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Judul Artikel : Anti-hyperglikemic of *Urena lobata* leaf extract by inhibition of Dipeptidyl peptidase-4 (DPP-4) on diabetic rats

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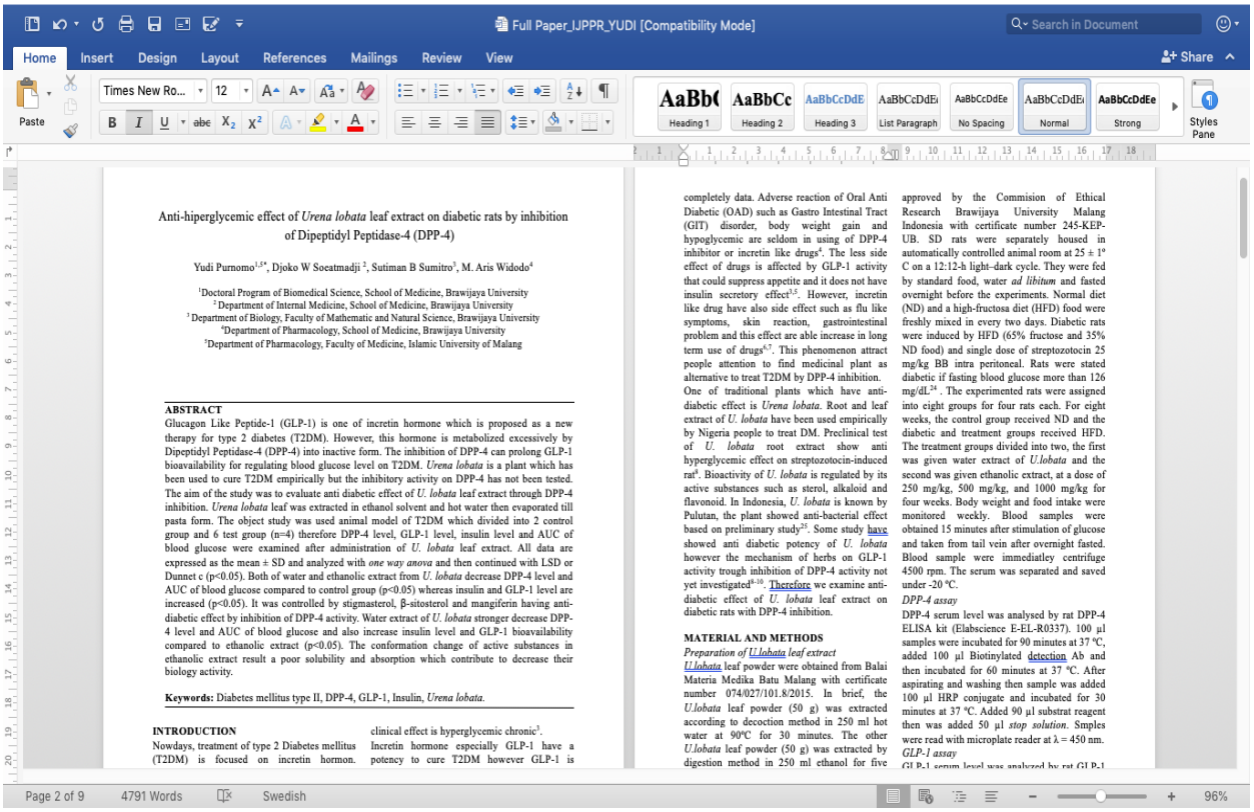

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Anti-hiperglycemic effect of *Urena lobata* leaf extract on diabetic rats by inhibition of Dipeptidyl Peptidase-4 (DPP-4)

