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EFFECTS OF PASSIFLORA FOETIDA ON FERTILITY AND SOME BIOCHEMICAL PARAMETERS IN SULFASALAZINE INDUCED REPRODUCTIVE TOXICITY AMONGST MALE ALBINO RATS

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ABSTRACT

Sulfasalazine (SASP) is a drug commonly used for the treatment of inflammatory bowel diseases (IBDs) such as Ulcerative colitis and crohn's disease, it's endorsed with changes in fertility and biochemical parameters in experimental animals. This study was designed to investigate the effect of *passiflorafoetida* on fertility and biochemical parameters on sulfasalazine inducedreproductive toxicity in male albino rats. Twenty-four male albino rats were divided into four groups with each group containing (n=6), normal control, positive control and test groups 1 and 2. Reproductive toxicity was induced with SASP at a dose of 600mg/kg body weight by gavageto positive control and test groups 1 and 2 once daily for 3 days. The test groups 1 and 2 were treated with *passiflorafoetida* extract (200-400mg/kg body weight) once daily for 15 days. Blood sample was taken directly from the heart for biochemical analysis while the testis were surgically removed for histological analysis. Biochemical analysis revealed that SASP resulted in significant ($p<0.05$) decrease in CAT and increase in GPx levels in the positive control group. Histological analysis showed few spermatogenic cell with distortion of cellular architecture inthe positive control compared with the normal control. Animals treated simultaneously with *passiflorafoetida* extract, which fairly restored the CAT and GPx levels in test groups 1 and 2. These study reveals that sulfasalazine causes deleterious effect on fertility and biochemicalparameters, while *passiflorafoetida* extract shows promising results in the effect caused bysulfasalazine. Thus the study suggests that *passiflorafoetida* extract might have protective effect against oxidative stress, lipid peroxidation and impaired fertility.

KEYWORDS: *Sulfasalazine, Passiflorafoetida, inflammatory, Reproductive, Spermatogenic cell.*

INTRODUCTION

Medicinal plants have been used for many centuries for the treatment of both physical andmental illnesses to improve health of many individuals (Bent and Ko, 2004). Infertility is defined as the inability to conceive even after 12months of unprotected sexual intercourse.

Before the introduction of potent drugs, plant remedies were widely employed for the treatment of infertility. There has been renewed interest in identifying new infertility drugs from natural sources.

Passiflora Foetida (passion stinking flower) which belongs to the family passfloraceae, is an exotic medicinal vine which is extensively used in the Ivorian folk medicine (Adjanohoun *et al* 1997). *P. Foetida* has been used traditionally in countries like India, Malaysia, Argentina, Brazil, French guuana and in Africa for the treatment of different illments (Bleu *et al* 2012). A large genus of herbaceous or woody tendril climber (the wealth of India), mostly distributed in the warm temperature and tropical regions of the world but they are much rarer in Asia, Australia and tropical African (Beninca *et al* 2007).

The purpose of this study is to evaluate the antifertility properties of the plant *P. Foetida* and biochemical parameters on sulfasalazine induced reproductive toxicity in male albino rats.

Http://www.google.com/search?q=image+of+passiflora+foetida&client=ms opera

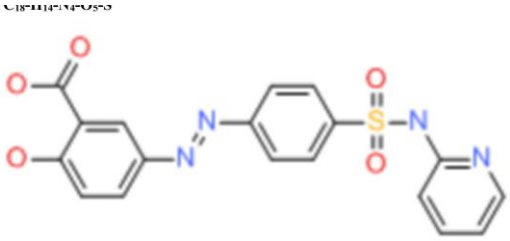
Passiflora foetida (passion stinking flower) belonging to the family passifloraceae, is an exotic medicinal vine which is extensively used in the Ivorian folk medicine (Adjanohoun *et al*, 1997). It is a fast growing and spreading vine found in riverbeds, dry forest floors and wayside thickets, covering the top of thorny shrubs. The extraction of leaves by boiling and unripe fruit is used to treat snakebites, women infertility, epilepsy, abscess and hysteria. The leaf paste is applied on the head for giddiness and headache, uteria, hepatitis, constipation and pains (Dhawan *et al* 2003).

In addition *P. Foetida* constitute of important phytochemicals such as passifloricins polyketides, alkaloids, phenols, glycoside flavoniods, cyanogenic compounds and alpha-pyrons (Dhawan *et al* 2004 and Echeverri *et al* 2001).

