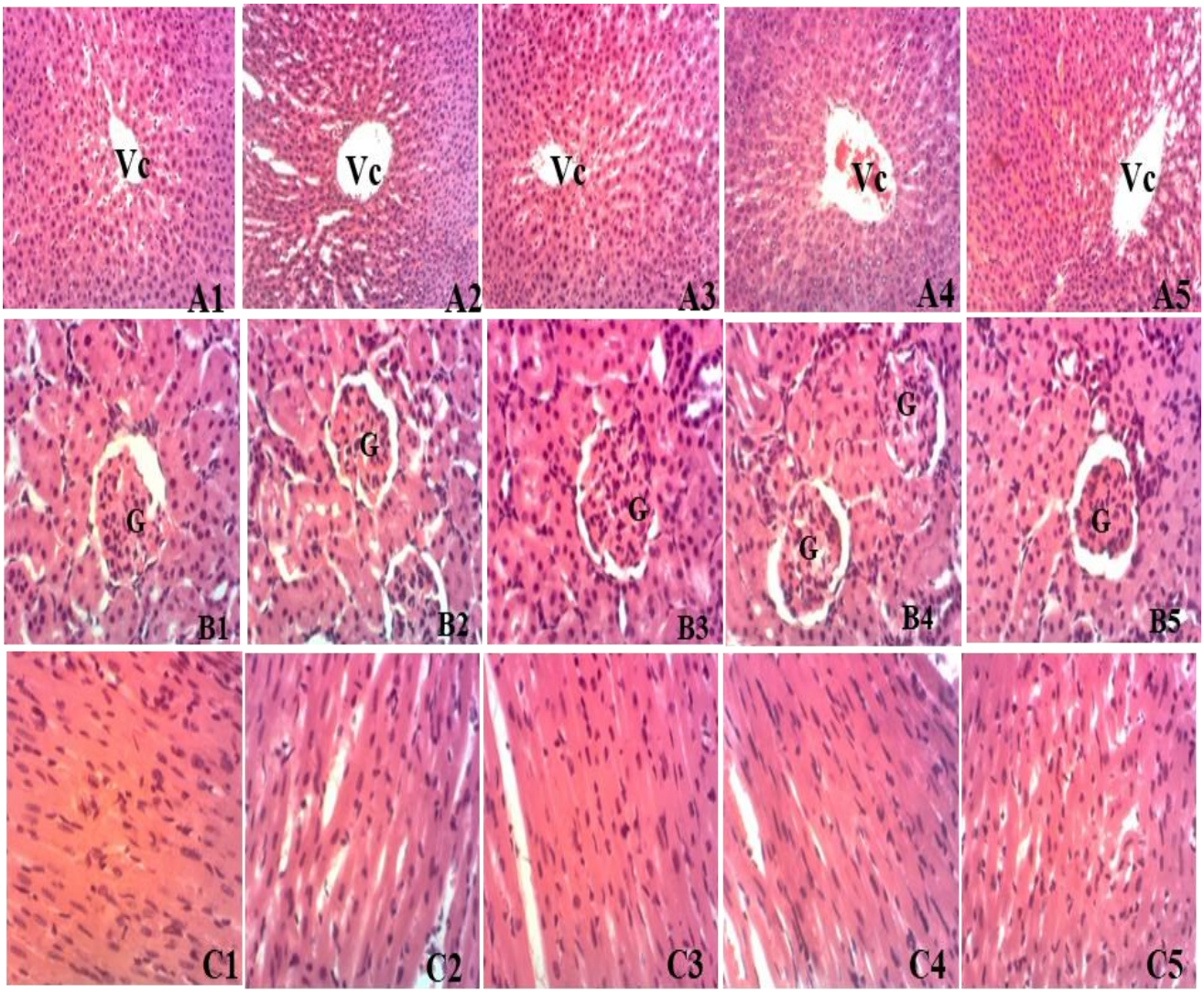

Figure 4. Histopathological examination of organs of male rats in a sub'chronic oraltoxicity study. (A–C) the liver, kidney, and heart respectively; and (1–5) the control, the low, middle, high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein.

outcomes show that there was no mortality or irregular conduct or indications of toxicity after the organization of the most noteworthy portion (2 ml/100 g BW) for as long as 14 days. This shows, as indicated by the naming and grouping of acute foundational toxicity prescribed by the OECD, the deadly portion might be over this portion limit, known as Class 5 status (OECD, 2001). Further examination was directed to assess the sub-chronic toxicity of rice husk bubbled water for 28 days in rodents.

Substances directed in chronic sickness conditions may require a toxicological assessment of rehashed dosages (sub-chronic toxicity study), since everyday use may bring about aggregation in the body with slow consequences for tissues and organs (Abotsi et al., 2011; Bariweni et al., 2018). Twenty-eight (28) days of oral toxicity study of water overflowed with rice husks, at portions of 0.5, 1 and 2 ml/100 g BW, did not give any antagonistic clinical indications or negative effects on conduct and mortality in the experimental groups. Changes in feed and water admission and body weight gain have been utilized as a marker of the general wellbeing status of exploratory creatures (El Hilaly et al., 2004). Feed utilization is directed through a few complex natural instruments that can guarantee generally consistent body weight over extensive stretches of time (Kuriyan et al., 2007). No critical distinction in body weight addition of the male and female rodents contrasted with the control was recorded. Once more, in toxicity contemplates, changes in the heaviness of organs are delicate markers of toxicity, consequences for compounds, physiologic unsettling influences and target organ injury (Michael et al., 2007). An expansion in organ weight recommends the event of hypertrophy while an abatement proposes corruption in the objective organ

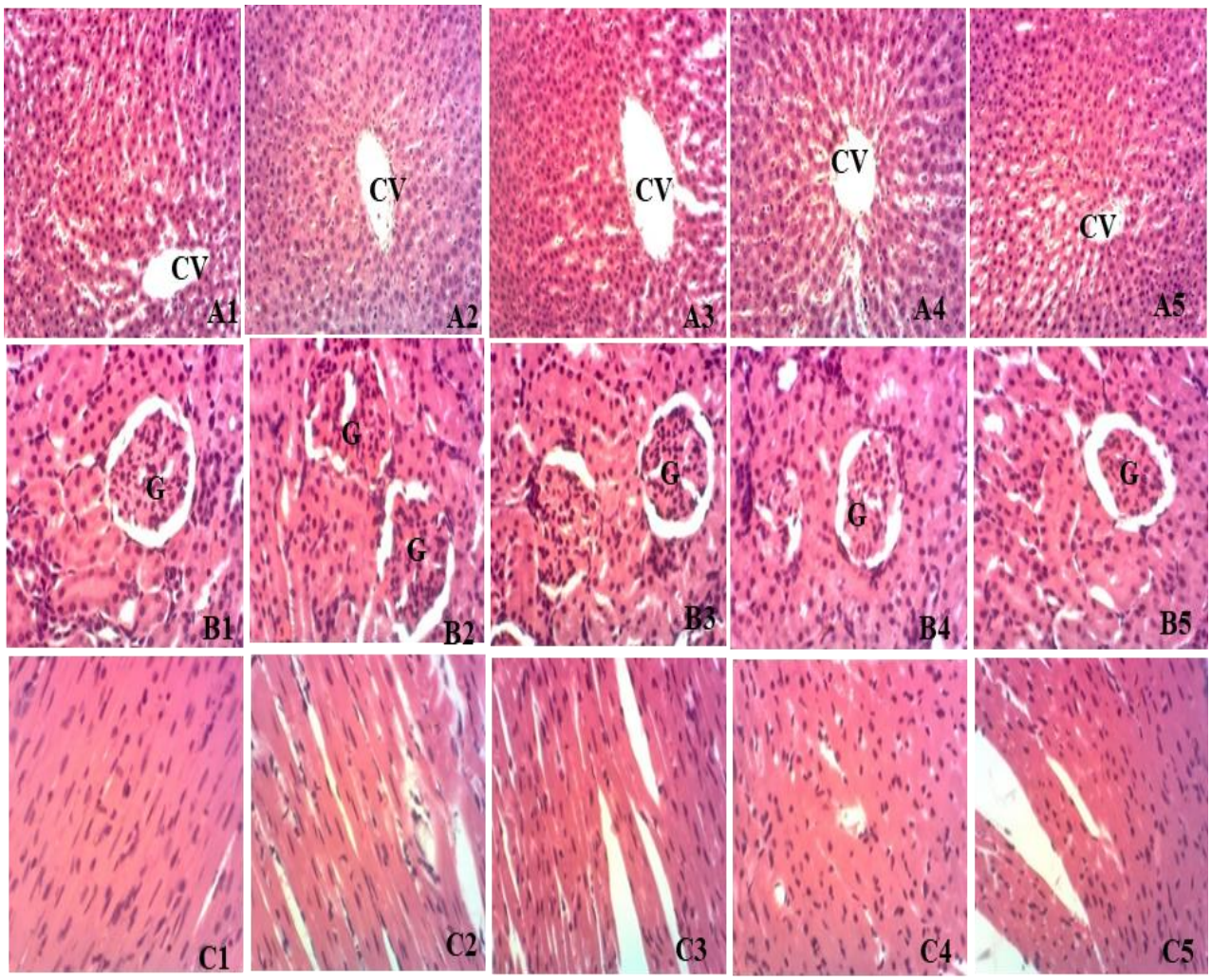

Figure 5. Histopathological examination of organs of female rats in a sub-chronic oral toxicity study: (A–C) the liver, kidney, and heart respectively; and (1–5) the control, the low, middle high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein

(Teo et al., 2002). Right now, relative organ weight of the liver and kidneys, heart, lungs, and spleen assessed in the experimental groups did not show a critical contrast in both genders at all dosages contrasted with the Control.