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ABSTRACT

Glucagon Like Peptide-1 (GLP-1) is one of incretin hormone which is proposed as a new therapy for type 2 diabetes (T2DM). However, this hormone is metabolized excessively by Dipeptidyl Peptidase-4 (DPP-4) into inactive form. The inhibition of DPP-4 can prolong GLP-1 bioavailability for regulating blood glucose level on T2DM. *Urena lobata* is a plant which has been used to cure T2DM empirically but the inhibitory activity on DPP-4 has not been tested. The aim of the study was to evaluate anti diabetic effect of *U. lobata* leaf extract through DPP-4 inhibition. *Urena lobata* leaf was extracted in ethanol solvent and hot water then evaporated till pasta form. The object study was used animal model of T2DM which divided into 2 control group and 6 test group (n=4) therefore DPP-4 level, GLP-1 level, insulin level and AUC of blood glucose were examined after administration of *U. lobata* leaf extract. All data are expressed as the mean ± SD and analyzed with *one way anova* and then continued with LSD or Dunnet c ($p<0.05$). Both of water and ethanolic extract from *U. lobata* decrease DPP-4 level and AUC of blood glucose compared to control group ($p<0.05$) whereas insulin and GLP-1 level are increased ($p<0.05$). It was controlled by stigmasterol, β-sitosterol and mangiferin having anti-diabetic effect by inhibition of DPP-4 activity. Water extract of *U. lobata* stronger decrease DPP-4 level and AUC of blood glucose and also increase insulin level and GLP-1 bioavailability compared to ethanolic extract ($p<0.05$). The conformation change of active substances in ethanolic extract result a poor solubility and absorption which contribute to decrease their biology activity.

Keywords: Diabetes mellitus type II, DPP-4, GLP-1, Insulin, *Urena lobata*.

INTRODUCTION

Nowdays, treatment of type 2 Diabetes mellitus (T2DM) is focused on incretin hormon. clinical effect is hyperglycemic chronic[3]. Incretin hormone especially GLP-1 have a potency to cure T2DM however GLP-1 is completely data. Adverse reaction of Oral Anti Diabetic (OAD) such as Gastro Intestinal Tract (GIT) disorder, body weight gain and hypoglycemic are seldom in using of DPP-4 inhibitor or incretin like drugs[4]. The less side effect of drugs is affected by GLP-1 activity that could suppress appetite and it does not have insulin secretory effect[3,5]. However, incretin like drug have also side effect such as flu like symptoms, skin reaction, gastrointestinal problem and this effect are able increase in long term use of drugs[6,7]. This phenomenon attract people attention to find medicinal plant as alternative to treat T2DM by DPP-4 inhibition. One of traditional plants which have anti-diabetic effect is *Urena lobata*. Root and leaf extract of *U. lobata* have been used empirically by Nigeria people to treat DM. Preclinical test of *U. lobata* root extract show anti hyperglycemic effect on streptozotocin-induced rat[8]. Bioactivity of *U. lobata* is regulated by its active substances such as sterol, alkaloid and flavonoid. In Indonesia, *U. lobata* is known by Pulutan, the plant showed anti-bacterial effect based on preliminary study[25]. Some study have showed anti diabetic potency of *U. lobata* however the mechanism of herbs on GLP-1 activity trough inhibition of DPP-4 activity not yet investigated[8-16]. Therefore we examine anti-diabetic effect of *U. lobata* leaf extract on diabetic rats with DPP-4 inhibition.

MATERIAL AND METHODS

Preparation of U.lobata leaf extract

U.lobata leaf powder were obtained from Balai Materia Medika Batu Malang with certificate number 074/027/101.8/2015. In brief, the *U.lobata* leaf powder (50 g) was extracted according to decoction method in 250 ml hot water at 90ºC for 30 minutes. The other *U.lobata* leaf powder (50 g) was extracted by digestion method in 250 ml ethanol for five approved by the Commision of Ethical Research Brawijaya University Malang Indonesia with certificate number 245-KEP-UB. SD rats were separately housed in automatically controlled animal room at 25 ± 1º C on a 12:12-h light–dark cycle. They were fed by standard food, water *ad libitum* and fasted overnight before the experiments. Normal diet (ND) and a high-fructosa diet (HFD) food were freshly mixed in every two days. Diabetic rats were induced by HFD (65% fructose and 35% ND food) and single dose of streptozotocin 25 mg/kg BB intra peritoneal. Rats were stated diabetic if fasting blood glucose more than 126 mg/dL[24] . The experimented rats were assigned into eight groups for four rats each. For eight weeks, the control group received ND and the diabetic and treatment groups received HFD. The treatment groups divided into two, the first was given water extract of *U.lobata* and the second was given ethanolic extract, at a dose of 250 mg/kg, 500 mg/kg, and 1000 mg/kg for four weeks. Body weight and food intake were monitored weekly. Blood samples were obtained 15 minutes after stimulation of glucose and taken from tail vein after overnight fasted. Blood sample were immediatley centrifuge 4500 rpm. The serum was separated and saved under -20 ºC.