Biological activities of *P. foetida* include; antidepressant (Santos *et al* 2011), antimicrobial and antibacterial (Mohanasundari *et al* 2007), antihypertensive (Ranganatha *et al* 2013). Sasikila *et al*(2011) reported the anti-inflammatory activities and analgesic of *P. foetida* which provides bases for the use of the plant traditionally for the treatment of inflammatory diseases.

Phytochemical screening of ethanol extract of *P. foetida* leaves yielded anthraquinones steroids, polyterpenes, flavonoids, tanins, saponins, alkaloids and cardiac glycosides (Echeverri *et al.*, 2001 and Dhawan *et al.*, 2004).

Studies evaluated a methanolic extract of leaves of *P. Foetida.* for antidepressant activity in mice. Result showed does-dependent decrease in immobility time in both tail suspension and forced swim tests, with effects comparable to fluoxetine and imipramine (Santos *et al* 2011), result show antidepressant effects on vivo, with therapeutic interest for the use in treatment of patients with depressive disorders.



Sulfasalazine, like other sulfonamides, causes a characteristic idiosyncratic liver injury that has features of drug-allergy or hypersensitivity. The typical onset is sudden development of fever and rash followed by jaundice within a few days or weeks of starting the medication. Common side effects include anorexia, headache, nausea, gastrointestinal upset, fever, arthralgias and rash, as well as hemolysis, are associated with high serum sulfapyridine levels (Cosentino *et al.* 1984). It consists of sulphapyridine (SP) linked to 5 amino salicylic acid (5-ASA) by an azo bond. 5-ASA is the active therapeutic moiety of SASP, while the most adverse effects are related to SP, one of those is SASP-induced male infertility (Horimoto *et al.*, 2000). The actual mechanism bywhich this drug work is yet to be known but Fukushima *et al.*, (2004) investigated mechanisms of SASP-induced infertility, they found that sperm motility and a chromosome reaction were reduced by SASP. The drug's mechanism of action may relate to its effects on prostaglandin synthesis or interference with arachidonic acid metabolism by the lipoxygenase pathway

C_{18}-H_{14}-N_4-O_5-S

Chemical formula and structure of sulfasalazine

METHODS

Twenty-four (24) male albino rats of Wistar strain weighing 104g to 160g were used in the study. They were obtained from the animal house of the department of biochemistry, university of Port Harcourt, River state. Nigeria. They were kept in standard case in the animal house, in the Department of Biochemistry, Niger Delta University, Wilberforce Island, Bayelsa State and allow free access to pelleted growers and distilled water for a period of 2 weeks to acclimatize to the new environment.

Fresh whole plant was gotten from Nature Pure Laboratory Farm, Igboghene, Yenagoa, Bayelsa State, Nigeria and identified at Nature Pure Laboratory by a Botanist Prof. Alikwo Philip from the department of agriculture Niger Delta University, Wilberforce Island, Bayelsa State with the voucher number of NDU/FA/CS/PF.001

The drug was gotten from the department of biochemistry, Niger Delta University with the assistance of Prof. Sule, O.J., of the Department of Biochemistry, Niger Delta University, Amassoma. Bayelsa state. Nigeria.

The whole plant was washed with clean water overtime to remove dirt and dried for 7 days. The dried whole plant was homogenized into powder form with the aid or an electric blender. 300g of the powdered whole plant was extracted with 1.5litres of ethanol then stirred and allowed to stand at room temperature for a period of 3days with agitation every 24hours. The mixture was strained and the Marc pressed and filtered using a double chess net. The filtrate was collected on a beaker then concentrated on a water bath (60°c) for 48hours to yield thick blackish green paste. 23g of the paste was mixed with 100ml of normal saline water and stored in the refrigerator for further use 1500mg of the tablet was meshed in a plastic mortar and transferred into a container containing 60ml of normal saline water and covered properly, then stored in the refrigerator for further use. 24 male albino rats were grouped into four after acclimatization for one week. Each group contained six animals.

Group A (Normal control)
Received 200mg/kg body weight of normal saline water from day 4 - day 18 orally by gavage and also received pellet growers and table throughout the period of experiment.

Group B (Positive control)
Received 600mg/kg body weight of sulfasalazine from day 1- 3 orally by gavage after fasting from 6am-12noon and allowed free access to table water for a period of 3hours before giving pellet grower.