The study of biochemical parameters are pointers of toxicity, raising the adequacy of the establishment of toxicity on imperative organs. Right now, as creatinine in male, all-out bilirubin, and uric corrosive in female rodents indicated critical contrasts between the experimental groups and controls. The portion of 1 ml/100g in guys altogether expanded creatinine and at 2 ml/100g bw directed in females, uric corrosive was additionally essentially expanded ($P<0.05$) yet all-out bilirubin fundamentally diminished. Creatinine is a discharge result of muscle movement, which circles in the blood. Its end is solely renal, so there is a relationship between's creatinine levels and renal capacity. Most creatinine that is disposed of by the kidneys is unreservedly separated in renal glomeruli, and a little part is sifted by the rounded segment, which is a decent pointer of renal-glomerular capacity (Bohinski, 1991; Raju et al., 2016; Ghorbel et al., 2016; Belhadj et al., 2018). The lessening of these parameters would show the hepatoprotective activity of the bubbled water test at this portion. Uric corrosive is the final result of nucleic corrosive digestion (Wallace et al., 2004). It is shaped by the liver and chiefly discharged by the kidneys (65-75%) and digestive organs (25-35%) (Álvarez and Macarron., 2010). In the present study, blood uric corrosive was high in female rodents (2 ml/100g), proposing kidney harm (Raju et al., 2015, 2016; Belhadj et al., 2018). The working of the liver was evaluated by the serum all-out protein, bilirubin and egg whites. Expansion in these parameters is generally observed in destructive conditions or following a high protein diet (Tietz et al., 1994). Our study demonstrated a noteworthy decline in

Table 3. Hematological profile of male rats given different doses of boiled water for 28 days.

Parameter	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/100 g)
Red blood cell (10^6/µL)	4.58±0.28	4.66±0.19	4.23±0.42	5.01±0.37	4.57±0.24
Haemoglobin (g/dL)	15.50±0.34	15.53±0.48	15.77±0.34	13.70±0.82	14.67±0.54
Hematocrit (%)	49,63±1,33	41.30±0.73	49.77±1.56	43.07±1.01	47.03±0.48
MCV (fL)	88.33±0.68	86.67±0.68	88.33±0.26	85.33±2.46	86.00±1.55
MCH (pg)	31.10±1.70	34.83±0.79	32.13±1.01	27.30±1.22	32.17±0.92
MCHC (g/dL)	31.87±0.16	33.93±0.61	33.70±1.28	32.70±0.95	32.90±0.99
White blood cell (10^3/µL)	7.97±0.14	7.93±0.25	8.30±0.35	8.83±0.96	7.93±0.29
Lymphocytes (10^3/µL)	2.63±0.36	3.73±0.52	3.57±0.29	4.77±1.12	4,00±0.65
Monocytes (10^3/µL)	0.25±0.05	0.37±0.20	0.30±0.18	0.27±1.31	0.37±0.05
Granulocytes (10^3/µL)	3.20±0.08	3.47±0.21	4.37±0.72	4.23±0.36	3.30±0.97
Platelets (10^3/µL)	356.0±19.3	360.7±4.5	362.3±29.4	426.3±3,2*	368.0±5,5

Table 4. Hematological profile of female rats given different doses of boiled water for 28 days.

Parameter	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/ 100 g)
Red blood cell (10^6/µL)	4.48±0.21	4.56±0.39	4.40±0.26	4.25±0.12	4.15±0.11
Haemoglobin (g/dL)	14.80±0.12	15.10±0.28	13.67±0.39	14.17±0.54	12.57±0.38
Hematocrit (%)	42.87±1.27	43.47±3.45	41.63±0.72	45.70±1.20	40.30±0.13
MCV (fL)	86.00±0.89	86.00±1,18	87.00±1.34	88.00±2.37	87.67±0.68
MCH (pg)	30.07±0.52	31.33±0.52	33.03±3,07	32.00±1.72	29.90±0.45
MCHC (g/dL)	31.83±0.68	32.40±1.95	32.23±1.19	31.10±0.85	31.90±0.18
White blood cell (10^3/µL)	7.80±0.20	7.77±0.23	7.63±0.09	8.33±0.35	7.95±0.31
Lymphocytes (10^3/µL)	2.07±0.49	2.87±0.42	3.13±0.63	2.97±0.23	2,50±0,24
Monocytes(10^3/µL)	0.37±0.14	0.32±0.08	0.30±0.11	0.49±0.31*	0.39±0.09
Granulocytes (10^3/µL)	1.80±0.29	2.70±0.76 *	2.53±0.07 *	2.47±0.30*	1.93±0.11
Platelets (10^3/µL)	393.7±22.76	455.7±21.99	341.3±3.36	436.0±24.19	399.3±56.66

Values are expressed as mean ± SD; * and ** = significantly different from the Control group ($p < 0.05$ and $p < 0.001$ respectively). MCV: Mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cell, n = 4. Control : healthy rats given distilled water; Sat: Rats that received 2 ml/100 g of boiled water and observed 14 days after the end of the experiment, BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5, 1 and 2 ml/100 g bw respectively.

complete bilirubin in the female rodents at the portion of 2 ml/100g recommending the lethal impact of the bubbled water on the liver of the creatures. The complete protein serum level did not contrast altogether from the benchmark group. This shows the impact on the liver could be a gentle harmful impact influencing just the female rodents. For the most part, it created the impression that the water influenced the female at a lower portion rather than the guys, which were not influenced.

Hematopoiesis is the procedure of platelet arrangement. Analysis of the hematological parameters is significant in evaluating the poisonous impacts of test substances, just as in deciding the physiological and obsessive status of the body, as varieties in these parameters may show toxicity related with the test substances and different illnesses and conditions, including frailty, leukemia, responses to aggravation and diseases (Olson et al., 2000; Martini et al., 2012). There was no noteworthy contrast in a few parameters, for example, red platelet checks, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin focus, white platelet tallies, lymphocytes, and platelet between the treated gatherings and the benchmark group, demonstrating that the bubbled water had no impact on the circling platelets of the tried creatures (Tables 3 and 4). For monocytes, the examinations indicated a huge increment at the portion of 2 ml/100 g, the number of granulocytes likewise essentially expanded ($P<0.05$) at all the dosages when contrasted with the control in females. Notwithstanding, these distinctions acquired at the moment did not show a hematological change since they are inside the ordinary scope of these parameters for good wellbeing (Giknis and Clifford, 2008).

These distinctions got between the tried creatures and

the control could be clarified by the nearness of suspended issues; for example, the rice husk debris (RHA) right now. This RHA is found in the bubbled water through the ventilation produced by the fan. As indicated by Xu et al. (2012), debris has the most elevated extent of silica content among all plant buildups. The normal organization of very much consumed RHA is 90% undefined silica. Our discoveries are in accordance with those of Wai et al. (2017) who explored the *in vivo* toxicity of Silica nanoparticles (SiNPs) of 150 nm in different measurements by means of intravenous organization in mice and demonstrated that SiNPs were biocompatible and ok for *in vivo* use in mice.

The histology of the kidneys, liver, and heart in the male and female rodents did not create any lethal changes, in spite of introducing a few changes in biochemical tests, the histological study proposes the wellbeing of the rice husk bubbled water in these organs. This shows rice husk utilized as a fuel in PO150 gasifier stove is non-harmful and therefore safe for cooking food.

Conclusion

The results obtained in this work suggested that rice husk used as fuel is not toxic at all the doses studied (0.5 -1 and 2 ml/ 100 gbw) and did not produce any evident symptoms in the acute and sub-chronic oral toxicity studies in both male and female rats. The histological examination revealed no changes in the internal organs, like kidneys, liver and heart of the rats, in both the control and test groups. However, more studies are required to evaluate the safety of using rice husk in long term.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of the effects of *Azadirachta indica* leaf on haematology, lipid profile, body weight and organ-system functions of streptozotocin-induced diabetic male rats

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This study was carried out to evaluate the effects of ethanol extract of *Azadirachta indica* leaf on haematological parameters, lipid profile, body weight, organ weight and histopathological functions of streptozotocin-induced diabetic rats. Diabetes was induced by a single intraperitoneal administration of streptozotocin (50 mg/kg bw.). The haematological parameters, lipid profile and histopathological investigations were performed using standard methods. Continuous administration of ethanol extract of *A. indica* leaf for a period of four weeks significantly ($p<0.05$) increased the bodyweight of the streptozotocin-induced diabetic rats compared with the diabetic-untreated control. There was a significant ($p<0.05$) increase in the haemoglobin concentration, packed cell volume, red blood cells, platelet count and a significant ($p<0.05$) reduction in the total serum cholesterol, low-density lipoprotein, triglycerides and very-low-density lipoprotein of the groups treated with ethanol extract of *A. indica* compared with the diabetic-untreated control. The result of the histopathological studies showed regeneration of the organs for the groups treated with 400 mg/kg bw of the extract compared with the diabetic-untreated control. These results suggest that the ethanol extract of *A. indica* can be considered as an excellent remedy for diabetes and an alternative to antidiabetic drugs in reducing the complications associated with type II diabetes mellitus.

Key words: Diabetes, *Azadirachta indica*, haematological parameters, Lipid profile, Bodyweight, Histopathological functions.

INTRODUCTION

Diabetes mellitus is characterized by the presence of hyperglycaemia. This is as a result of the body's inability to produce required amounts of insulin (the hormone that regulates blood sugar) known as type 1 diabetes or to

efficiently use the insulin it produces; in other words, reduced sensitivity of the cells of the body to insulin. In this case, it is known as type 2 diabetes (WHO, 2019). It is known to be the fifth leading cause of death (Kazi, 2014). Diabetes mellitus (DM) is a silent killer that is not only assuming pandemic proportions worldwide but also poses threats to the economies of low-income countries of the world much more than their high-income counterparts. It is one of the leading causes of death worldwide. Diabetes mellitus has been known for many centuries as far back as the fifth century (Karamanou et al., 2016). It was derived from the Greek word "Diabetes" meaning "a siphon" while the "Mellitus" mean "sweet" (Piero, 2015).

Type 1 diabetes mellitus often times occur in childhood, and its onset can happen in adults. It has been estimated that about 84% of people living with type 1 diabetes mellitus are adults. Type 1 diabetes mellitus is caused by autoimmune destruction of pancreatic β cells in genetically predisposed individuals and results in severe insulin deficiency. It is usually regarded as a disease of childhood and adolescence, but its onset can happen at any age (Ziegler and Neu, 2018). Accurate diagnosis of type 1 diabetes in young individuals (less than 20 years) responsible for about 85% of diabetes mellitus cases in that population and it is responsible for less than 5% of all diabetes cases (Diaz-Valencia et al., 2015). This type of diabetes requires treatment with insulin (DeWitt and Hirsch, 2003).