DPP-4 assay

DPP-4 serum level was analysed by rat DPP-4 ELISA kit (Elabscience E-EL-R0337). 100 µl samples were incubated for 90 minutes at 37 ºC, added 100 µl Biotinylated detection Ab and then incubated for 60 minutes at 37 ºC. After aspirating and washing then sample was added 100 µl HRP conjugate and incubated for 30 minutes at 37 ºC. Added 90 µl substrat reagent then was added 50 µl *stop solution*. Smples were read with microplate reader at λ = 450 nm.

GLP-1 assay

GLP-1 serum level was analyzed by rat GLP-1

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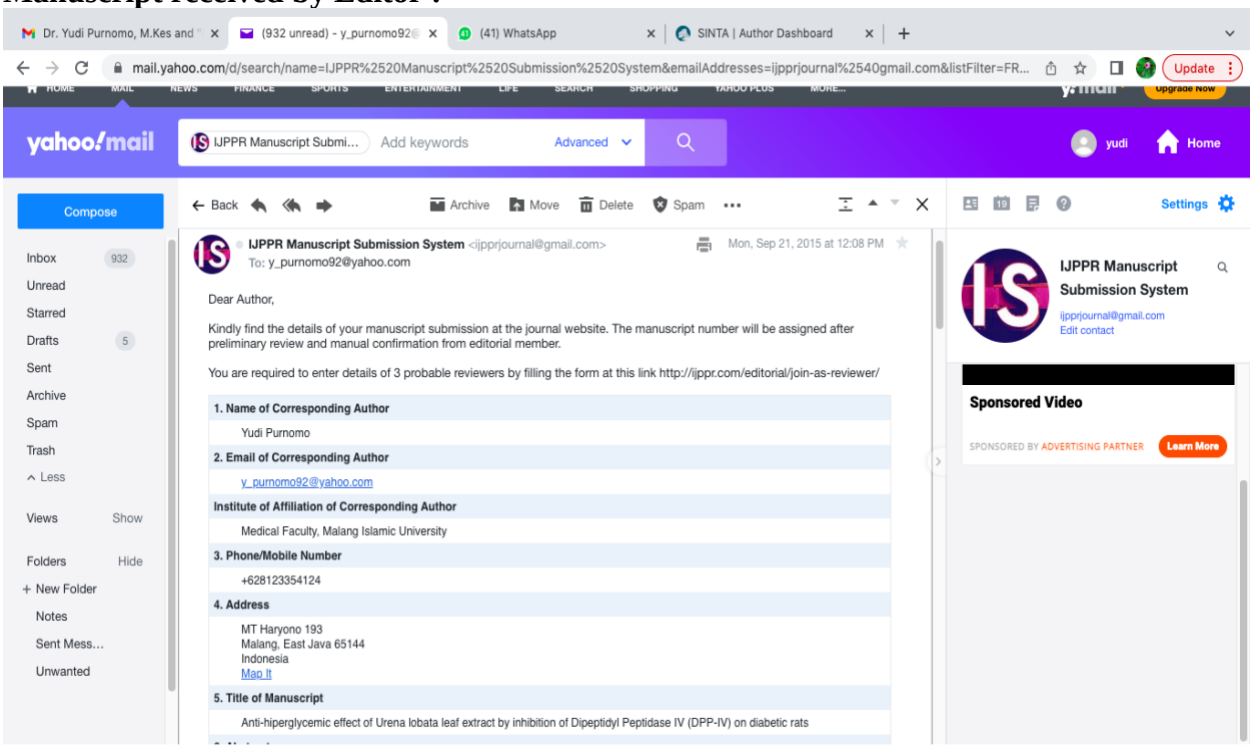