From day4-18 animals received 200mg/kg body weight of normal saline water at 12noon and allowed free access to pellet growers and table water without fasting throughout the period of experiment.

Group C (Test group 1)
Received 600mg/kg body weight of sulfasalazine from day 1- 3 orally by gavage after fasting from 6am- 12noon and allowed free access to table water for a period of 3hours before giving pellet grower.

From day 4-18 animals received 200mg/kg body weight of *passiflora foetida* orally by gavage at 12noon and allowed free access to pellet growers and table water without fasting throughout the period of experiment.

Group D (Test group 2)
Received 600mg/kg body weight of sulfasalazine from day 1- 3 orally by gavage after fasting from 6am- 12noon and allowed free access to table water for a period of 3hours before giving pellet grower.

From day4-18 animals received 400mg/kg body weight of *passiflora Foetida* orally by gavage at 12noon and allowed free access to pellet growers and table water without fasting throughout the period of experimented.

At the end of the experiment the body weight of each animal was recorded. The testes and epididymis from each animal were carefully dissected out and weighed independently.

After euthensing the animal with general anesthesia (chloroform), animals were dissected and blood removed directly from the heart with a 5ml syringe into plain bottle and stand overnight and centrifuged at 2500 rpm for 15 minutes, the serum was separated into another plain bottle, corked and stored in the freezer for biochemical and hormonal assays.

Determination of biochemical parameters
Catalase
Principle: Catalase is present in nearly all animal cells, plant and bacteria and acts to prevent accumulation of noxious H_2O_2 which is converted to O_2 and H_2O
I.e. $H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$

Peroxidase which are less widely distributed catalyze the above reaction.

NB. At high concentrations of low molecular weight alcohols or formaldehyde and low peroxide concentration, catalase exhibits peroxidative activity.

Procedure

	Sample	Blank	Spectrophotometer Standard
Sample	0.5	-	-
30mMH202	5.0	5.0	-
Distilled water	-	0.5	-

Mix by inversion, stand for 3 minutes

6M H2S04	**1.0**	**1.0**	**1.0**
0.05M PhosphateBuffer (pH7.4)	_	_	5.5

Mix by inversion

0.01M KMn04	7.0	7.0	7.0

Mix by inversion

Absorbance is read at 480nm 30 – 60 seconds against distilled H20.

CALCULATION

Abs of blank = Abs**B** Abs of spec std = Abs_{std} Abs of test = Abs_T

K

S_o = Abs_{std} - Abs_B S_3 = Abs_{std} - Abs_T S_o = [S] at zero time

S_3 = [S] at t = 3 minute

K_C = inverse log of $\frac{S_o}{S_3}$ x $\frac{203}{1}$

Glutathione Peroxidase (EC 1.11.1.7)

Principle

H_2O_2 + Pyrogallol $\xrightarrow{\text{Peroxidase}}$ $2H_2O$ + Purpurogallin

(donor) (oxidized donor)

Conditions: T = 20°C, pH = 6.0, A420nm, Light path = 1 cm

Method: Continuous Spectrophotometric Rate Determination

Reagents

A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 20°C (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 20°C with 1.0 M KOH.)

B. 0.50% (w/w) Hydrogen Peroxide Solution (H_2O_2) (Prepare 50 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **Prepare Fresh.**)

C. 5% (w/v) Pyrogallol Solution (Prepare 10 ml in deionized water using Pyrogallol, Sigma Prod. No. P-0381. **Prepare Fresh and Keep From Light**)
Peroxidase Enzyme Solution (Immediately before use, prepare a solution containing 0.4-0.7unit/ml of Peroxidase in cold Reagent A.)

Procedure

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Deionized Water	2.10	2.10
Reagent A (Buffer)	0.32	0.32
Reagent B (H_2O_2)	0.16	0.16
Reagent C (Pyrogallol)	0.32	0.32

Mix by inversion and equilibrate to 20°C. Monitor the A 420nm until constant, using a suitably

Thermostatted spectrophotometer.		
Then add: Reagent A (Buffer)	------	0.10
Reagent D (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in rA420nm for approximately 5 minutes. Obtain the rA420nm/20 seconds using the maximum linear rate 2 for both the Test and Blank.