Type 2 diabetes mellitus is a progressive condition in which the cells of the body become resistant to insulin action and/or gradually the pancreas loses its capacity to produce adequate insulin (American Diabetes Association, 2019). It is a disease of adulthood but affects both old and young people with females and patients aged 61-65 years mostly affected (Debrah et al., 2020). The driving factors for the production of type 2 diabetes include obesity, sedentary lifestyle, increased consumption of energy-dense diets, sugar-sweetened beverages (Yan et al., 2018). Type 2 diabetes mellitus accounts for over 90% of diabetes mellitus cases (Holman et al., 2015). Research has shown that type 2 diabetes could be prevented and managed by maintaining healthy body weight, engaging in a healthy diet, exercising daily for at least 30 min, avoiding smoking and consuming alcohol in moderation (Schellenberg et al., 2013). Type 2 diabetes mellitus (T2DM) is sometimes undiagnosed at an early stage because hyperglycemia gradually develops a year before its symptoms could be noticed (American Diabetes Association, 2019).

Apart from the two classifications of diabetes, there is gestational diabetes mellitus, which is characterized by glucose intolerance during pregnancy (Coustan, 2013). Women with gestational diabetes mellitus have an increased risk of developing T2DM when compared to normoglycaemic pregnancy (Bellamy et al., 2009). Pregnant women with gestational diabetes mellitus are always at risk of birth complications for both the mother and the baby because they can have babies that are large. Diabetes mellitus causes complications like diabetic peripheral neuropathy (nerve damage) (Said, 2007), diabetic retinopathy, most common blindness among working-age individuals (Klein et al., 2006), diabetic nephropathy (kidney disease) (Jain, 2012). Diabetes mellitus also increases the morbidity and mortality associated with cardiovascular disease (Chiha et al., 2012; Lee et al., 2000). Diabetic patients are more likely to die after myocardial infarction (Donahoe et al., 2007).

Globally, 463 million people were estimated to be living with diabetes, and the number is predicted to be 578 million by the year 2030. If the trend is continued, by the year 2045 700 million people will be living with diabetes mellitus. Over 4 million people between the ages of 20-79 years were estimated to die of diabetes-related complications (IDF Diabetes Atlas, 2019). In 2019 over one million children and adolescent have type 1 diabetes and 231.9 million of the 463 million adults living with type 2 diabetes with women most affected. About 20.4 million live births are estimated to be affected by high blood glucose in pregnancy (IDF Diabetes Atlas, 2019). Research conducted shows that diabetes prevalence is increasing in sub-Saharan Africa, with a regional prevalence of 2–3% in the mid-1990s rising to about 4.6% in 2010 (Mbanya et al., 2010).

According to the IDF Diabetes Atlas 9th Edition, 19.4 million adults (20-79 years) are living with diabetes representing a regional prevalence of 3.9%. Africa has the highest number of undiagnosed diabetes cases, with 60% of adults living with diabetes. In Nigeria, the South-South region has the highest pooled prevalence of T2DM at 8.5% followed by the North–East and South-East regions, at 4.6 and 3.7% respectively. The North-Central had the lowest pooled prevalence at 2.0%. The highest prevalence of T2DM was observed in the period 2000-2009 and 2010-2015 at 6.9 and 4.0% respectively (Adeloye et al., 2017).

Diabetes mellitus does not only affect an individual, or the society but also, the economy of a country. It has caused a regional economic loss of about 25.5 billion US$ (about $3633 per diabetic case) in Africa as of the year 2000. Insulin and other medications were responsible for the bulk amount of money spent on diabetes (Kirigia et al., 2009).

Globally, the yearly health expenditure on diabetes is estimated to be US$ 760 billion. It is predicted that

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spending will reach US$ 825 billion by 2030 and US$ 845 billion by 2045 (IDF Diabetes Atlas, 2019). The global increase of diabetes mellitus and its adverse effect on the individual, economy and the world at large has attracted the need for more research on treatment options for the disease and its complications, especially as the cost of insulin continues to rise (Robert, 2019). Medicinal Plants are vastly used in the treatment of various diseases as they exhibit essential phytochemicals that are therapeutic with lesser or no side effect and are cost-effective. *Azadirachta indica* is a member of the Meliaceae family prevalent in India, Bangladesh and Nepal. It possesses a therapeutic property in the treatment and prevention of diseases due to its rich source of antioxidant and phytochemicals. *A. indica* has both antidiabetic and antidiabetogenic effects and could be of great use in the treatment and management of diabetes mellitus, controlling blood sugar level as well as in preventing or delaying the onset of diabetes mellitus. Pre-treatment with the aqueous extract of *A. indica* at a dose of 100 mg/kg bw for fourteen days showed significant protection from alloxan-induced diabetogenic effect in rats resulting in a 39.5% reduction in blood glucose level when diabetes was induced (Ezeigwe et al., 2015).

The various components in *A. indica*, including Nimbin, Nimbidin, Nimbolide, and limonoids aid in disease treatment through modulation of different genetic pathways and other activities. Nimbolide displays anticancer activity by selective modulation of multiple cell signaling pathways that are related to inflammation, survival, growth, invasion, angiogenesis and metastasis (Bodduluru and Sistla, 2014). It is a chemotherapeutic agent for bladder cancer as it inhibits the proliferation of bladder cancer cells via Chk2-mediated (antibodies against checkpoint kinase) G2/M phase cell cycle arrest (Shin et al., 2019). A team of scientists reported that Nimbolide isolated from *A. indica* can stop pancreatic cancer from growing and spreading without harming normal, healthy cells. In their, report Nimbolide induces the excessive generation of reactive oxygen species (ROS), thereby regulating both apoptosis and autophagy in pancreatic cancer cells (Subramani et al., 2016). Quercetin and ß-sitosterol were first polyphenolic flavonoids extracted from the leave of *A. indica*, and they have antifungal and antibacterial activities (Govindachari et al., 1998). Nathan et al. (2005) reported that azadirachtin and other limonoids components of *A. indica* extracts are active on malaria vectors. Seed kernel of unripe *A. indica* reduces about 30% proportion of red blood cell infected with the malaria parasite in C57BL/6 mice. There was a high level of TNF (Tumour necrosis factor) and MMP-9 (Matrix metalloproteinase-9 Mmp9), establishing a pro-inflammatory effect of the plant (Habluetzel et al., 2019).

A. indica inhibits the growth of *Aspergillus flavus* and *Alternaria solani* (Shrivastava and Swarnkar, 2014). In a study conducted by Anjali et al. (2013), it was reported that aqueous extracts of neem inhibit the spore germination of fungi such as *C. lunata*, *H. pennisetti*, and *C. gloeosporioides*. *A. indica* also exhibit antiulcer activity. In a clinical study, the lyophilized powder of *A. indica* extract controls gastric hypersecretion to about 77%. The bark extract almost completely healed a duodenal ulcer at the dose of 30-60 mg twice daily for 10 weeks. One case of oesophagal ulcer and the gastric ulcer was healed completely when administrated at the dose of 30 mg twice daily for 6 weeks (Bandyopadhyay et al., 2004). The antidiabetic property and antioxidant potentials of ethanol extract of *A. indica* leaf in streptozotocin-induced diabetic rats have earlier been reported (Ezeigwe et al., 2020). This study was carried out to evaluate the effects of *A. indica* Leaf on haematology, lipid profile, bodyweight, organ weight and organ-system functions of streptozotocin-induced diabetic male rats. This study aims to produce a more reliable alternative treatment to type II diabetes mellitus.

MATERIALS AND METHODS

Collection and Identification of plant materials

The leaves of *A. indica* were collected from Nnamdi Azikiwe University, Awka, Anambra State. The sample was identified by a botanist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is 14.

Preparation of ethanol extract of *A. indica* leaf

The leaves were washed and air-dried at room temperature. The dried leaves were pulverized into powder using Corona manual grinding machine. Then 1 kg of the ground leaves powder of *A. indica* was soaked in 5 L of 80% ethanol for 24 h for complete extraction. The ethanol extraction was sieved using a muslin cloth and filtered using Whatman number 1(125 mm) filter paper. The filtrate was evaporated to dryness using a rotary evaporator. The extract was stoppered in a universal bottle and preserved in the refrigerator for use. The extract was solubilized with distilled water on a daily basis and administered to the experimental animals (extract-treated groups) for a period of 28 days.

Chemicals

Streptozotocin was manufactured by Sigma, Germany. All other chemicals used in this study were analytical grade.

Experimental animals

A total of 30 male albino rats of Wistar strains were bred within the animal house of Chris Experimental Animals Farm, Awka, Anambra State, Nigeria. They were maintained and housed in aluminum cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka with optimum condition and were allowed to acclimatize with the environment freely for one week before use. The animals were allowed free access to guinea growers mash pellets (Vital feed, Agro products) and water *ad libitum.* The floors of the cage were filled with saw specks of dust and cleaned daily.

Table 1. Weekly body weight (g) of the rats treated with the graded doses of ethanol leaf extract of A. indica used for antidiabetic studies expressed as mean ±SD.

Time (days)	Normal rats	Diabetic untreated control	100 mg/kg Metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
Initial weight	137.4±0.894	136.8±1.789	133.6±2.510	128.4±4.391	135.8±3.564	135.4±2.607
Day 0	141.3±2.060	113.7±3.03[a]	118.3±4.21[a]	120.0±6.72[a]	119.2±7.02[a]	125.1±2.03[a]
Day 7	145.8±2.168	115.3±7.37[a]	123.6±3.97[a]	123.5±3.10[a]	121.4±6.06[a]	128.4±1.3[ad]
Day 14	155.0±4.062	110.5±3.53[a]	122.8±1.92[a]	128.3±9.9[ad]	137.3±9.9[ad]	139.0±8.5[ad]
Day 21	164.4±4.220	120.0±4.24[a]	131.8±4.55[a]	137.3±9.9[ad]	137.3±9.9[ad]	139.0±8.5[ad]
Day 28	176.8±7.259	126.5±4.95[a]	137.8±2.49[a]	146.0±7.0[ad]	146.8±2.6[ad]	149.0±7.0[ad]

[A]Significant reduction with respect to normal control; [b]significant increase with respect to normal control; [c]significant reduction with respect to diabetic untreated control; [d]significant increase with respect to diabetic untreated control.