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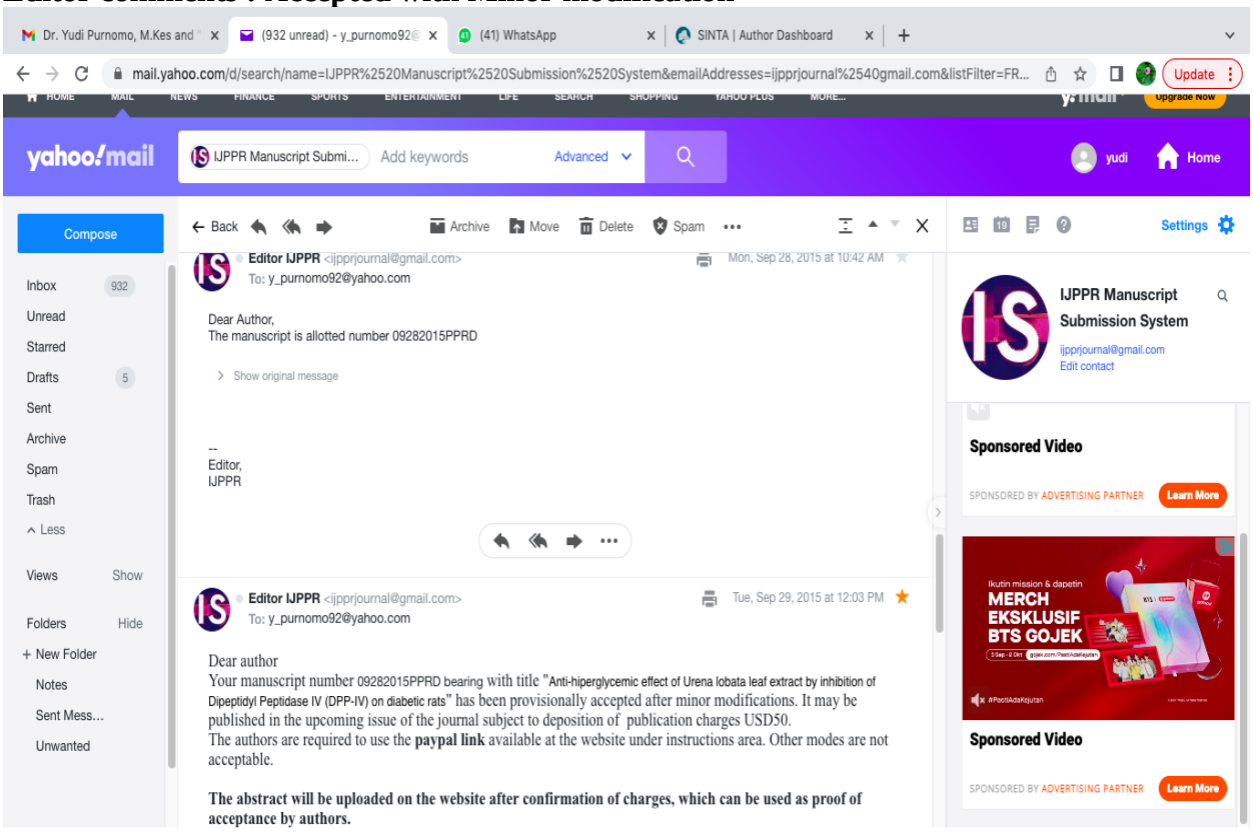