CALCULATION

Units/ml enzyme (0.1) = (r A420nm/20 s Test - rA420nm/20 s Blank) (3) (df)
(12)
s = seconds
3 = Volume (in milliliters) of assaydf = Dilution factor
12 = Extinction coefficient 3 of 1 mg/ml of Purpurogallin at 420 nm
0.1 = Volume (in milliliters) of enzyme used Units/mg solid = units/ml enzyme
mg solid/ml enzyme Units/mg protein = units/ml enzyme mg protein/ml enzyme

Determination of hormonal profile

Follicle stimulating hormone (FSH)

Serum samples for FSH were measured for each rat using the enzyme-linked immunosorbent assay (ELISA) technique using the fortress kit at the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa state. Nigeria.

Testicular histology

Testes were removed, weighed and immediately fixed in 10% formalin for histological studies. The tissue samples were cleared in xylene and embedded in paraffin wax and sections were cut using 5-micron in a rotary microtome. The sections were then examined using the light microscope after staining with haematoxylin and eosin dyes and interpreted by an expert histologist.

Statistical analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparisons between the normal control, positive control and the treated groups were done using the student'st-test and $P<0.05$. Was set as significant.

RESULTS

The body weight of the animals, the testes and both epididymis were recorded in all control and test groups and result showed non-significant ($p>0.05$) decrease in body weight, testes and epididymis compared to positive control.

Table 1: Effects of sulfasalazine (SASP) and *Passiflora foetida* on body weight, testes weight and epididymis weight of male albino rats.

	Normal control	Positive control (SASP)	Test group 1 (SASP & PF)	Test group 2 (SASP &PF)
Body weight (g)	149.91±69.81[a]	150.38±69.89[b]	131.86±61.30[a]	132.48±61.63[a]
Testes weight (g)	2.6875±34.30[a]	2.62±34.24[a]	2.46±30.21[a]	2.27±30.45[b]
Epididymis weight (g)	0.96±35.51[a]	1.12±35.64[a]	1.21±31.90[a]	1.23±31.18[a]

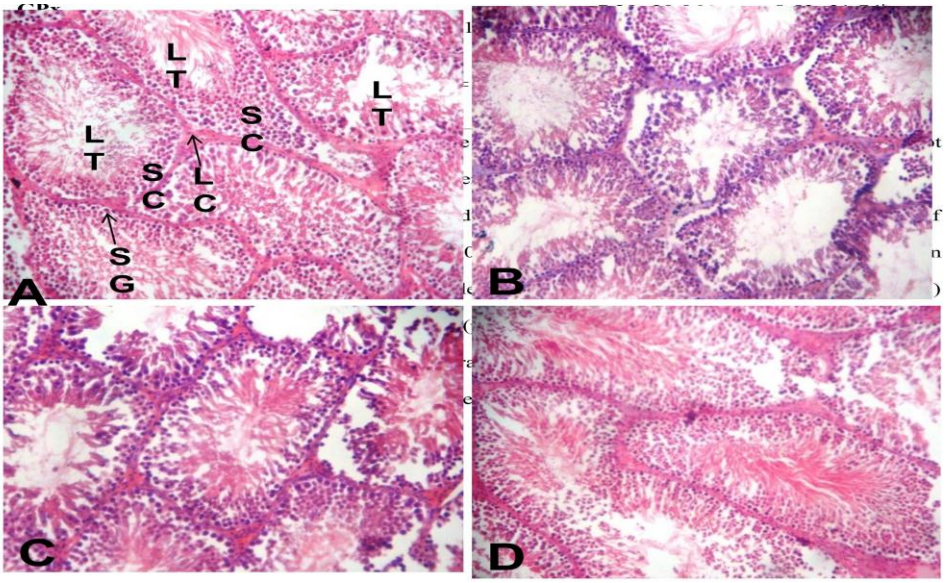
Values are recorded as MEAN±SD of triplicate determinations. Means with the same superscript letters on the same row are not statistically different at 95% confidence level ($p\leq0.05$).

Lipid peroxidation marked and antioxidant enzymes in blood serum of all groups shown in table 2. There was non-significant increase ($p>0.05$) of Catalase activities from 65.38±20.02 to 64.13±20.13. The GPx values of the positive control increases significantly when compared to the normal control while the test groups decreases non significantly when compared to positive control which shows promising result of plant *passiflora foetida* extract in reversing the effect ofsulfasalazine.

Table 2: Effect of sulfasalazine (SASP) and *passiflora foetida* Linn on catalase and glutathione peroxidase levels in male albino rats.