Animal grouping and extract administration

Thirty Albino rats of Wistar strains weighing between 120 and 150 g were randomly grouped into six (Groups A-F). Group A was not induced. In groups B to F diabetes was induced by giving an intraperitoneal injection using 50 mg/kg bodyweight of streptozotocin. Group B was diabetic but didn't receive treatment, Group C was treated with 100 mg/kg bodyweight metformin (a standard antidiabetic drug used for the treatment of diabetes), Groups D to F were treated with 100, 200 and 400 mg/kg bodyweight of the ethanol extract respectively. The treatment was carried out by oral gavage daily for a period of 28 days. At the end of the treatment period, the animals were anesthetized and blood was collected by cardiac puncture before the organs were harvested.

Determination of body and organ weight

The weight of the rats and their organs (liver, kidney, heart, lungs, pancreas and brain) were determined using a compact electronic scale (Alpha-SRS 130).

Hematological analysis

Hematological parameters that were analyzed include Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV) and Platelets. They were determined using automated hematology analyzer (Mindray-BC-28000).

Lipid profile

The lipid profile (Total Cholesterol, Triglycerides, HDL, LDL and VLDL) were determined using Randox test kits (Trinder, 1969; Tietze et al., 1990). Low-density Lipoprotein-Cholesterol (LDL-C) was calculated using a standard formula (Friedewald et al., 1972). The procedure used was according to the manufacturer's instructions.

Histopathological studies

Immediately the animals were sacrificed, the organs were eviscerated and fixed in 10% buffered formalin. The tissues were grossed and processed after 48 h of fixation. Tissue procession involved: Dehydration using graded alcohol concentration (starting with 70% alcohol, to 80, to 90 and 95% alcohol, and finally absolute alcohol). The clearing was done with xylene. Molten paraffin wax was subsequently used for infiltration and embedding. Microtomy was done, and the slides were stained using Haematoxylin and Eosin method (Titford, 2009). The slides were interpreted by a Histopathologist.

Statistical analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for Windows version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results obtained was performed by using one-way analysis of variance test to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

RESULTS

Body weight

The result of the body weights of the animals shows a significant ($p<0.05$) decrease in all the weights of the animals after the induction of diabetes with the exception of the normal control group which was not induced (Table 1). The weight of the normal rats increased gradually but consistently for the period of twenty-eight days. The weight of the groups treated with graded doses of *A. indica* leaf extract slightly increased. However, the weight of the extract-treated groups and the group treated with standard antidiabetic drug increased in the second, third and fourth week of treatment although the increase was not statistically significant ($p>0.05$) when compared with the weights before the induction of diabetes. The weight of the diabetic-untreated rats remained low in the course of treatment, although there was a slight increase on day 21 and 28. The weight of the treated groups was observed to be more than their initial weights while the weight of the diabetic-untreated rats was lower than the initial weight before the induction of diabetes (Table 1).

Organ weight

Induction of diabetes caused a significant ($p<0.05$) decrease in the weight of the pancreas. Treatment with

Table 2. Organ (pancreas, liver, right kidney, left kidney, heart, brain and lungs) weight of the rats treated with ethanol extract of *A. indica* leaf expressed as mean ± SD.

Organs	Normal rats	Untreated diabetic control	100 mg/kg Metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
Pancreas	1.316 ± 0.03	0.815 ± 0.04[a]	1.114 ± 0.05[d]	1.195 ± 0.13[d]	1.120 ± 0.16[d]	1.113 ± 0.09[d]
Liver	5.908 ± 0.02	6.830 ± 0.01[b]	5.408 ± 0.02	5.897 ± 0.04	6.587 ± 0.02	6.625 ± 0.01
Right Kidney	0.548 ± 0.03	0.810 ± 0.07[b]	0.558 ± 0.08[c]	0.670 ± 0.05[c]	0.720 ± 0.02	0.712 ± 0.05
Left Kidney	0.468 ± 0.05	0.630 ± 0.01[b]	0.522 ± 0.02	0.570 ± 0.09	0.585 ± 0.09	0.538 ± 0.03
Heart	0.672 ± 0.07	0.685 ± 0.00	0.496 ± 0.08[c]	0.543 ± 0.02[c]	0.538 ± 0.01[c]	0.500 ± 0.03[c]
Brain	1.510 ± 0.08	1.480 ± 0.09	1.510 ± 0.01	1.480 ± 0.04	1.470 ± 0.06	1.470 ± 0.04
Lungs	1.536 ± 0.08	1.680 ± 0.01	1.516 ± 0.07	1.560 ± 0.01	1.545 ± 0.09	1.505 ± 0.08

[a]Significant reduction with respect to normal control; [b]significant increase with respect to normal control; [c]significant reduction with respect to diabetic untreated control; [d]significant increase with respect to diabetic untreated control.

the graded doses of *A. indica* extracts significantly ($p<0.05$) increased the weight of the pancreas compared to the diabetic-untreated control (Table 2). Diabetes caused a significant ($p<0.05$) decrease in the weight of the liver when compared to that of the normal control group, which was not induced. Treatment with the different doses of ethanol extract of *A. indica* leaf and metformin restored the weight of the liver close to normal when compared with the nondiabetic control group. The results revealed that the right kidney weigh more than the left kidney (Table 2). Induction of diabetes significantly ($p<0.05$) increased the weight of the right kidney compared to that of nondiabetic control. Continuous treatment for twenty-eight days significantly ($p<0.05$) reduced the weight of the kidney for the groups treated with Metformin (100 mg/kg b.w.) and *A. indica* leaf extract (100 mg/kg b.w.). Induction of diabetes did not cause a marked difference in the weight of the heart. However, continuous treatment for twenty-eight days significantly, ($p<0.05$) reduced the weight of the heart in all the treatment groups. The brain and the lungs did not show a significant difference in their weight after the induction of diabetes and in the cause of treatment (Table 2).

Haematological analysis

The result of the twenty-eight day's treatment with ethanol leaf extract of *A. indica* on the haematological parameters is reported in Table 3. The ethanol extract of *A. indica* leaf triggered significant ($p<0.05$) increases in haemoglobin concentration, packed cell volume, red blood cells and platelets compared with the diabetic untreated and non-diabetic groups. This effect is dose-dependent. The white blood cells significantly ($p<0.05$) increased in the diabetic-untreated group compared with the extract-treated groups (Table 3). There was a significant ($p<0.05$) increase in haemoglobin concentration and the packed cell volume of the groups that were administered the graded doses of ethanol extract of *A. indica* leaf compared with the group that was treated with 100 mg/kg bw metformin.

There was an increase ($p<0.05$) in haemoglobin (HGB) concentration and packed cell volume (PCV) levels of rats administered *A. indica* leaf extracts compared with both normal non-diabetic and diabetic untreated rats. While diabetes appears to increase WBC, administrations of *A. indica* leaf extract appear to have normalized the WBC count in tested groups. RBC also appears to be normalized in diabetic rats treated with *A. indica* leaf extracts. Appreciable recovery in platelet counts in tested animals was observed (Table 3).

Result of the lipid profile test

The effect of treatments with ethanol leaf extract of *A. indica* on the lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and very-low-density lipoprotein) are shown in Table 4. It is apparent from table 4 that diabetes upsets the lipid profile of the experimental animals, increasing the TCH, LDL, TRIG and VLDL while reducing HDL. The administration of *A. indica* extract modulated these profiles to differing degrees. The diabetic-untreated group showed a significant ($p<0.05$) increase in the total cholesterol level compared to the normal non-diabetic group. There was a significant ($p<0.05$) decrease in serum total cholesterol level in all instances of treatment compared with the untreated diabetic rats. The modulatory effect of *A. indica* extract on total cholesterol is dose-dependent, declining with increasing concentration of extract and approximating the concentration in normal non-diabetic groups at 400 mg/kg bw. Results show a dose-dependent increase in serum HDL-Cholesterol for groups treated with ethanol extract of *A. indica* leaf. This marked increase shows a significant ($p<0.05$) difference when compared with the diabetic-untreated group. The diabetic-untreated group showed a significant ($p<0.05$) decrease in the

Table 3. The effect of treatment with different doses of ethanol extract of *A. indica* for a period of twenty-eight days on the haematological parameters expressed as mean ±SD.

Haematological parameter	Normal rats	Diabtetic untreated control	100 mg/kg Metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
WBC (x109/L)	16.30±1.302	21.75±2.475	17.74±0.7635	18.23±2.786	19.50±2.876	17.73±1.159
HGB(g/dl)	11.78±0.3962	8.200±0.990	15.18±0.795[d]	14.13±0.61[d]	14.85±0.59[d]	15.58±0.29[d]
PCV (%)	40.52±2.213	25.30±3.25[a]	45.58±2.425[d]	42.38±1.85[d]	44.55±1.79[d]	46.83±0.97[d]
RBC(x10^{12}/L)	7.652±0.1865	5.515±0.149	7.422±0.3920	6.093±0.210	6.468±0.296	6.980±0.159
Platelete (x10^{9}/L)	405.4±32.54	200.5±83.3[a]	369.4±34.2[d]	354.5±53.2[d]	391.8±81.4[d]	470.3±38.3[d]

[a]Significant reduction with respect to normal control; [b]significant increase with respect to normal control; [c]significant reduction with respect to diabetic untreated control; [d]significant increase with respect to diabetic untreated control.

Table 4. The effect of treatment with different doses of ethanol extract of *A. indica* for a period of twenty-eight days on the lipid profile expressed as mean ± SD.

Lipid profile parameter (mg/dl)	Normal rats	Diabetic untreated control	100 mg/kg Metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
TCH	70.01±5.941	93.88±6.046[b]	79.72±3.737	75.15±4.101[c]	73.00±3.393[c]	71.53±4.471[c]
HDLC	49.58±6.313	29.79±1.654[a]	40.89±5.383[d]	40.57±4.492[d]	43.41±5.817[d]	43.12±3.242[d]
LDL-C	5.386±2.685	26.12±6.300[b]	17.88±9.601	15.58±4.119	8.973±7.213[c]	9.888±6.613[c]
TRIG	83.71±3.018	189.9±9.546[b]	104.11.31[c]	94.98±4.231[c]	102.9±5.010[c]	92.60±5.319[c]
VLDL	16.74±0.601	37.97±1.909[b]	20.94±2.261[c]	19.00±0.8467[c]	20.58±1.003[c]	18.52±1.065[c]

[a]significant reduction with respect to normal control; [b]significant increase with respect to normal control; [c]significant reduction with respect to diabetic untreated control; [d]significant increase with respect to diabetic untreated control.