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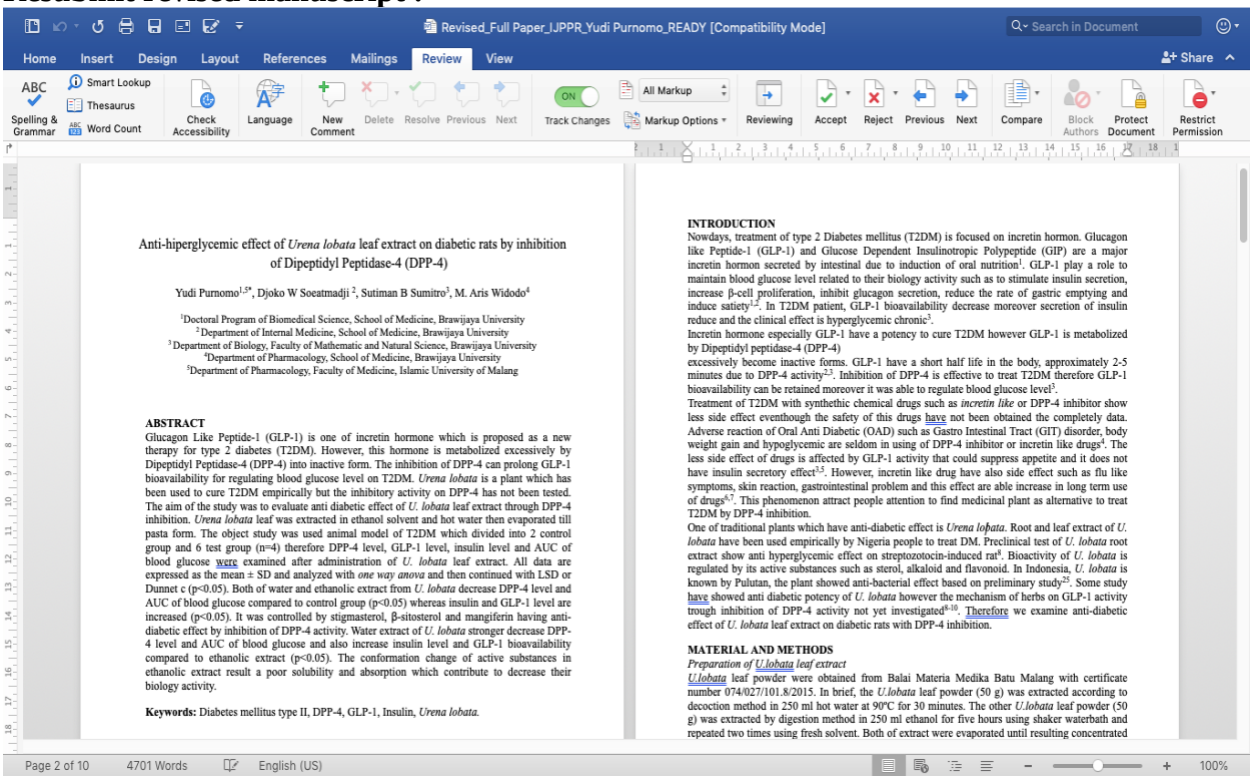

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Glucagon Like Peptide-1 (GLP-1) is one of incretin hormone which is proposed as a new therapy for type 2 diabetes (T2DM). However, this hormone is metabolized excessively by Dipeptidyl Peptidase-4 (DPP-4) into inactive form. The inhibition of DPP-4 can prolong GLP-1 bioavailability for regulating blood glucose level on T2DM. *Urena lobata* is a plant which has been used to cure T2DM empirically but the inhibitory activity on DPP-4 has not been tested. The aim of the study was to evaluate anti diabetic effect of *U. lobata* leaf extract through DPP-4 inhibition. *Urena lobata* leaf was extracted in ethanol solvent and hot water then evaporated till pasta form. The object study was used animal model of T2DM which divided into 2 control group and 6 test group (n=4) therefore DPP-4 level, GLP-1 level, insulin level and AUC of blood glucose were examined after administration of *U. lobata* leaf extract. All data are expressed as the mean ± SD and analyzed with *one way anova* and then continued with LSD or Dunnet c ($p<0.05$). Both of water and ethanolic extract from *U. lobata* decrease DPP-4 level and AUC of blood glucose compared to control group ($p<0.05$) whereas insulin and GLP-1 level are increased ($p<0.05$). It was controlled by stigmasterol, β-sitosterol and mangiferin having anti-diabetic effect by inhibition of DPP-4 activity. Water extract of *U. lobata* stronger decrease DPP-4 level and AUC of blood glucose and also increase insulin level and GLP-1 bioavailability compared to ethanolic extract ($p<0.05$). The conformation change of active substances in ethanolic extract result a poor solubility and absorption which contribute to decrease their biology activity.