	Normal control	Positive control(SASP)	Test group 1 (SASP & PF)	Test group 2(SASP &PF)
CAT (unit/gram protein)	65.38±20.02[a]	44.13±20.06[b]	64.13±20.13[a]	39.35±21.75[a]
GPx (unit/gram protein)	8.75±19.87[a]	7.39±19.54[a]	7.39±20.06[a]	8.53±21.74[b]
FSH (uIU/ml)	0.58 ± 0.08[a]	0.70 ± 0.09[a]	1.07 ± 0.08[a]	1.22 ± 0.08[a]

Values are recorded as MEAN±SD of triplicate determinations. Means with the same superscript letters on the same row are not statistically different at 95% confidence level ($p\leq0.05$).



FSH levels are shown in table 2. The induction of rats with 600mg/kg bodyweight of sulfasalazine caused significant decrease ($p>0.05$) in serum FSH levels of positive control when compared with normal control. While FSH levels of the treated groups (test groups 1and2) showed dose dependent significant increase ($p>0.05$) compared to both normal and positive control. Therefore, it is clear that the administration of *Passiflora foetida* extract (200-400mg/kg bodyweight) can restore FSH levels in experimental rats.

Histopathology

Result showing testicular histology.

Photomicrographs of Hematoxylin and Eosin stained testis section. Magnification ×100.

Group A (normal Control): micrograph of tissue from rats administered with 200mg/kg bodyweight, slide shows normal lumen of seminiferous tubules (LT) with spermatogenic cells (SC), (Spermatogonia (SG) and Leydig cell (LC).

Group B (positive control): micrograph of testis from rats administered with 600mg/kg bodyweight of sulfasalazine shows few spermatogenic cells with distortion of cellular architecture when compared with the normal control.

Group C (test group1): micrograph of testis from rats administered with 600mg/kg of bodyweight sulfasalazine and 200mg/kg bodyweight of *Passiflora foetida* shows a mild improvement over Group B.

Group D (test grouo2): micrograph of testis from rats administered with 600mg/kg bodyweight of sulfasalazine and 400mg/kg bodyweight of *Passiflora foetida* is similar to the control.

DISCUSSION

The use of medicinal plants as a fundamental component of African traditional healthcare system is perhaps the oldest and most assorted of all therapeutic systems.

It is evident that FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogonia process. The androgens are necessary to induce meiosis formation and development of spermatids in response to FSH (Hall 1995). The study reveal that the *Passiflora foetida* reduced the effect caused by sulfasalazine in treated groups 1and 2 with a average of 1.0 and 1.2 while the positive control reduced to 0.7 compared to the normal controlof 0.6.

Free oxygen radicals produce by various agents such as LPS cause oxidative stress and cellular damage. Thiobarbituric acid reactivesubstances (TBARS) are detected in the cellular damage (Dik *et al*, 2016. and Tvarijonaviciute *et al*, 2012).

Notwithstanding the short period of exposure of animals to sulfasalazine, catalase activity in the positive control group was observed in this study. The normal control and test groups showed no significant difference which is attributed to the antioxidant and phytochemical constituents of *Passiflora foetida* (Antonio *et al*, 2019). Catalase in cultured cells and whole animals hasprovided protection against the deleterious effects of a wide range of oxidative stress paradigms (Warner 1994).

Showing the significant decrease ($p<0.05$) of glutathione peroxidase in the test groups compared to positive control suggests that the ethanol extract of *Passiflora foetida* is rich in antioxidants able to revive the effect of reactive species caused by sulfasalazine. Overexpression of GPx has been shown to be protective against oxidative stress in cultured cells and whole animals (Mirault *et al*, 1994. and Comhair *et al*, 2005). The decrease in bodyweight and organ weight observed in this study

correlate with the report of Baysal *et al*., (2017). The observed reduction in bodyweight is as a result of decrease in food intake of rats (table1).

Histopathology is the examination of biological tissues in order to observe the appearance of diseased cells in microscopic details. Ethanoic extract of *Passiflora foetida* shows promising results in increased sperm cell proliferation within the lumen in group B of testicular histology. Histological results from this study revealed that sulfasalazine is capable of causing infertility in rats even on short exposure since it affects leydigs cell production and the ethanoic extract of *Passiflora foetida* showed little protective effect at low dosage compared to the higher dose.

CONCLUSION

The findings from this study revealed that sulfasalazine is capable of causing deleterious effecton reproductive parameters in male albino rats and *Passiflora foetida* shows promising result in recovering the effect caused by sulfasalazine.

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