HDL-cholesterol compared to the normal non-diabetic group. The HDL cholesterol concentration of the extract-treated groups maintained a close level with that of the normal non-diabetic rats. A dose-dependent decrease in serum LDL-Cholesterol was observed for groups treated with ethanol extract of *A. indica* leaf. The observed decrease in the treatment groups is significant ($p<0.05$) compared with the diabetic-untreated group. The LDL-cholesterol of the diabetic-untreated group significantly ($p<0.05$) increased compared to the extract administered groups and the normal non-diabetic group. There was a significant ($p<0.05$) decrease in the serum triglyceride level of the group of rats treated with ethanol extracts of *A. indica* leaf compared with the diabetic-untreated rats. The diabetic-untreated group showed a significant ($p<0.05$) increase in the triglyceride level compared with the groups treated with the ethanol extract of *A. indica* leaf and normal non-diabetic group. There was a significant ($p<0.05$) decrease in the serum VLDL level of the group of rats treated with ethanol extract of *A. indica* leaf compared with the diabetic-untreated rats though the values remain significantly higher than the VLDL values observed in the normal non-diabetic (Table 4).

Histopathological analysis

Macroscopy of the pancreas

Normal non-diabetic rat: A lobulated yellowish tissue weighing 1.34 g and measuring 0.5 cm × 0.4 cm x 0.2 cm. Cut sections show normal lobulated appearance.

Diabetic-untreated rat: A lobulated yellowish tissue weighing 0.85 g and measuring 0.5 cm × 0.4 cm x 0.2 cm. Cut sections show normal lobulated appearance.

Diabetic-treated rat: A lobulated yellowish tissue weighing 1.12 g and measuring 0.4 cm × 0.3 cm × 0.1 cm. Cut sections show normal lobulated appearance.

Macroscopy of the Liver

Normal non-diabetic rat: Mahogany colored liver weighing 5.32 g and measuring 3 cm × 2 cm × 1.5 cm.

Diabetic-untreated rat: Enlarged yellowish liver tissue weighing 6.96 g and measuring 3.3 cm x 2.1 cm x 1.6 cm.

Cut sections show acute congestion with focal yellowish discolouration suggesting fatty change.

Diabetic-treated rat: Moderately enlarged yellowish liver tissue weighing 6.67 g and measuring 3 cm x 2 cm x 1.5 cm. Cut sections show mild congestion.

Macroscopy of the kidney

Normal non-diabetic rat: Brownish right and left kidneys weighing 0.56 and 0.45 g respectively. The kidneys measure 0.9 × 0.7 × 0.4 cm and 0.8 × 0.6 × 0.3 cm respectively. Cut sections show normal corticomedullary differentiation.

Diabetic-untreated rat: Brownish right and left kidneys weighing 0.86g and 0.64g respectively. The kidneys measure 0.9 × 0.7 × 0.4 cm and 0.8 × 0.6 × 0.3 cm respectively. Cut sections show normal corticomedullary differentiation.

Diabetic-treated rat: Brownish right and left kidneys weighing 0.72 and 0.55 g respectively. The kidneys measure 0.9 × 0.7× 0.4 cm and 0.8 × 0.6 × 0.3 cm respectively. Cut sections show normal corticomedullary differentiation.

Macroscopy of the heart

Normal non-diabetic rat: A heart tissue weighing 0.76 g and measuring 1 × 1 × 0.8 cm. Cut sections show no focal lesion, and the left and right ventricles appear normal. Wall thickness is normal.

Diabetic-untreated rat: A heart tissue weighing 0.67 g and measuring 1 × 1 × 0.8 cm. Cut sections show no focal lesion, and the left and right ventricles appear normal. Wall thickness is normal.

Diabetic-treated rat: A heart tissue weighing 0.49 g and measuring 1 × 1 × 0.8 cm. Cut sections show no focal lesion, and the left and right ventricles appear normal. Wall thickness is normal.

Macroscopy of the brain

Normal non-diabetic rat: A brain weighing 1.41 g, measuring 1 × 0.7 × 0.2 cm. The cerebral hemispheres are symmetrical. Coronal sections through the cerebral hemispheres appear normal. Radical sections through the cerebellum also appear normal. Transverse sections through the brain are also normal.

Diabetic-untreated rat: A brain weighing 1.34 g, measuring 1 × 0.7 × 0.2 cm. The cerebral hemispheres are symmetrical. Coronal sections through the cerebral hemispheres appear normal. Radical sections through the cerebellum also appear normal. Transverse sections through the brain are also normal.

Diabetic-treated rat: A brain weighing 1.44 g, measuring 1 × 0.7 × 0.2 cm. The cerebral hemispheres are symmetrical. Coronal sections through the cerebral hemispheres appear normal. Radical sections through the cerebellum also appear normal. Transverse sections through the brain are also normal.

Macroscopy of the lungs

Normal non-diabetic rat: Left and right lungs weighing 1.32 g and measuring 2.5 × 2 × 1.8 cm. Cut sections show no focal lesion. Floatation tests are negative on the lobes.

Diabetic-untreated rat: A moderately heavy lung is weighing 1.80 g and measuring 2.6 x 2 x 1.8 cm. Cut sections show patchy areas of consolidation with positive floatation test.

Diabetic-treated rat: 1.82 g and measuring 2.5 × 2 × 1.8 cm. Floatation tests are negative on the lobes.

Microscopy of the pancreas, liver, kidney, heart, brain and lung

The normal non-diabetic control shows a normal pancreas. *The diabetic-untreated control* shows features of insulitis, as evidenced by a focal aggregate of Chronic Inflammatory Cells (CIC). This can cause inflammatory infiltration of the islets of Langerhans. The infiltrate classically consists of cytotoxic T cells, macrophages, T helper cells and B cells. *The standard drug (100 mg/kg b.w. metformin) treated group* shows centroacinar cells which are inconspicuous small cells with minimal cytoplasm and oval nuclei. A large Congested Vascular Channel (CVC) is seen running through the lobular formation of the acini. *The group treated with 400 mg/kg b.w. The extract* shows nuclei with a Stippled Chromatin Pattern (SCP), and there is moderate amphophilic cytoplasm (Plate 1).

The normal non-diabetic control shows normal liver tissue. The diabetic-untreated control *shows* hypoxic injury evidenced by foci of micro and Macrovesicular Steatosis (MS). The central vein shows Vascular Congestion (VC). This is the primary cause of liver injury during transplantation. The standard drug (100 mg/kg b.w. metformin) treated group shows liver tissues with

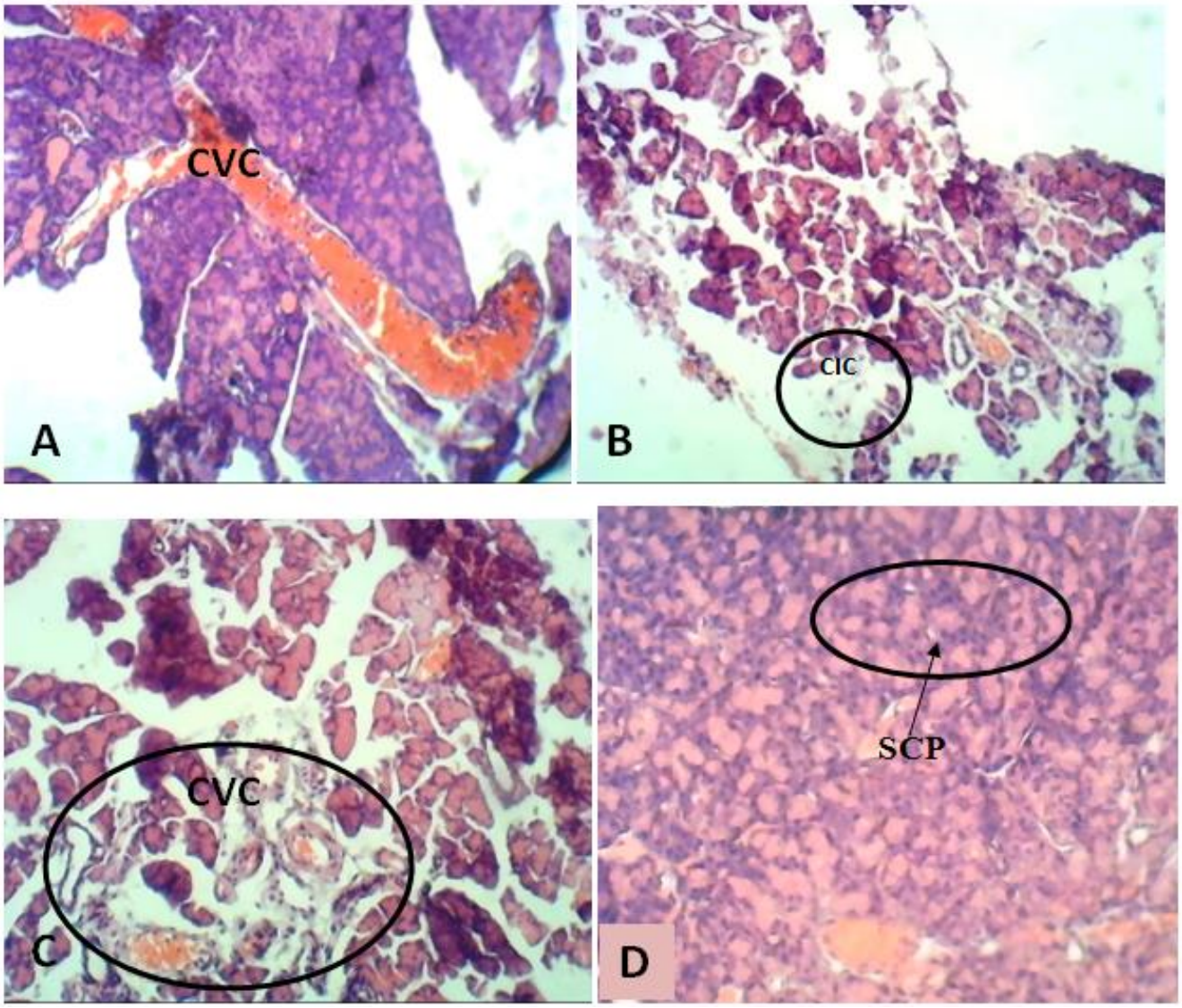

Plate 1. Light micrographs pancreas tissue **A:** (Normal Control/Non-diabetic). **B:** (Diabetic-untreated). **C:** (100 mg/kg b.w. metformin). **D:** (400 mg/kg b.w. ethanol extract of *A. indica*).