Keywords: Diabetes mellitus type II, DPP-4, GLP-1, Insulin, *Urena lobata*.

INTRODUCTION

Nowdays, treatment of type 2 Diabetes mellitus (T2DM) is focused on incretin hormon. Glucagon like Peptide-1 (GLP-1) and Glucose Dependent Insulinotropic Polypeptide (GIP) are a major incretin hormon secreted by intestinal due to induction of oral nutrition[1]. GLP-1 play a role to maintain blood glucose level related to their biology activity such as to stimulate insulin secretion, increase β-cell proliferation, inhibit glucagon secretion, reduce the rate of gastric emptying and induce satiety[1,2]. In T2DM patient, GLP-1 bioavailability decrease moreover secretion of insulin reduce and the clinical effect is hyperglycemic chronic[3].
Incretin hormone especially GLP-1 have a potency to cure T2DM however GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4)
excessively become inactive forms. GLP-1 have a short half life in the body, approximately 2-5 minutes due to DPP-4 activity[2,3]. Inhibition of DPP-4 is effective to treat T2DM therefore GLP-1 bioavailability can be retained moreover it was able to regulate blood glucose level[3].
Treatment of T2DM with synthethic chemical drugs such as *incretin like* or DPP-4 inhibitor show less side effect eventhough the safety of this drugs have not been obtained the completely data. Adverse reaction of Oral Anti Diabetic (OAD) such as Gastro Intestinal Tract (GIT) disorder, body weight gain and hypoglycemic are seldom in using of DPP-4 inhibitor or incretin like drugs[4]. The less side effect of drugs is affected by GLP-1 activity that could suppress appetite and it does not have insulin secretory effect[3-5]. However, incretin like drug have also side effect such as flu like symptoms, skin reaction, gastrointestinal problem and this effect are able increase in long term use of drugs[6,7]. This phenomenon attract people attention to find medicinal plant as alternative to treat T2DM by DPP-4 inhibition.
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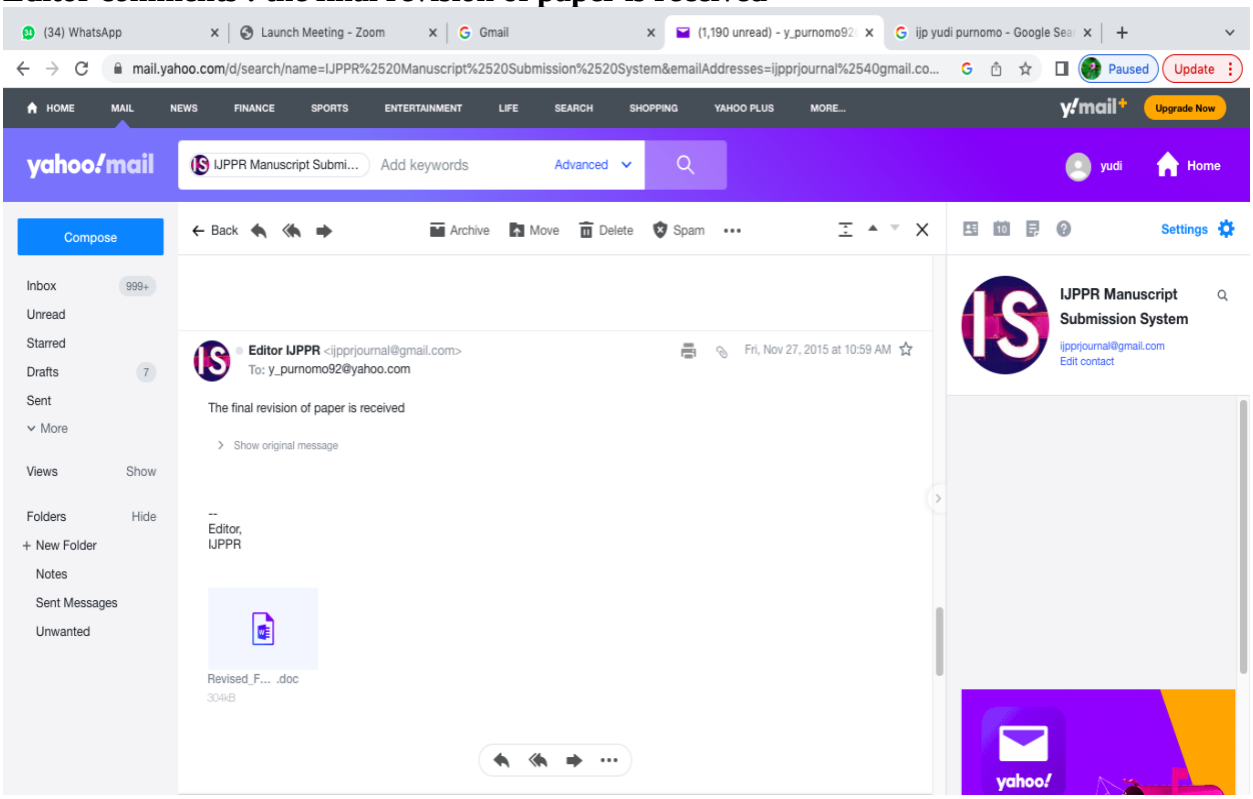