Focal Mild Steatohepatosis (FMS). The implication of this is a severe fatty liver disease if not diagnosed early and treated. The group managed with 400 mg/kg b.w. *The extract* shows liver tissue with mild Microvesicular Steatosis (FMS) portal triaditis (PT). It is often associated with characteristics of the metabolic syndrome and is considered to be the hepatic manifestation of the metabolic syndrome (Plate 2).

The normal non-diabetic control is showing normal kidney tissue. *The diabetic-untreated control* shows Focal Interstitial Nephritis (FIN) with normal glomeruli, tubules and vascular channels. Interstitial nephritis is a kidney condition characterized by swelling in between the kidney tubules. Swelling of these tubules can cause some kidney symptoms that range from mild to severe conditions. *The standard drug (100 mg/kg b.w. metformin) treated group* shows normal glomerular on light microscopy. *The group treated with 400 mg/kg b.w. The extract* shows normal kidney tissue (Plate 3).

The normal non-diabetic control shows a normal heart. *The diabetic-untreated control* shows pulmonary trunk displaying Fat Embolism (FE) that is partly attached to the vascular wall. Fat embolism obstructs the pulmonary blood flow completely. It can also cause endothelial damage and respiratory failure. *The standard drug (100 mg/kg b.w. metformin) treated group* shows the myocardium of the heart, which appears normal. *The group treated with 400 mg/kg b.w. The extract* shows a myocardium of the heart, which appears like normal issue (Plate 4).

The normal non-diabetic control shows a normal cerebellum. *The diabetic-untreated control* shows evidence

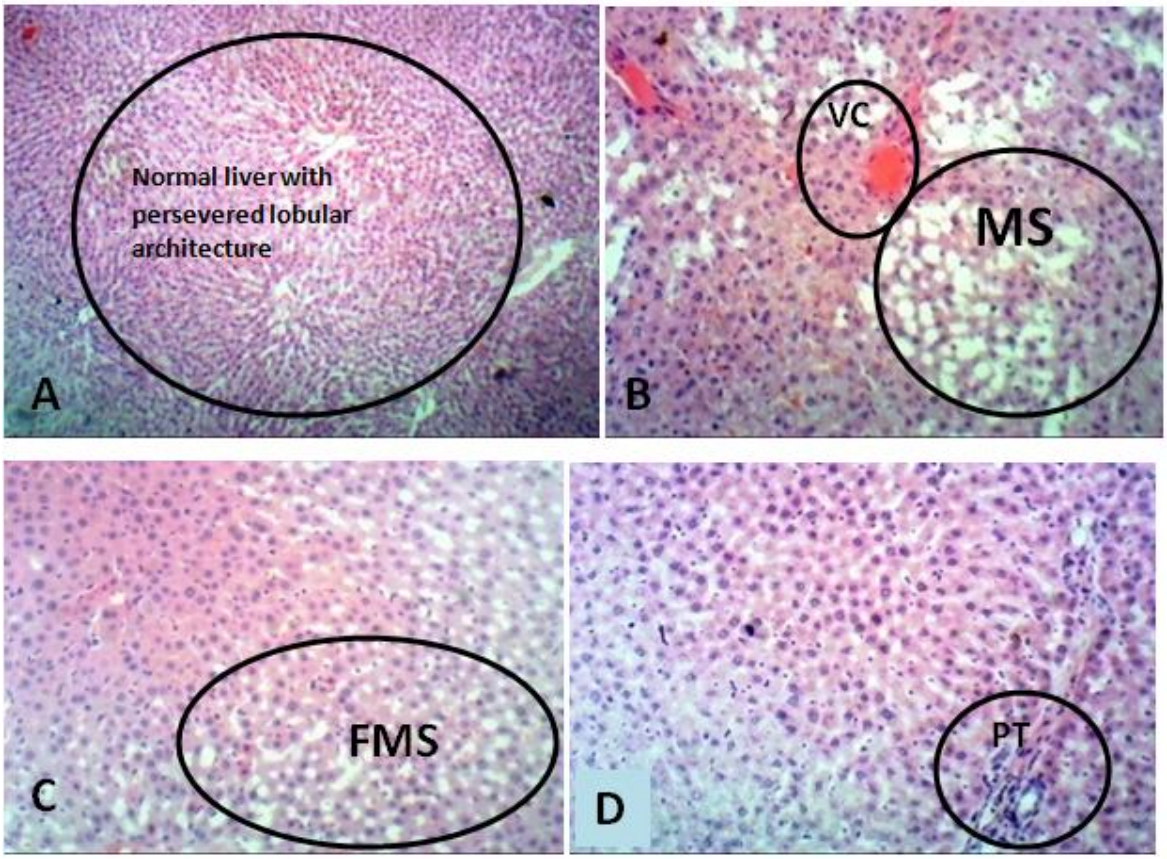

Plate 2. Light micrographs of Liver tissue **A:** (Normal Control/Non-diabetic). **B:** (Diabetic-untreated). **C:** (100 mg/kg b.w. metformin). **D:** (400 mg/kg b.w. ethanol extract of *A. indica*).

of Cerebral Hypoxia (CH) and acute ischaemic injury, which causes increased eosinophilia of neuron with *treated group* shows normal brain with normal villi lining the third ventricle. *The group treated with 400 mg/kg b.w. the extract* shows a normal brain (Plate 5).

The normal non-diabetic control *shows a normal lung.* The diabetic-untreated control *shows* lung having features of Interstitial Pneumonia (IP) with intense infiltration of the stroma by lymphoid cells, forming follicles with germinal centres. *The standard drug (100 mg/kg b.w. metformin) treated group* shows lung with evidence of a diffuse Interstitial Pneumonia (IP), causing damage to the interstitium. The interstitium provides support to the lungs microscopic air sacs (alveoli). *The group treated with 400 mg/kg b.w. extract* shows a normal lung (Plate 6).

DISCUSSION

The result of the body weights of the animals shows a significant ($p<0.05$) decrease in the body weights after the induction of diabetes except for the normal control group, which was not induced. The study is in agreement evidence of shrinkage, creating vacuoles around the neurons. *The standard drug (100 mg/kg b.w. metformin)* with Alese et al. (2013), Daye et al. (2013) and Zafar and Naqvi, 2010) that observed reduction in body weight of rats after administration of streptozotocin (STZ). STZ-induced Diabetes goes along with weight loss (Akbarzadeh et al., 2007). STZ-induced loss of body weight is as a result of its alkylation of DNA (Zafar and Naqvi, 2010). The weight of the groups treated with graded doses of *A. indica* leaf extract slightly increased. This is consistent with the study of Das et al. (2010), which observed an increase in the weight of the diabetic rat at 250 mg/kg body weight of *A. indica* extract. Gupta et al. (2017) stated that *A. indica* extract prevents weight loss. The maintenance of high blood glucose level requires a regular breakdown of structural proteins of the body which affect the body weight, resulting in weight loss in the diabetic rat (Irfan et al., 2016).

The white blood cells significantly ($p<0.05$) increased in the diabetic-untreated group compared with the extract-treated groups and the non-diabetic control. It is apparent from the results that the ethanol extract of *A. indica* leaf triggered significant ($p<0.05$) increases in haemoglobin concentration, packed cell volume, red blood cells and

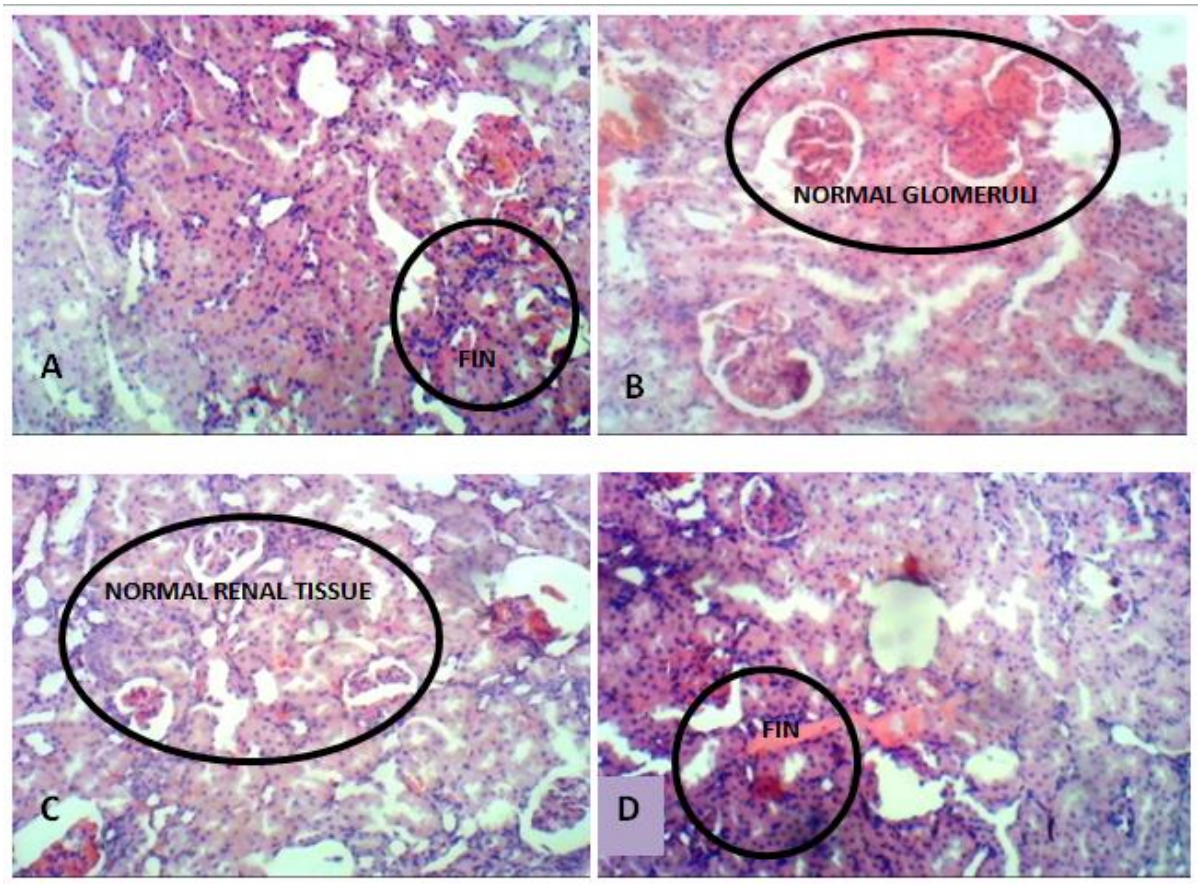

Plate 3. Light micrographs of Kidney tissue **A:** (Normal Control/Non-diabetic). **B:** (Diabetic-untreated). **C:** (100 mg/kg b.w. metformin). **D:** (400 mg/kg b.w. ethanol extract of *A. indica*).

platelets compared with the diabetic untreated and non-diabetic groups. This result is in agreement with Iyare and Obaji (2014), stating that *A. indica* is a hematopoietic agent with the potential of improving anaemia during pregnancy. The rise in the blood parameters could be traced to its components (flavonoids and quercetin) that have hematopoietic properties (Raja et al., 2011). It has also been reported to increase the body's macrophage response, which stimulates the lymphatic system and also increase the production of WBCs (Ray et al., 1996; Sen et al., 1992).

Diabetes upsets the lipid profile of the experimental animals, increasing the TCH, LDL, TRIG and VLDL while reducing HDL. The reports of Ebaid et al. (2019) and Erukainure et al. (2013) were in agreement with the present result. Insulin resistance and insulin deficiency observed in type 2 diabetic patients are likely to contribute to these lipid changes, as insulin functions in regulating lipid metabolism, a significant factor for the risk of cardiovascular diseases (Bruno, 2015). Several studies have established the fact that increased hyperlipidaemia increases lipid peroxidation and reduces the hepatic antioxidant defence mechanism in rats fed with high cholesterol diet for 30 days (Oh et al., 2006; Kumar et al., 2007).

There was a significant ($p<0.05$) decrease in the serum triglyceride, LDL, and VLDL level of the group of rats treated with ethanol extracts of *A. indica* leaf compared with the diabetic-untreated rats. In a research study conducted by Adekunle et al. (2016) in other to determine the hypoglycemic, antihyperglycemic, antihyperlipidemic and antioxidative properties of 2 different doses of *A. indica* (100 and 200 mg/kg) in comparison with glibenclamide (a reference drug), the researchers reported a significantly ($p<0.05$) reduction in total cholesterol, triglyceride and LDL-cholesterol concentrations when compared with corresponding values in the untreated diabetic groups after 21 days. The HDL cholesterol concentration of the extract-treated groups maintained a close level with that of the normal non-diabetic rats. The increased HDL-cholesterol facilitates the transport of triglyceride cholesterol from serum to liver through reverse cholesterol transport, where it is catabolized and excreted. HDL-cholesterol transports cholesterol from peripheral tissues to the liver for catabolism, causing a significant reduction in TCHOL,

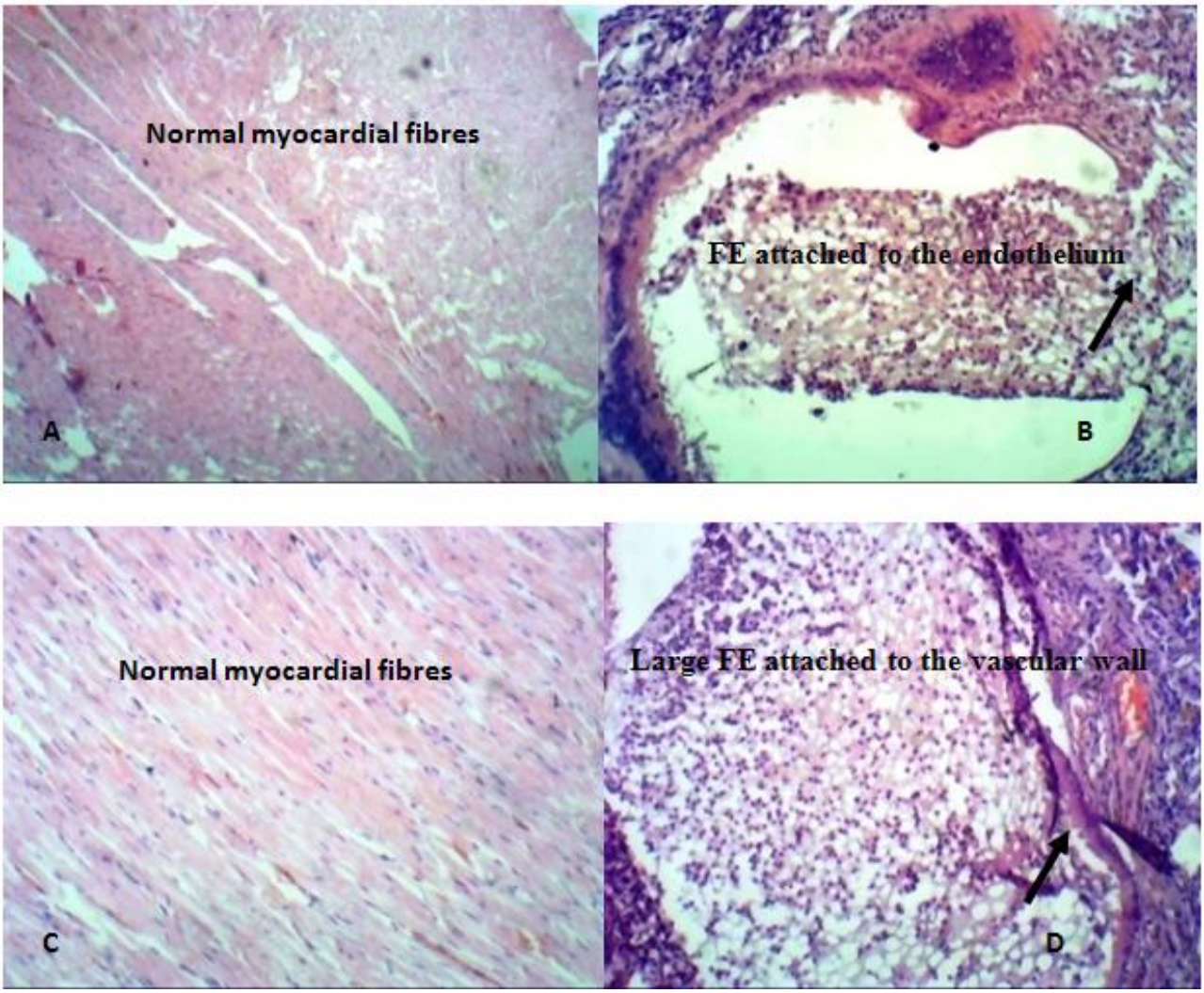

Plate 4. Light micrographs of Heart and pulmonary vessel **A,** (Normal Control). **B,** (Diabetic-untreated/Non-diabetic). **C,** (100 mg/kg b.w. metformin). **D,** (400 mg/kg b.w. ethanol extract of *A. indica*).

TRIG, and VLDL-cholesterol, (Srinivasan-Rao and Saileela, 2013). It also exacts antioxidative and anti-inflammatory capacities (Femlak et al., 2017).

Induction of diabetes caused a significant ($p<0.05$) decrease in the weight of the pancreas. The diabetogenic action of STZ led to the irreversible destruction of the pancreatic beta cells resulting in degranulation and loss of its ability to secrete insulin leading to the loss of weight of pancreases (Kim et al., 2006; Heidari et al.; 2008). The untreated diabetic group revealed a breakdown of micro-anatomical features including necrotic changes, β-cell degranulation and severe vacuolation in the islet when viewed under the microscope. The islet cells show an irregular shape. This is in line with the observations of Alese et al. (2013). STZ-induced diabetic mice show severe damage to the pancreas, liver and kidney as well as glomerular proliferation, cell necrosis and hypochromatosis (Zhang et al., 2017).

Treatment with the graded doses of *A. indica* extracts significantly ($p<0.05$) increased the weight of the pancreas compared with the diabetic-untreated control. The increase in the weight of the pancreatic tissue could be as a result of the antioxidative properties of the extract. Oxidative stress occurs when free radicals act on biological molecules causing damage to the cell by pulling an electron from the molecule destabilizing the molecule and turning it into a free radical. An excessive amount of free radicals causes oxidative damage on proteins, lipids and nucleic acid. It is the primary source of inflammation and is associated with diseases like cancer, atherosclerosis, myocardial infarction and many others (Katerji et al., 2019). According to Biney et al. (2020), *A. indica* contains phenol a principal phytochemical constituent that exhibits a significant role in reducing free radicals found in the body. This phytochemical is responsible for the increase in antioxidant