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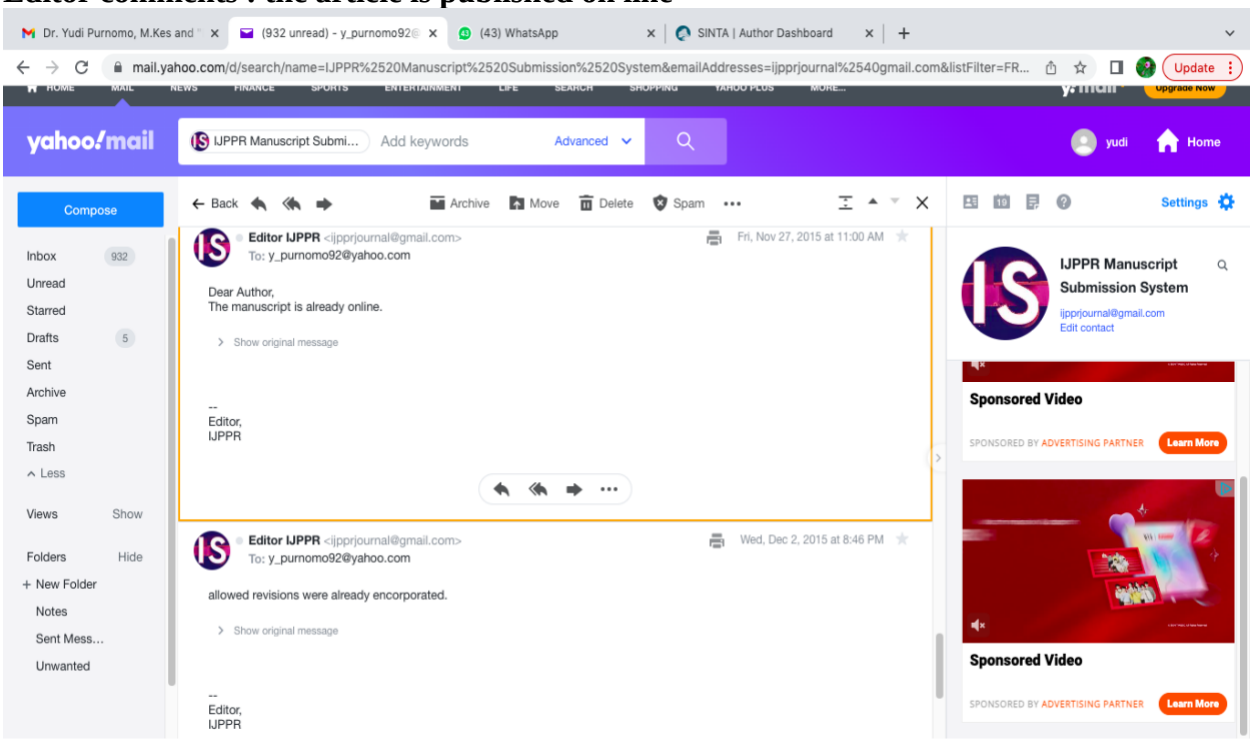

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Research Article

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