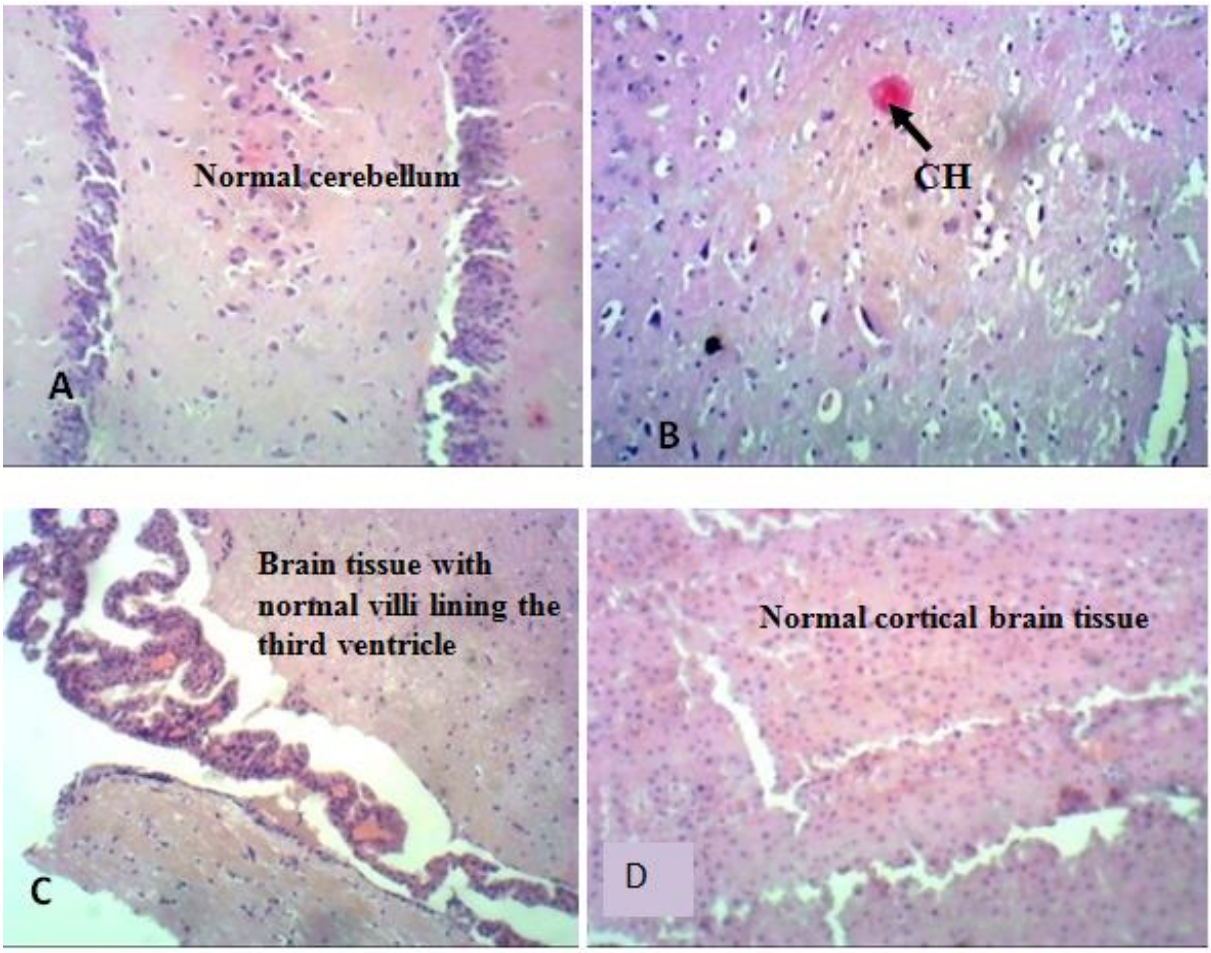

Plate 5. Light micrographs of brain tissue **A:** (Normal Control). **B:** (Diabetic-untreated/Non-diabetic). **C:** (100 mg/kg b.w. metformin). **D:** (400 mg/kg b.w. ethanol extract of *A. indica*).

enzyme activity in experimental animals (Ezeigwe et al., 2020). This explains the increase in weight of the pancreas. *A. indica* reduces the free radicals in the body thereby reducing the rate at which proteins and lipids are broken down, which in turn causes an increase in weight of the pancreas.

Diabetes caused a significant ($p<0.05$) decrease in the weights of the liver and kidney when compared with the normal control group, which was not induced. Treatment with 400 mg/kg bodyweight of the ethanol extract of *A. indica* leaf and standard antidiabetic drug restored the weight of the liver close to normal when compared to the normal control group. This is in line with the researches carried out by Shailey and Basir (2012). In the report, it stated that both *A. indica* leaf extract and *A. indica* bark extract increased the weight of the kidney of diabetic rats.

Histological investigation of the organs of the normal non-diabetic rats, untreated diabetic rats, rats treated with 100 mg/kg bw. metformin and the rats treated with 400 mg/kgb.w ethanol extracts of *A. indica* leaf respectively revealed important changes in the photomicrograph of the organs. The pancreas of untreated diabetic rats showed evidence of insulitis as evidenced by focal aggregate of chronic inflammatory cells. This can cause inflammatory infiltration of the islets of Langerhans if not treated. The groups treated with the extract did not show any sign of insulitis revealing that the extract may be responsible for restoring the pancreas to normal. The liver of the untreated diabetic rats showed evidence of hypoxic injury with focal micro and macro-vascular steatosis with associated mild steato hepatitis. The implication of this is serious fatty liver disease if not diagnosed early and treated. The liver of the extract treated groups appeared normal with mild microvesicular steatosis. The kidney of the untreated diabetic rats showed focal interstitial nephritis. Interstitial nephritis is characterized by swelling in between the kidney tubules. Swelling of these tubules can cause a number of kidney symptoms that range from mild to severe. The photomicrographs of the diabetic rats treated with the extract showed normal kidney tissue.

The heart of the untreated diabetic rats showed fat

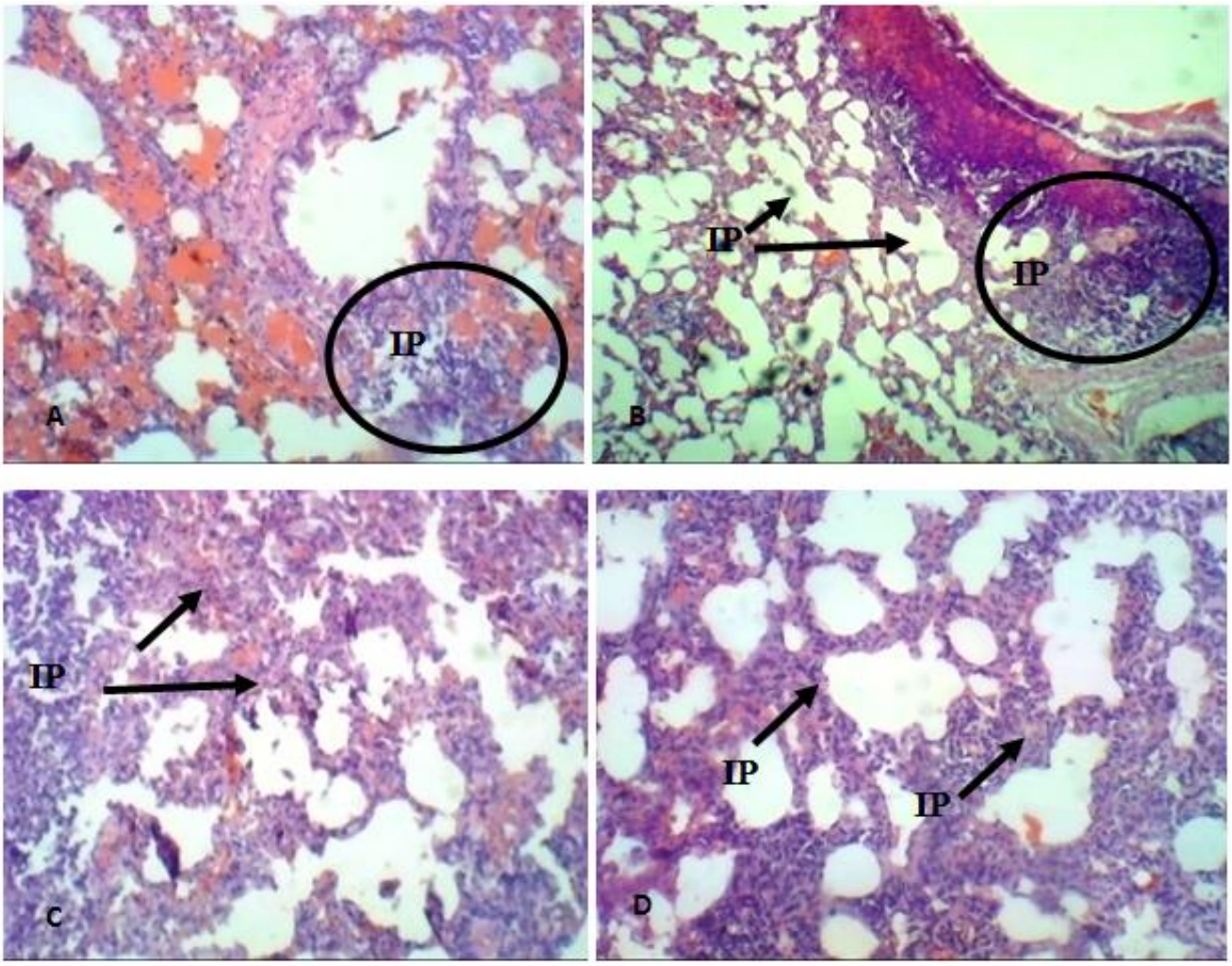

Plate 6. Light micrographs of Lung tissue **A:** (Normal Control). **B:** (Diabetic-untreated/Non-diabetic). **C:** (100 mg/kg b.w. metformin). **D:** (400 mg/kg b.w. ethanol extract of *A. indica*).

embolism that is partly attached to the vascular wall. Fat embolism causes obstruction of the capillary blood flow completely with associated endothelial damage and respiratory failure. The photomicrographs of the diabetic rats treated with the extracts did not show any evidence of fat embolism. The brain of the untreated diabetic rats had cerebral hypoxic, acute ischaemic injury, increased eosinophilia of neurone with evidence of shrinkage, creating vacuoles around the neurons. The brain of the diabetic rats treated with the extracts appeared normal. The lung of the untreated diabetic rats had evidence of interstitial pneumonia with intense infiltration of the stroma by lymphoid cells, forming follicles with germinal centers. These abnormalities were observed to be mild in the photomicrographs of the lung of diabetic rats treated with the extracts. These observations made from the histological studies of the organs of the diabetic untreated rats could be responsible for the high mortality rate of the rats in the diabetic untreated group compared with the group of rats that were treated with extracts and a reference drug in the course of the experiment. The ethanol extract of *A. indica* leaf ameliorated some of these observations made in the organs of the diabetic untreated rats. The rats treated with the A. indica leaf extract showed evidence of recovery from the abnormalities created by the induction of diabetes.

Conclusion

Some of the major complications of diabetes mellitus are perturbations in haematological parameters, aberrant lipid profile, weight loss, and organ damage. The result of this study shows that *A. indica* in addition to having hypoglycemic effects; it also protects against the adverse effects of diabetes mellitus and thus can be used as a remedy for the treatment and management of diabetes mellitus and its complications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Ethical approval

The study was carried out in strict compliance with the recommendations in the guide for the Institutional Animal Care and Use Committee (IACUC) of Nnamdi Azikiwe University, Awka, Nigeria in line with the detailed protocols of Animal Care and Use in Research, Education and Testing (ACURET).

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